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Effects of Varieties, Cultivation Methods, and Origins of *Citrus sinensis* 'hongjiang' on Volatile Organic Compounds: HS-SPME-GC/MS Analysis Coupled with OPLS-DA

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Abstract: Volatile organic compounds (VOCs) in *Citrus sinensis* 'hongjiang' oranges significantly vary depending on the fruit variety, cultivation mode, and cultivation location. The effect of these three factors on VOCs was experimentally determined in this study. In total, 102 VOCs were separated via headspace solid-phase microextraction and identified via gas chromatography-mass spectrometry, and the differential components were analyzed by partial least-squares-discriminant analysis (OPLS-DA). The VOCs of 'hongjiang' mainly comprised alkenes, alcohols, aldehydes, and ketones. They were well clustered in OPLS-DA and principal component analysis (PCA), and the seven groups were distinctly differentiated. The results of the S-plot, variable importance in projection (VIP), and heatmap analyses showed that these factors had a significant impact on VOCs in 'hongjiang'. The characteristic VOCs between the two pairs were significant, while the net room cultivation mode had the most substantial effect on VOCs.

Keywords: *Citrus sinensis* 'hongjiang'; gas chromatography-mass spectrometer; volatile organic compound; species

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1. Introduction

Citrus sinensis 'hongjiang' is a genus of citrus in the Rutaceae family, a unique variety of sweet orange in China. It is native to Hongjiang Farm, located in Lianjiang City, Guangdong Province, and its planting area has exceeded 600 hectares. Because of its large shape, thin and smooth skin, orange-red flesh, tender flesh, juicy dregs, moderate sweetness and acidity, and unique flavor, this fruit (hereinafter referred to as 'hongjiang') is known as the "Fairy Peach on Earth" in China and "The King of Chinese Oranges" abroad [1].

Citrus is popular among consumers for its unique flavor, color, and nutritional composition, where the flavor is one of the most important characteristics affecting the quality of the fruit and the most significant quality parameter of natural food or processed products [2]. Volatile aroma components (VOCs) are secondary metabolites formed during fruit ripening, most of which are derived from a series of enzymatic reactions during the growth and development of fruits, with fatty acids, amino acids, carbohydrates, and other precursors. Olefins, esters, aldehydes, alcohols, and ketones are the main components of the fruit free-state aroma. Factors such as fruit development [3,4], variety [5,6], ripeness [7], processing [8], and storage process [9] can have a considerable influence on the type and content of orange aroma. Tang et al. [10] studied the large variability in volatile aroma components of five commercially available navel oranges, namely Fengjie New Holland, Fengjie Navel, Gannan Navel, Changhong Navel, and Beibei Navel. Arena et al. [11] isolated and identified 22 odoriferous active compounds from Moro, Tarocco, Washington navel, and Valencia late oranges, and revealed differential active compounds in these varieties. Zhang et al. [12] reported that 'Olinda' summer orange showed major differences in the aroma of d-limonene, β-Laurene, decanal, octanal, and valencene during low-temperature refrigeration at 7 °C. Among them, d-limonene, β-Laurene, and decanal showed a significant decrease in aroma content after three months of storage in summer oranges. It was also determined that the preservation measures of three preservatives (bacodyl, imipramine, and 2,4-dichlorophenol sodium salt), combined with nanopreservation bags, effectively preserved the basic quality of summer oranges during storage, significantly inhibiting any changes in their main aroma components within the first month of storage. However, the volatile aroma characteristics of 'hongjiang' have rarely been reported.

Liberobacterasiaticum is a fatal factor limiting the development of the orange industry, and once it is contracted, it tends to spread over a large area of orange orchards1 [13–15]. After years of research and development, the Scientific Research Institute of Zhanjiang State Farms, China, has effectively developed various techniques, such as a net room cultivation mode, to curb the spread of liberobacterasiaticum [16]. However, there are few reports on whether the new cultivation methods impact the quality of oranges. In addition, preliminary experiments revealed that the differential performance of the cultivation location (Red River Orange Farm and Qingping Town) led to differences in the flavor of 'hongjiang'. Still, few reports have revealed their differential characteristics. In this study, HS-SPME-GC-MS combined with the OPLS-DA method was used to assess the effects of three different factors (variety, cultivation mode, and cultivation location) on the VOCs of 'hongjiang', and to explore the differential components to provide basic data support for the development of the 'hongjiang' orange industry.

2. Materials and Methods

2.1. Information on the Samples

This study's main 'hongjiang' varieties were multinuclear and oligonuclear species, collected from three cultivation locations: Red River Orange Team 38, Qingping Township, and Chengyu Township. The following four cultivation modes were used: open-air cultivation, "half-net room" (i.e., "open-air" mode for one year after net room storage), "little net room", and "big net room". Samples of 'hongjiang' were collected in clean sealed bags after picking and stored at 4 °C in the refrigerator for use, and three parallel samples were collected from each group.

The following designations of samples were adopted:

PRHN (1,2,3), PRGN (1,2,3), and PRNN (1,2,3) are multinuclear species from the Red River Farm Team 38 cultivation location, produced by half-net room, little-net room, and big-net room cultivation methods, respectively. MRNN indicates that the sample's cultivation location was Red River Orange Team 38, and the cultivation mode was 'big net room'. PROY (1,2,3), PQOY (1,2,3), and PCON (1,2,3) indicate that the varieties were multinuclear species, that their cultivation mode was open-air cultivation, and that their cultivation locations were Red River Orange Team 38, Qingping Town, and Chengyu Town, respectively.

2.2. Volatile Organic Compound Composition Analysis

Sample pretreatment and injection conditions. Only uniform and undamaged 'hongjiang' samples were selected, and the juice was extracted after peeling and removing the core. A volume of 10 mL of juice was pipetted into a 20 mL headspace vial, sealed, and mixed well. The 50/30 μm DVB/CAR/PDMS extraction headspace was extracted at 55 °C for 30 min with an equilibration time of 5.0 min, and thermal desorption was performed at 220 °C for 5.0 min, which was started together with the acquisition. The data were collected together with the start-up.

GC-MS analysis conditions. The gas chromatograph was equipped with an HP-5ms column (30 m × 0.25 mm, 0.25 μm) and analytical parameters referenced by Tu et al. with some modifications [17]. The inlet temperature was 250 °C; the sample was passed through the column under the action of helium using a ramp-up procedure of starting at 40 °C, holding for 3 min, ramping up to 160 °C at 3 °C/min, holding for 3 min, ramping up to 240 °C at 8 °C/min, and holding for 3 min. The injection mode was non-split, with a total flow rate of 10.0 mL/min, a column flow rate of 1.11 mL/min, a linear velocity of 38 cm/s, a purge flow rate of 3.0 mL/min, and a shunt ratio of −1.0. The analytical conditions of the mass spectrometer were 230 °C for the ion source, 280 °C for the interface, 3.50 min for the solvent delay time, and 35~350 amu for the mass scan range (m/z).

