agriculture

MDPI

Article

The Flavor Characterization of 'Huyou' (*Citrus changshanensis*) Essential Oils Extracted by Conventional and Novel Methods

Huan Cheng [1,2,3,4,*], Feifei Liu [1,2], Yanru Zhang [1], Zhiyue Ye [1], Jin Chen [1], Shiguo Chen [1,2,3,4] and Xingqian Ye [1,3,4]

[1] National-Local Joint Engineering Laboratory of Intelligent Food Technology and Equipment, Zhejiang Key Laboratory for Agro-Food Processing, Zhejiang International Scientific and Technological Cooperation Base of Health Food Manufacturing and Quality Control, Fuli Institute of Food Science, College of Biosystems Engineering and Food Science, Zhejiang University, Hangzhou 310058, China; ffliu@zju.edu.cn (F.L.); zhangyanru@zju.edu.cn (Y.Z.); zhiyueye@zju.edu.cn (Z.Y.); jin_chen@zju.edu.cn (J.C.); hendle@zju.edu.cn (S.C.); psu@zju.edu.cn (X.Y.)
[2] Innovation Center of Yangtze River Delta, Zhejiang University, Jiaxing 314102, China
[3] Ningbo Innovation Center, Zhejiang University, Ningbo 315100, China
[4] Zhongyuan Institute, Zhejiang University, Zhengzhou 450000, China
* Correspondence: huancheng@zju.edu.cn

Abstract: The aroma of citrus is among the most appealing natural flavors. 'Huyou' (HY) is a hybrid citrus with a unique flavor compared to grapefruit (GF), but few studies have analyzed its characteristic flavor comprehensively. In this study, we extract six essential oils (EOs) from HY and GF peels by cold pressing (CP) and microwave-assisted hydrodistillation (MADH) and spinning cone column (SCC). Further, the flavor of six EOs was investigated by using flavoromics analysis, including e-nose, GC-MS and GC-O combined with chemometric approaches. The results showed that CP EOs exhibited a stronger citrus characteristic flavor, while MADH and SCC EOs contained more diverse volatiles. A total of 23 key odorants were identified in the GC-O-MS analysis, 12 of which were specific to HY. The flavor wheel and partial least squares regression (PLSR) revealed that floral, sweet and fruity odors were positively correlated with linalool, α-terpineol and geraniol, while fatty, green and woody odors with germacrene D, germacrene B and nootkatone. Additionally, based on orthogonal partial least squares discriminant analysis (OPLS-DA), six aroma-active compounds were screened as aroma markers to distinguish HY from GF; i.e., γ-terpinene, D-limonene, germacrene D, nootkatone, germacrene B and terpinolene. The extraction methods and citrus varieties both impact the flavor characterization of citrus EOs, and our study provides guidance on the extraction and application for citrus EOs.

Keywords: 'Huyou' (*Citrus changshanensis*); essential oil; flavor; aroma-active compounds

Citation: Cheng, H.; Liu, F.; Zhang, Y.; Ye, Z.; Chen, J.; Chen, S.; Ye, X. The Flavor Characterization of 'Huyou' (*Citrus changshanensis*) Essential Oils Extracted by Conventional and Novel Methods. *Agriculture* **2024**, *14*, 131. https://doi.org/10.3390/agriculture14010131

Academic Editor: Mariusz J. Stolarski

Received: 13 December 2023
Revised: 9 January 2024
Accepted: 12 January 2024
Published: 16 January 2024

1. Introduction

Citrus is one of the most widely produced fruits in the world, which mainly includes mandarin (*Citrus reticulate*), orange (*Citrus sinensis*), grapefruit (*Citrus paradisi*) and lemon (*Citrus limon*) [1]. During processing, large quantities of citrus peel waste is generated, which is an important source of bioactive compounds such as essential oils (EOs), flavonoids, carotenoids and vitamins [2]. Among them, citrus EOs are widely beloved for their delightful and invigorating aroma, making them in great demand in food, pharmaceutical, cosmetic and perfumery industries [2] and having promising market potential. Hence, the extraction of EOs is an effective way for the reclaimed utilization of citrus peel.

The traditional extraction methods of EOs are cold pressing (CP) and steam distillation. The CP EO presents natural citrus aroma characteristics, since no chemical reactions occur in the extraction process that alter flavor composition. However, it contains some impurities such as pigments and waxes and has a low yield [3]. Hydrodistillation (HD) is also a commonly used method, with several shortcomings like long time consumption and

losses of volatile compounds [4]. Therefore, some novel techniques were introduced such as microwave-assisted extraction [4,5] and ultrasound-assisted extraction [6]. For example, microwave-assisted hydrodistillation (MADH) is one of the highly efficient and low energy consumption methods, as an excellent alternative for HD [7]. Anyway, noteworthy is the spinning cone column (SCC), an efficient counter-current liquid–gas contact extraction technology which emerged in recent years. It provides the advantages of high extraction efficiency, low residence time and a wide range of temperature control, thus greatly reducing the damage degree of heat-sensitive flavor compounds [8]. SCC has been applied in the food industry, including but not limited to the aroma recovery of tea and coffee [9], alcohol adjustment of wine [10] and extraction of essential oils [8,11]. However, the application of SCC in citrus is still empty.