2.3. Data Processing and Analysis

Retention time (RI) Calculation. The C7–C30 normal paraffin standards were prepared with n-hexane as the solvent. The retention time of each substance was determined by a solution with an integral of 0.1%. The RI of each component was calculated as follows:

$$RI = 100_n + 100 \times (t_x - t_n)/(t_{n+1} - t_n) \quad (1)$$

where t_x is the retention time of the outflow peak of the analyzed component/min, t_n is the retention time/min of the outflow peak of n-alkanes with carbon atoms, t_{n+1} is the retention time/min of the outflow peak of n + 1 carbon atoms, and $t_n < t_x < t_{n+1}$.

All samples were taken three times in parallel and then measured, and compounds that appeared in at least two parallel tests were selected for analysis. The data obtained were analyzed qualitatively using the NIST Chemical Structures library (2014) and the Wiley Library library (9), and the VOC components were quantified using the peak area normalization method. OriginPro 2021 (Northampton, MA, USA), SIMCA 14.1 (Goettingen, Germany), and Photoshop 2019 (Los Angeles, CA, USA) were used for plotting, data processing, and statistical analysis.

3. Results and Discussion

3.1. Effect of Different Factors on the Composition and Relative Content of Volatile Organic Compounds in 'hongjiang'

To reduce the interfering effect of volatile essential oils in the peel of 'hongjiang', the juice in the pulp of 'hongjiang' after peeling was selected for this experiment. As shown in Tables 1 and S1, a total of 102 VOCs were identified from the juice of 'hongjiang' by HS-SPME-GC/MS, mainly consisting of alkenes, alcohols, aldehydes, and ketones, which were consistent with the typical characteristics of citrus flavor components [18]. In total, 71, 60, 74, 70, 73, 72, and 56 VOCs were identified from PRHN, PRGN, PRNN, MRNN, PROY, PQOY, and PCON, respectively. As shown in Figure 1A, the highest number of alkene and alcohol compounds was found in PRNN, the highest number of esters was in MRNN, PROY and PQOY, and the highest number of ketones was observed in PRHN. Figure 1B shows that the PRHN sample was the richest in aldehydes, PCON in alcohols, and PRGN in alkenes, ketones, and esters, proving that cultivation modes significantly affected the types and contents of VOCs in 'hongjiang'.

The results of the significant analysis of VOCs in the 'hongjiang' juice samples showed that the differences in the proportion of esters, alkenes, and ketones in 'hongjiang' between the half-net room modes (PRHN), little-net room (PRGN), and big-net room (PRNN) cultivation modes were not significant, in contrast to that of alcohols and aldehydes.

The difference in the proportion of alcohols, esters, alkenes, and ketones in 'hongjiang' samples from Red River Orange Farm Team 38 (PROY) and Qingping Town (PQOY) was not significant, in contrast to that of aldehydes.

The difference in the proportion of aldehydes, esters, alkenes, and ketones was insignificant between the varieties with multinuclear species (PRNN) and oligonuclear species (MRNN), and the difference in the proportion of alcohols was significant. In addition, as shown in Figure 1A,B, all three factors affected the number of species and content of VOCs in 'hongjiang' juice. Still, there was no significant correlation between the trends of the species and the content of VOCs.

Table 1. The composition and relative contents of volatile organic compounds (VOCs) among *Citrus sinensis* 'hongjiang'.

No	Name	PRHN	PRGN	PRNN	MRNN	PROY	PQOY	PCON	Identification Method	Classification
1	Hexanal	0.686 ± 0.036	0.524 ± 0.227	0.565 ± 0.029	0.346 ± 0.028	0.417 ± 0.002	0.341 ± 0.015	0.333 ± 0.019	RI, MS	Aldehydes
2	ethyl butanoate	0.093 ± 0.005	0.073 ± 0	0.035 ± 0.001	0.034 ± 0.002	0.058 ± 0.001	0.043 ± 0.007	0.044 ± 0.006	RI, MS	Esters
3	Hex-2-enal	0.293 ± 0.02	0.442 ± 0.242	0.308 ± 0.009	0.191 ± 0.008	0.311 ± 0.008	0.165 ± 0.036	0.152 ± 0.007	RI, MS	Aldehydes
4	Hex-3-en-1-ol	0	0	0	0	0.028 ± 0.001	0	0	RI, MS	Alcohols
5	Hexan-1-ol	0.292 ± 0.018	0.152 ± 0.052	0.103 ± 0.014	0.11 ± 0.009	0.131 ± 0.014	0.075 ± 0.02	0.087 ± 0.007	RI, MS	Alcohols
6	Heptanal	0.07 ± 0.004	0.054 ± 0.024	0.029 ± 0.001	0.033 ± 0.002	0.166 ± 0.207	0.033 ± 0.003	0.035 ± 0	RI, MS	Aldehydes
7	α-Thujene	0.048 ± 0.007	0	0.085 ± 0.006	0	0.045 ± 0.004	0.078 ± 0.015	0	RI, MS	Alkenes
8	α-Pinene	0.61 ± 0.015	0.279 ± 0.138	0.418 ± 0.02	0.407 ± 0.022	0.372 ± 0.003	0.617 ± 0.204	0.523 ± 0.058	RI, MS	Alkenes
9	Sabinene	0.169 ± 0.054	0	0.316 ± 0.011	0.275 ± 0.01	0.143 ± 0.027	0.271 ± 0.127	0.198 ± 0.074	RI, MS	Alkenes
10	β-Myrcene	1.839 ± 0.02	1.743 ± 1.189	2.581 ± 0.074	2.974 ± 0.136	2.529 ± 0.026	3.664 ± 0.077	3.617 ± 0.112	RI, MS	Alkenes
11	Octanal	0.489 ± 0.025	0.4 ± 0.039	0.405 ± 0.025	0.343 ± 0.013	0.428 ± 0.007	0.611 ± 0.073	0.656 ± 0.015	RI, MS	Aldehydes
12	Car-3-ene	0.353 ± 0.022	0.21 ± 0.101	0.409 ± 0.013	0.452 ± 0.022	0.401 ± 0.004	0.527 ± 0.18	0.446 ± 0.022	RI, MS	Alkenes
13	α-Terpinene	0.092 ± 0.013	0	0	0	0	0	0	RI, MS	Alkenes
14	Limonene	34.709 ± 7.491	31.316 ± 0.929	50.189 ± 1.159	50.159 ± 1.187	48.438 ± 0.427	61.694 ± 7.259	58.505 ± 1.011	RI, MS	Alkenes
15	β-Ocimene	0.106 ± 0.017	0.23 ± 0.346	0.104 ± 0.009	0.109 ± 0.005	0.061 ± 0.001	0.072 ± 0.01	0.066 ± 0.001	RI, MS	Alkenes
16	γ-Terpinene	0	0	0.367 ± 0.005	0.42 ± 0.02	0.325 ± 0.003	0.176 ± 0.269	0	RI, MS	Alkenes
17	2-Octen-1-ol	0.038 ± 0.001	0	0	0	0	0.347 ± 0.281	0.025 ± 0.001	RI, MS	Alcohols
18	1-Octanol	0.315 ± 0.003	0.179 ± 0.069	0.287 ± 0.006	0.324 ± 0.011	0.273 ± 0.01	0.643 ± 0.277	0.831 ± 0.03	RI, MS	Alcohols
19	7-methyloct-3-yne	0	0	0	0	0.027 ± 0.001	0	0	RI, MS	Alkenes
20	α-Terpinolene	0.246 ± 0.017	0.324 ± 0.198	0.336 ± 0.038	0.26 ± 0.023	0.352 ± 0.014	0.383 ± 0.064	0	RI, MS	Alkenes
21	Linalool	1.991 ± 0.025	0.584 ± 0.067	1.954 ± 0.025	2.144 ± 0.031	2.043 ± 0.023	2.339 ± 0.533	2.732 ± 0.127	RI, MS	Alcohols
22	1-Nonanal	0.464 ± 0.015	0.488 ± 0.047	0.235 ± 0.005	0.175 ± 0.004	0.261 ± 0.006	0.355 ± 0.063	0.428 ± 0.038	RI, MS	Aldehydes
23	*p*-Mentha-1,5,8-triene	0	0	0.028 ± 0.003	0.04 ± 0.002	0.038 ± 0.001	0.034 ± 0.005	0.042 ± 0.004	RI, MS	Alkenes
24	4,8-Dimethylnona-1,3,7-triene	0.038 ± 0	0.113 ± 0.064	0.068 ± 0.005	0.067 ± 0.002	0.04 ± 0.002	0.029 ± 0.005	0	RI, MS	Alkenes
25	1-methyl-4-(1-methylethenyl)-2-Cyclohexene-1-ol	0.059 ± 0	0	0.045 ± 0.001	0.053 ± 0.003	0.051 ± 0.003	0.042 ± 0.005	0.048 ± 0.001	RI, MS	Alcohols
26	Ethyl 3-hydroxyhexanoate	0.429 ± 0.018	0	0	0.055 ± 0.005	0.215 ± 0.001	0.04 ± 0.021	0.092 ± 0.01	RI, MS	Esters
27	4-Acetyl-1-methylcyclohexene	0	0.101 ± 0.007	0.07 ± 0.043	0.07 ± 0.004	0	0.053 ± 0.005	0.069 ± 0.003	RI, MS	Alkenes
28	*cis-p*-Mentha-2,8-dien-1-ol	0.048 ± 0.011	0	0.024 ± 0.006	0	0.045 ± 0	0	0.046 ± 0.003	RI, MS	Alcohols
29	Citronellal	0	0	0.058 ± 0.015	0	0.045 ± 0.002	0	0	RI, MS	Aldehydes
30	6-Nonenal	0.131 ± 0.012	0.086 ± 0.149	0.041 ± 0.001	0.045 ± 0.001	0.097 ± 0.01	0.04 ± 0.002	0	RI, MS	Aldehydes
31	2-Nonenal	0	0.06 ± 0.079	0	0	0	0	0	RI, MS	Aldehydes
32	2-None-1-ol	0.043 ± 0.003	0	0	0	0	0	0	RI, MS	Alcohols
33	nonan-1-ol	0.14 ± 0.006	0.166 ± 0.036	0.052 ± 0.008	0.092 ± 0.003	0.107 ± 0.004	0.171 ± 0.048	0.224 ± 0.011	RI, MS	Alcohols
34	4-methyl-1-(1-methylethyl)-3-Cyclohexen-1-ol	0.502 ± 0.01	0.323 ± 0.108	0.437 ± 0.048	0.656 ± 0.012	0.511 ± 0.01	0.704 ± 0.118	0.917 ± 0.06	RI, MS	Alcohols
35	Octanoic acid	0	0	0	0	0	0	0.05 ± 0.002	RI, MS	Acids
36	Isogeranial	0	0	0.041 ± 0.001	0.044 ± 0.001	0	0.03 ± 0.005	0	RI, MS	Alcohols
37	Cryptone	0	0	0	0	0.056 ± 0.003	0	0	RI, MS	Ketones
38	α-Terpineol	0.394 ± 0.02	0.122 ± 0.055	0.414 ± 0.019	0.402 ± 0.007	0.376 ± 0.018	0.429 ± 0.061	0.508 ± 0.003	RI, MS	Alcohols
39	Ethyl octanoate	0.199 ± 0.002	0.12 ± 0.05	0.21 ± 0.005	0.089 ± 0.005	0.122 ± 0.002	0.066 ± 0.011	0.082 ± 0.001	RI, MS	Esters
40	2-methyl-5-(1-methylethenyl)-Cyclohexanone	0.09 ± 0.003	0	0.076 ± 0.014	0.069 ± 0.001	0.117 ± 0.001	0.053 ± 0.004	0.091 ± 0.004	RI, MS	Ketones
41	Decanal	0.489 ± 0.02	0.833 ± 0.704	0.631 ± 0.021	0.345 ± 0.008	0.598 ± 0.018	0.975 ± 0.171	0.815 ± 0.063	RI, MS	Alkenes
42	2,4-Dodecadienal	0	0	0	0	0	0.104 ± 0.016	0	RI, MS	Ketones
43	2,4-Nonadienal	0.158 ± 0.002	0.113 ± 0.01	0	0.121 ± 0.004	0.108 ± 0.001	0	0	RI, MS	Aldehydes
44	Carveol	0.163 ± 0.003	0.089 ± 0.037	0.094 ± 0.008	0.154 ± 0.005	0.152 ± 0.001	0.176 ± 0.008	0.199 ± 0.005	RI, MS	Alcohols
45	β-Citronellol	0.802 ± 0.026	0.386 ± 0.074	0.756 ± 0.046	1.165 ± 0.041	0.672 ± 0.009	0.685 ± 0.225	0.856 ± 0.048	RI, MS	Alcohols
46	Z-Citral	1.147 ± 0.035	0	1.022 ± 0.018	1.349 ± 0.039	1.001 ± 0.028	1.128 ± 0.039	0	RI, MS	Aldehydes
47	Carvone	0.629 ± 0.033	0	0.421 ± 0.011	0.6 ± 0.015	0.681 ± 0.035	0.538 ± 0.098	0.495 ± 0.012	RI, MS	Ketones
48	Geraniol	0.214 ± 0.011	0.192 ± 0.071	0.177 ± 0.023	0.294 ± 0.016	0.17 ± 0.007	0.195 ± 0.007	0.209 ± 0.011	RI, MS	Alcohols
49	7-methoxy-3,7-dimethyl-Octanal	0.154 ± 0.004	0	0	0	0	0.052 ± 0.027	0	RI, MS	Aldehydes
50	3,7-dimethyl-2,6-Octadienal	1.877 ± 0.014	0	1.706 ± 0.017	2.179 ± 0.095	1.737 ± 0.014	2.291 ± 0.357	0	RI, MS	Aldehydes

Table 1. *Cont.*

No	Name	PRHN	PRGN	PRNN	MRNN	PROY	PQOY	PCON	Identification Method	Classification
51	*p*-Mentha-1(7),8(10)-dien-9-ol	0	0	0	0	0	0.026 ± 0.008	0.032 ± 0.006	RI, MS	Alcohols
52	2,4-Decadienal	0.183 ± 0.004	0.296 ± 0.161	0.202 ± 0.011	0	0.149 ± 0.002	0.058 ± 0.019	0	RI, MS	Aldehydes
53	Undecanal	0.072 ± 0.003	0.127 ± 0.097	0.074 ± 0.003	0.059 ± 0.002	0.088 ± 0.001	0.118 ± 0.015	0.1 ± 0.011	RI, MS	Alkenes
54	Methyl geranate	0	0	0	0	0.03 ± 0.003	0.038 ± 0	0.037 ± 0.003	RI, MS	Esters
55	1-methyl-4-(1-methylethenyl)-1,2-Cyclohexanediol	0	0	0.051 ± 0.009	0	0	0.03 ± 0.008	0	RI, MS	Alcohols
56	α-Copaene	0.128 ± 0.025	0.159 ± 0.057	0.218 ± 0.016	0.176 ± 0.002	0.087 ± 0.005	0.134 ± 0.025	0.133 ± 0.007	RI, MS	Alkenes
57	Citronellyl acetate	0	0	0.032 ± 0.001	0.039 ± 0.001	0	0	0	RI, MS	Esters
58	2-Undecenal	0	0	0	0.04 ± 0.009	0	0.033 ± 0.006	0.035 ± 0.003	RI, MS	Aldehydes
59	8-undecen-1-al	0.083 ± 0.003	0.083 ± 0.033	0	0	0.072 ± 0.003	0	0	RI, MS	Aldehydes
60	Neryl acetate	0	0	0.037 ± 0.013	0.037 ± 0.003	0.028 ± 0.001	0.021 ± 0.002	0	RI, MS	Esters
61	*n*-Decanoic acid	0	0	0	0	0	0.016 ± 0.028	0.045 ± 0.003	RI, MS	Acids
62	β-Element	0.363 ± 0.144	0.403 ± 0.165	0.555 ± 0.114	0.261 ± 0.003	0.207 ± 0.011	0.117 ± 0.015	0	RI, MS	Alkenes
63	Ethyl decanoate	0	0	0	0	0	0.024 ± 0.004	0.025 ± 0.001	RI, MS	Esters
64	Dodecanal	0.145 ± 0.006	0.331 ± 0.257	0.203 ± 0.013	0.151 ± 0.011	0.228 ± 0.016	0.387 ± 0.06	0.388 ± 0.032	RI, MS	Aldehydes
65	*trans*-Caryophyllene	0	0	0.694 ± 0.028	0	0.976 ± 0.023	0	0	RI, MS	Alkenes
66	Caryophyllene	0.955 ± 0.024	1.104 ± 0.449	0	0.796 ± 0.018	0	0.473 ± 0.097	0.422 ± 0	RI, MS	Alkenes
67	Germacrene D	0	0.055 ± 0.027	0	0	0	0.103 ± 0.012	0	RI, MS	Alkenes
68	1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl)-azulene	0	0.045 ± 0.02	0.029 ± 0.007	0	0	0	0	RI, MS	Alkenes
69	decahydro-1,1,7-trimethyl-4-methylene-1H-Cycloprop-azulene	1.215 ± 0.035	0.745 ± 0.979	0.937 ± 0.028	0.86 ± 0.038	1.057 ± 0.019	0.508 ± 0.118	0.45 ± 0.007	RI, MS	Alkenes
70	6,10-dimethyl-5,9-Undecadien-2-one	0.465 ± 0.017	0.826 ± 0.199	0	0.382 ± 0.027	0	0.31 ± 0.033	0.292 ± 0.001	RI, MS	Ketones
71	linalyl acetate	0	0	0.3 ± 0.007	0	0.369 ± 0.001	0	0	RI, MS	Esters
72	Farnesene	0.151 ± 0.013	0.107 ± 0.063	0.237 ± 0.01	0.276 ± 0.012	0.09 ± 0.007	0.191 ± 0.067	0.2 ± 0	RI, MS	Alkenes
73	Eremophila-1(10),8,11-triene	0	0	0.052 ± 0.009	0	0	0	0	RI, MS	Alkenes
74	Farnesene epoxide	0.094 ± 0.009	0	0	0	0	0	0	RI, MS	Others
75	8-Cedren-13-ol	0	0	0	0.053 ± 0	0	0	0	RI, MS	Alcohols
76	4a-dimethyl-6-(prop-1-en-2-yl)-1,2,3,4,4a,5,6,7-octahydronaphthalene	0.099 ± 0.01	0.085 ± 0.036	16.604 ± 0.028	0.07 ± 0.004	0.075 ± 0.002	0.026 ± 0.015	0	RI, MS	Others
77	α-selinene	1.403 ± 0.026	1.701 ± 0.564	1.209 ± 0.009	1.035 ± 0.045	1.24 ± 0.022	0.596 ± 0.135	0.522 ± 0.002	RI, MS	Alkenes
78	Selina-4,11-diene	4.278 ± 0.111	4.885 ± 1.636	3.538 ± 0.047	0	0	0	0	RI, MS	Alkenes
79	decahydro-1,1,3a-trimethyl-7-methylene-1H-Cyclopropa-naphthalene	0	0	0	3.026 ± 0.12	3.417 ± 0.048	1.795 ± 0.316	1.647 ± 0.008	RI, MS	Others
80	Valencene	22.136 ± 0.411	28.898 ± 1.371	0	11.048 ± 9.522	18.952 ± 0.214	8.787 ± 7.057	0.685 ± 0.021	RI, MS	Alkenes
81	α-Bulnesene	1.5 ± 0.062	1.76 ± 0.641	1.212 ± 0.069	1.189 ± 0.072	1.259 ± 0.028	0	0	RI, MS	Alkenes
82	α-Panasinsen	2.971 ± 0.032	3.654 ± 1.453	2.264 ± 0.172	2.066 ± 0.086	2.595 ± 0.041	1.243 ± 0.221	1.142 ± 0.005	RI, MS	Alkenes
83	δ-Cadinene	0.318 ± 0.013	0.326 ± 0.004	0.425 ± 0.01	0.374 ± 0.01	0.255 ± 0.005	0.266 ± 0.034	0.237 ± 0.002	RI, MS	Alkenes
84	11-Tridecyn-1-ol	0.359 ± 0.022	0.354 ± 0.174	0.243 ± 0.023	0.37 ± 0.016	0.435 ± 0.027	0.341 ± 0.141	0.43 ± 0.027	RI, MS	Alcohols
85	Nerolidol	0.12 ± 0.004	0.09 ± 0.033	0.088 ± 0.013	0.085 ± 0.008	0.071 ± 0.008	0.045 ± 0.006	0.039 ± 0.003	RI, MS	Alcohols
86	Diethyl phthalate	0.088 ± 0.011	0.8 ± 0.49	0.144 ± 0.072	0.104 ± 0.016	0.128 ± 0.006	0.532 ± 0.331	0.187 ± 0.021	RI, MS	Esters
87	Caryophyllene oxide	0.134 ± 0.035	1.198 ± 0.987	0.312 ± 0.12	0.626 ± 0.123	0.323 ± 0.229	0.134 ± 0.023	0.114 ± 0.013	RI, MS	Others
88	Neointermedeol	0.073 ± 0.007	0.083 ± 0.027	0.562 ± 0.435	0.055 ± 0.003	0.418 ± 0.353	0.286 ± 0.057	0	RI, MS	Others
89	13-nor-Eremophil-1(10)-en-11-one	0.102 ± 0.011	0.177 ± 0.06	0	0	0	0	0	RI, MS	Ketones
90	Labdadienedial	0	0	0.089 ± 0.004	0	0	0	0	RI, MS	Aldehydes

Table 1. *Cont.*

No	Name	PRHN	PRGN	PRNN	MRNN	PROY	PQOY	PCON	Identification Method	Classification
91	cadin-4-en-1β-ol	0	0	0.104 ± 0.012	0	0	0.037 ± 0.032	0	RI, MS	Alcohols
92	Himbaccol	0.101 ± 0.026	0	0	0	0.081 ± 0.01	0	0	RI, MS	Alcohols
93	11,11-dimethyl-, 4,8-bis(methylene)-Bicyclo [7.2.0] undecan-3-ol	0.142 ± 0.009	0.195 ± 0.097	0.159 ± 0.004	0.13 ± 0.008	0.113 ± 0.004	0.044 ± 0.011	0.04 ± 0.004	RI, MS	Alcohols
94	Viridiflorol	0.067 ± 0.009	0.1 ± 0.033	0.09 ± 0.012	0.067 ± 0.006	0.055 ± 0.004	0	0	RI, MS	Alcohols
95	Intermedeol	0	1.101 ± 0.536	0.641 ± 0.357	0.873 ± 0.082	0	0	0	RI, MS	Alcohols
96	decahydro-1,5,5,8a-tetramethyl-1,4-Methanoazulen-9-one	0.19 ± 0.015	0.278 ± 0.151	0.308 ± 0.022	0.2 ± 0.026	0.133 ± 0.003	0.053 ± 0.022	0.043 ± 0.001	RI, MS	Ketones
97	1,5-diethenyl-2,3-dimethyl-Cyclohexane	0	0	0.051 ± 0.01	0	0	0	0	RI, MS	Alkenes
98	α-Cyperone	0.052 ± 0.001	0.12 ± 0.083	0.068 ± 0.011	0.063 ± 0.007	0.034 ± 0	0	0	RI, MS	Ketones
99	Nootkatone	0.371 ± 0.026	0.513 ± 0.249	0.514 ± 0.017	0.353 ± 0.032	0.245 ± 0.007	0.144 ± 0.02	0.12 ± 0.001	RI, MS	Ketones
100	Solavetivone	0.045 ± 0.006	0.07 ± 0.037	0.071 ± 0.008	0.047 ± 0.005	0.03 ± 0.001	0	0	RI, MS	Ketones
101	geranylgeranyl acetate	0	0	0	0.051 ± 0.001	0	0	0	RI, MS	Esters
102	Corymbolone	0.066 ± 0.023	0.114 ± 0.077	0.054 ± 0.001	0.056 ± 0.006	0.066 ± 0.004	0.033 ± 0.005	0	RI, MS	Ketones

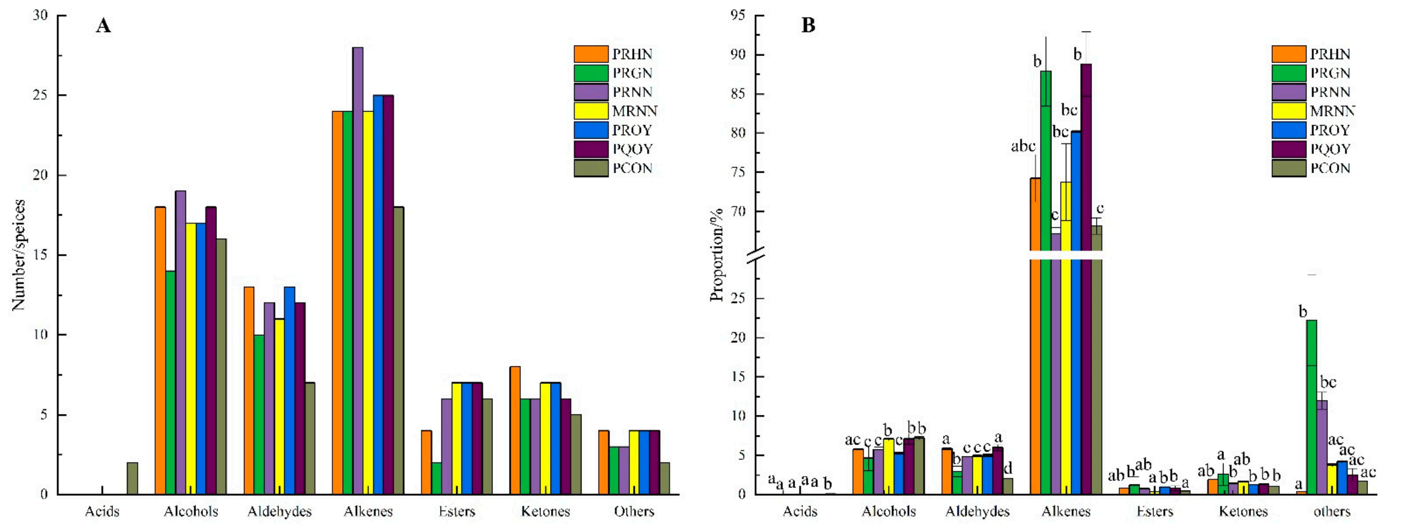

Figure 1. Analysis of species (**A**) and proportion (**B**) of VOCs among *Citrus sinensis* 'hongjiang'. a, b, c: Different letters represents a significant difference.

3.2. Modeling and Model Evaluation of VOCs in 'hongjiang' Obtained from Different Factors

PCA is a multidimensional data statistical analysis method for unsupervised pattern recognition, widely used for variety identification [19,20], processing method discrimination [21], and other analytical purposes. As shown in Figure 2A, a PCA model ($R^2X = 0.885$, $Q^2 = 605$) was constructed with the VOC data obtained from 'hongjiang' samples from different locations, varieties, and cultivation modes, and six principal components were extracted with a cumulative contribution rate of 88.5%, representing major data. As shown in Figure S1, the contribution values for PC 1, PC2, PC3, PC4, PC5, and PC6 were 0.320, 0.181, 0.145, 0.096, 0.087, and 0.056, respectively.

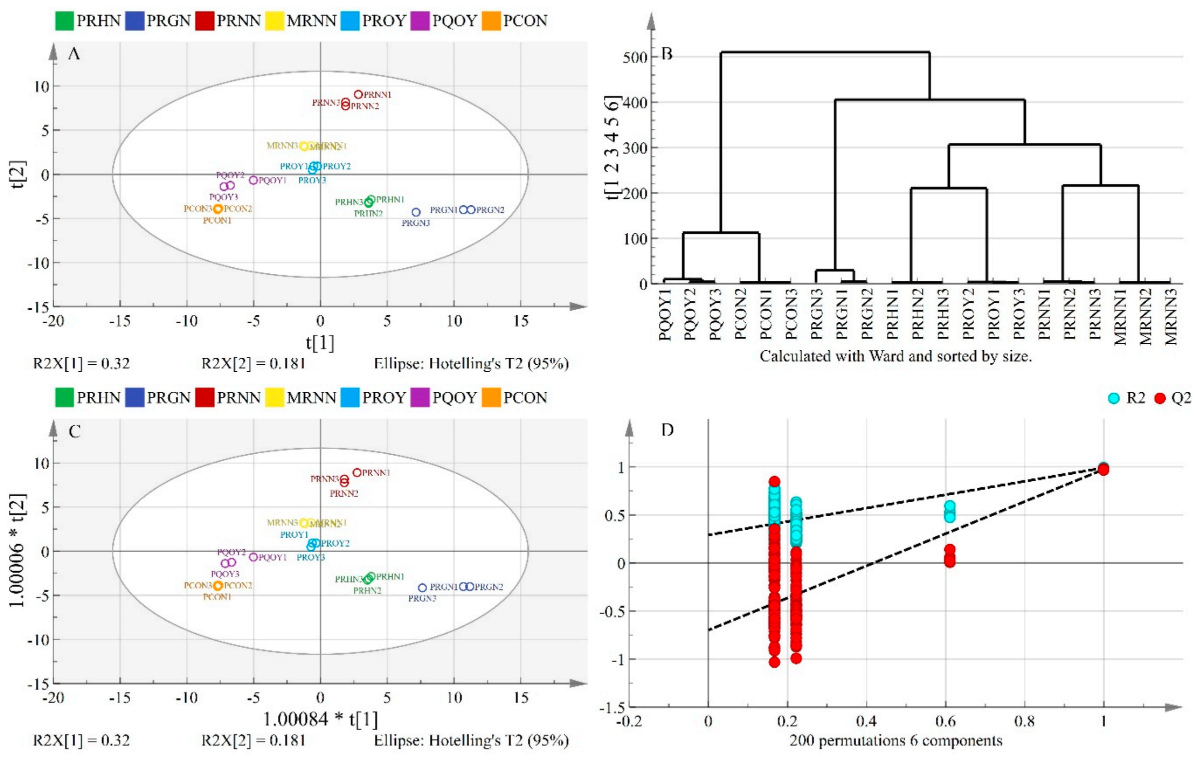

Figure 2. Score plot of the PCA-X model (**A**), HCA (**B**), OPLS-DA model (**C**), and cross-validation of the OPLS-DA model (**D**) for VOCs among *Citrus sinensis* 'hongjiang'.

As shown in Figure 2A, the 21 samples were distributed in the first, second, third, and fourth quadrants. PRGN was distributed in the fourth quadrant with a significant difference compared to other samples. There was a large difference between PRNN and PRHN distributed in the first quadrant and MRNN, PQOY, and PCON distributed in the third quadrant. HCA was performed based on the six principal components extracted by PCA, as shown in Figure 2B. The results of the HCA analysis showed that the 21 'hongjiang' samples clustered into four classes, of which PQOY and PCON clustered into the first class, PRGN into the second class, PRHN and PROY into the third class, and PRNN and MRNN into the fourth class. This indicated that the VOC characteristics of the location, variety, and cultivation mode varied greatly.

Similar to PCA, OPLS-DA is also a multidimensional vector analysis method based on dimensionality reduction. Still, the difference is that OPLS-DA is a supervised discriminant analysis method widely used for the quality evaluation of fruit and vegetable varieties [22] and origins [23]. As shown in Figure 2C, an OPLS-DA model with strong cumulative explanatory and predictive power and good stability ($R^2X = 0.884$, $R^2Y = 0.989$, $Q^2 = 0.964$) was established with the VOC component data of 'hongjiang' under three factors. In addition, the model was analyzed for reliability using 200 cross-validations and the replacement test ($R^2 = 0.2913$, $Q^2 = -0.6988$), as shown in Figure 2D, and negative values of Q^2 indicated that the model was reliable [24], had no overfitting, and was applicable for discriminant analysis of 21 samples under study.

3.3. Potential Differences in VOCs in 'hongjiang' Obtained by Different Factors

This study used S plots to identify chemical differences between two groups of samples and identify metabolites of statistical and potential biochemical significance, with the points at the ends of the "S" indicating the variables with the largest contributions to the model. In contrast, the variables that contributed less were clustered around the origin [25]. To more deeply explore the differential VOCs in the samples obtained from the three factors regarding the results of the OLPS-DA model analysis, this study focused on the comparative study of the differences between PRHN and PRGN, PRGN and PRNN, PROY and PQOY, and PRNN and PCON, as shown in Figure 3. The red dots in the graph indicate metabolites with VIP values exceeding 1. Larger VIP values correspond to larger differences between the two factors.

As shown in Figure 3, there were 17 differential VOCs in 'hongjiang' obtained from half-net room (PRHN) and little-net room (PRGN) cultivation modes, of which four compounds had VIP > 2, namely, valencene (80), limonene (14), 3,7-dimethyl-2,6-octadienal (50), and intermedeol (95). Nine differential VOCs were present in the little-net room (PRGN) and big-net room (PRNN) oranges, of which the 3 compounds with VIP > 2 were valencene (80), limonene (14), 4a-dimethyl-6-(prop-1-en-2-yl)-1,2,3,4,4a,5,6,7-octahydronaphthalene (76). Ten differential VOCs were present in Red River Orange Team 38 (PROY) and Qingping Township (PQOY), of which two compounds with VIP > 2 were limonene (14) and valencene (80). Twelve differential VOCs were present in the large-net room (PRNN) and open-air cultivation (PCON) products, of which three compounds with VIP > 2 were 4a-dimethyl-6-(prop-1-en-2-yl)-1,2,3,4,4a,5,6,7-octahydronaphthalene (76), limonene (14), and Selina-4,11-diene (78).

3.4. Analysis of the Differences in the Content of VOCs in 'hongjiang' Obtained by Different Factors

To visualize and accurately analyze the differences between location, variety, and cultivation modes, the differences in VOCs in 'hongjiang' obtained for different factors were analyzed by VIP analysis and heatmap analysis in OPLS-DA. Fifty-one differential VOCs with VIP > 1 in different factors were obtained in OPLS-DA and were used to make a heatmap in Figure 4 and Table S2. As shown in Figure 4, the content of VOCs in 'hongjiang', obtained under different locations and methods, was analyzed. For example, α-terpinene (13) was detected only in PRHN. In addition, 1,5-diethenyl-2,3-dimethyl-

Cyclohexane (97), Eremophila-1(10),8,11-triene (73), Labdadienedial (90) were detected only in PRNN. Geranylgeranyl acetate (101), and 8-Cedren-13-ol (75) were detected in MRNN only. Cryptone (37) and 7-methyloct-3-yne (19) were detected in PROY only, 2,4-dodecadienal (42) was detected in PQOY only, and octanoic acid (35) was detected in PCON only.

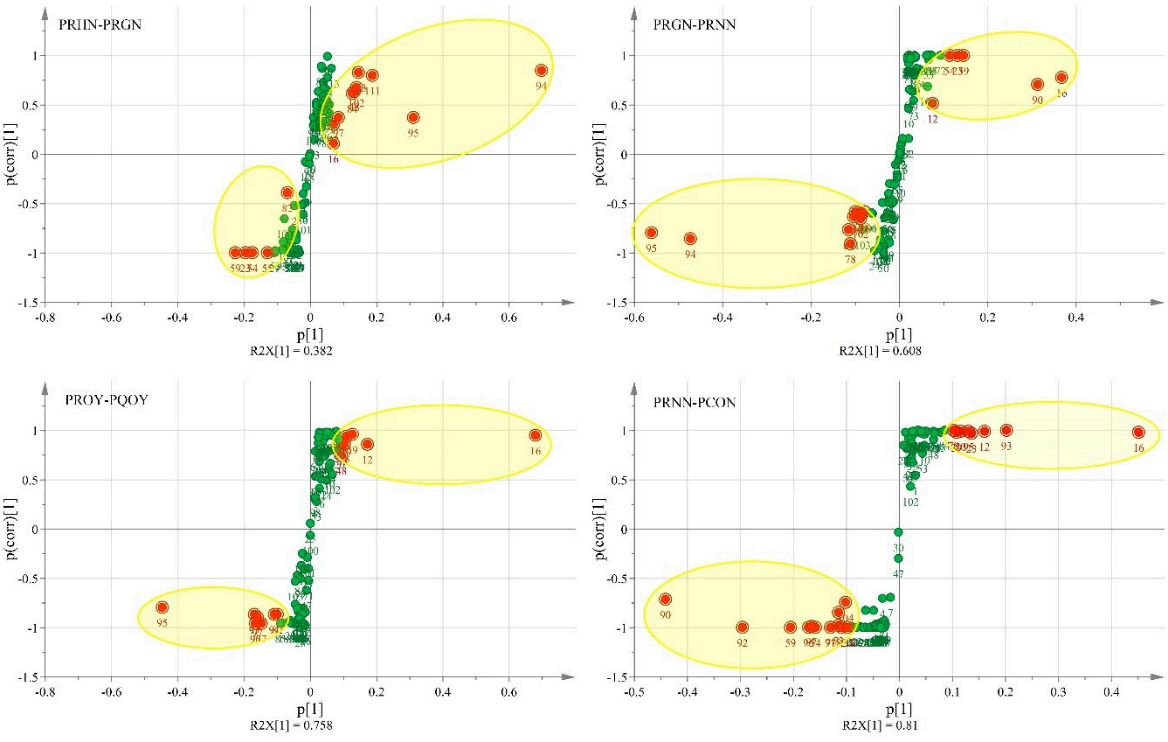

Figure 3. S plot of VOC-based OPLS-DA model analysis among *Citrus sinensis* 'hongjiang'.

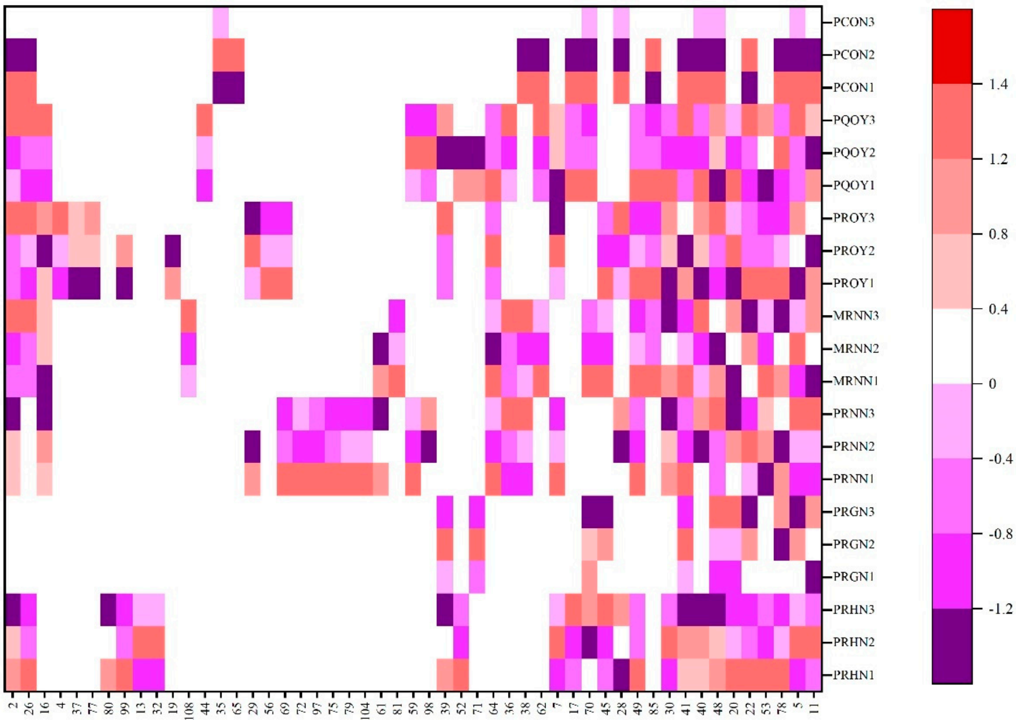

Figure 4. Heatmap of different metabolites among *Citrus sinensis* 'hongjiang'.

1-Hexanol (5), octanal (11), 1-nonanal (22), ethyl octanoate (39), 2-methyl-5-(1-methylethenyl) -cyclohexanone (40), decanal (41), β-citronellol (45), geraniol (48), farnesene (72), and δ-cadinene (83) were detected in 21 groups of samples. Methyl geranate (54) was absent in PRHN, PRGN, and PRNN (half-net room, little-net room, and big-net room cultivation modes) and present in PROY, PQOY, and PCON (open-air cultivation modes).

4. Discussion

VOCs are essential factors controlling the quality of fresh and storage-processed oranges. Studies related to VOCs in oranges have mainly focused on varieties such as brocade orange, sour orange, and navel orange varieties, while little has been reported on 'hongjiang'. Hong et al. [26] detected a total of 127 VOCs from ten orange juice samples by HS-SPME-GC-MS, mainly consisting of alkenes, alcohols, esters, aldehydes, and ketones, with alkenes and alcohols accounting for a relatively proportion of their large content. Consistent with the results of this study, it was further confirmed that alkenes, alcohols, esters, aldehydes, and ketones were the typical aroma compounds constituting orange juice. In addition, this study revealed that two factors (location and cultivation mode) had strong effects on the percentage of orange VOC composition: largest share of aldehydes was found in half-net room cultivation (PRHN) oranges, the largest shares of ketones, esters, and alkenes were found in little-net room cultivation (PRGN) oranges, and the largest percentage of alcohols was found in Chengyue town (PCON) oranges.

Tang et al. [10] used the HS-SPME-GC-MS technique to isolate and identify 65 VOCs from five commercially available navel orange fruits, which were dominated by limonene, β-watercressene, β-laurelene, and linalool, with limonene accounting for 70–81%. This study proved that limonene, valencene, α-panasinsen, linalool, and Selina-4,11-diene were the main VOCs in 'hongjiang', with limonene and valecene ranging from 30–60% and 1–30%, respectively. The composition of limonene in 'hongjiang' was much lower than that in navel oranges. Still, the composition of valencene in red river orange was higher than that of navel orange, indicating that the aroma characteristics of red river orange and navel orange differed significantly.

Navel orange fruits were dominated by limonene, β-watercressene, β-laurelene, and linalool, among which limonene accounted for 70–81%. At the same time, this study showed that limonene, valencene, α-Panasinsen, linalool, and Selina-4,11-diene were the main VOCs of 'hongjiang', with limonene accounting for 35–67% and valencene accounting for 10–20%. The composition of limonene in 'hongjiang' was much lower than that of navel oranges, while that of valencene and other components in 'hongjiang' exceeded that of navel oranges, indicating that the aroma characteristics of 'hongjiang' and navel oranges were different.

Net room cultivation is one of the important measures for green vegetable and fruit production. It plays a pivotal role in integrated pest management, and the results have been applied to cultivating fruits and vegetables, flowers, and so on [27,28]. Yuan et al. [29] showed that the intrinsic quality (vitamin C, soluble solids, acidity, solid–acid ratio, and juice rate), extrinsic quality (fruit shape index, peel thickness, fruit hardness, peel rate, and residue rate), and some coloring qualities (yellow value b, color saturation C, and brightness value L) of the fruits cultivated in net room cultivation were not significantly different from those of the open-air cultivation ($p > 0.05$). Still, the red value of citrus fruits in the net room cultivation was considerably smaller than that in the open-air cultivation area ($p < 0.05$), and the hue angle (describing the relative. amounts of redness and yellowness) was significantly larger than that in the open-air cultivation ($p < 0.05$). Liu et al. [30] revealed the effect of net room cultivation on the quality of late-ripening citrus in Guinan, with a significant decrease in the total sugar content of wogon and a substantial increase in the vitamin C content of Maugu tangerine compared to open-air cultivation, with no substantial changes in other quality indicators. In this study, twelve different VOCs were identified from the samples obtained from net room cultivation and open-air cultivation, among which methyl geranate (54) was only present in the open-air cultivation, and selina-4,11-diene was only present in the net room cultivation. The differences in VOC composition might be due to the influence of net room cultivation on indoor temperature and humidity pests, budding, flowering, and physiological fruit drop phenology [31–33].

5. Conclusions

In this study, a total of 102 volatile organic compounds were identified from seven groups of 'hongjiang' samples using headspace solid-phase microextraction-gas chromatography-mass spectrometry. These compounds consisted mainly of alkenes, alcohols, aldehydes, and ketones, with the largest number being alkenes (28) and the largest proportion (81%) being alkenes too. Different cultivation methods of PRNN, PRGN, and PCON, various origins of PROY and PQOY, and other varieties of PRNN and MRNN all influenced the VOCs and differential aroma components in 'hongjiang', with the largest number of VOCs in 'hongjiang' from PRNN (74) and the largest number of VOCs in 'hongjiang' from PQOY (81). The largest proportion of VOCs was 94% in PQOY. This study proved that the OPLS-DA, VIP distribution map, S plot, and heatmap effectively identified differential VOC components of 'hongjiang' of different varieties, cultivation locations, and methods. This technique can be extended to other fruits' varieties, cultivation methods, and cultivation origins.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture12101725/s1, Figure S1: Scores plot of PC1/PC3 (A) and PC2/PC3 (C), loading plot of PC1/PC2 (E), PC1/PC3 (B) and PC2/PC3 (D) for VOCs among *Citrus sinensis*. 'hongjiang', Table S1: The composition and relative contents of volatile organic compounds (VOCs) among *Citrus sinensis* 'hongjiang', Table S2: the differences in VOCs in 'hongjiang' obtained from different factors were analyzed by VIP analysis and heat map analysis in OPLS-DA.

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