'Huyou' (HY) is a unique citrus variety which is hybridized by *Citrus grandis* (L.) O Sbeck and *Citrus sinensis* (L.) O Sbeck [12]. It is mainly produced in Changshan country, Zhejiang Province, China. Compared to many other citrus species, HY EO exhibits stronger antibacterial activity, which may be related to the coexistence and content of D-limonene, α-pinene, β-pinene, myrcene and ocimene [13]. Meanwhile, it shows strong antioxidant activity and has a potential to be used as a natural food preservative [13]. However, most studies on HY are focused on chemical constituents and its functional evaluation [14,15]; there are very few studies on its flavor [13,16]. Even though HY and grapefruit (GF) are both hybrids of pomelo and orange, their flavors still differ significantly. Yet, no comprehensive investigation has been conducted on the unique flavor of HY; thus, it remains unclear which specific aroma-active compounds contribute to the aroma differences between HY and GF.

The flavoromics approach is an improvement over traditional flavor studies, providing a comprehensive and unbiased analysis of food flavor [17]. Moreover, the application of flavoromics enables flavor compounds to be correlated with sensory properties through high-resolution instrumental analysis and chemometrics [17,18]. Thus, the objectives of this study are to (1) investigate the flavor difference of HY and GF EOs extracted by three different methods, (2) screen out the differential aroma-active compounds between HY and GF EOs using GC-O combined orthogonal partial least squares discrimination analysis (OPLS-DA) and (3) investigate the relationship between aroma-active compounds and sensory properties using partial least squares regression (PLSR).

2. Materials and Methods

2.1. Materials and Chemicals

Citrus were harvested at the ripening stage. HY was collected from Changshan Country, Quzhou city, Zhejiang Province, China in January 2022. GF ('Star Ruby') was purchased from South Africa in June 2022. The harvested citrus fruits were washed and then dried. The peel was obtained by a fruit peeler (SH-607, Guangzhou Juis Trading Co., Ltd., Guangzhou, China) for subsequent oil extraction.

The mixture of n-alkanes (C_8-C_{20}) for liner retention index (LRI) calculation was supplied by Sigma Chemical Co. (St. Louis, MO, USA). n-Hexane (GC grade) and cyclohexanone (internal standard, GC grade) were purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). Sodium sulfate (anhydrous, analytical grade) was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

2.2. Extraction of Citrus Essential Oils

2.2.1. CP

The CP EOs were extracted according to the method of Ou et al. [19]. Fresh citrus peels were soaked in 0.50% calcium chloride solution for 2 h and then squeezed through a three-roller pressing machine. The obtained mixture was collected and centrifuged at 6000 rpm for 20 min, and then the upper essential oil was collected in brown vials, dehydrated with anhydrous sodium sulfate and stored at 4 °C for subsequent analysis.

2.2.2. MAHD

MAHD was performed according to the method of Golmakani [7] with modifications. The peels were crushed and immersed in ultrapure water at a 1:5 material-to-liquid ratio. The oven power and operating time were set at P40 (650 W, 18 s break for every 12 s operation) and 15 min, respectively. The essential oils were collected and preserved as 2.2.1.

2.2.3. SCC

The extraction of SCC was carried out in the workshop. The citrus slurry flowed downwards through the column, while simultaneously, the steam flowed up and striped volatile compounds. Finally, the steam containing citrus aroma extract exited the top of the column and then passed through the condenser, resulting in an essential oil and hydrosol mixture. The product flow rate was 500 L/H and the heat temperature was 87 °C. Approximately 2.5 kg of citrus peels were used for each batch, with about 5 kg of aroma extract collected.

2.3. Quantitative Descriptive Sensory Analysis

Quantitative descriptive sensory analysis (QDA) was conducted by 18 trained assessors (12 females and 6 males, aged 20–30) from the College of Biosystems Engineering and Food Science, Zhejiang University. Based on the previous research [20,21] and joint discussion, the aroma descriptors of citrus essential oil were determined as follows: fruity (crushed strawberries), sweet (honey), floral (phenylethanol), green (cis-3-hexenol), fatty (decanal) and woody (oak wood chips). Each 100 µL essential oil was placed in a 5 mL brown vial and the assessors were asked to evaluate the aroma profiles of six samples on a 10-point scale from 0 (not perceivable) to 9 (strongly perceivable). The average scores of each descriptor from 18 panelists were presented in the spider diagram.

2.4. Heracles NEO e-Nose Analysis

The comparative analysis of flavor in six citrus EOs was conducted using Heracles NEO e-nose (Alpha M.O.S., Toulouse, France), which was equipped with an automatic sampling device, two columns of different polarity (MXT-5 and MXT-1701) and two flame ionization detectors (FIDs). The samples were analyzed according to the method of Chen et al. [22] with modifications. Briefly, each 20 mL vial containing 100 µL EO was heated to 40 °C for 15 min with oscillation at 500 rpm. Then, 500 µL headspace gas was injected into the GC port at 200 °C and the temperature of two FIDs were maintained at 260 °C. The carrier gas (hydrogen) was circulated at a constant flow rate of 1.0 mL/min with a split mode of 10 mL/min. The oven heating procedure was as follows: increased from 40 °C to 180 °C at a rate of 6 °C/s for 60 s, then to 250 °C at a rate of 3 °C/s and held for 120 s. Each sample was analyzed in six replicates.

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

The analysis of volatile compounds was carried out by GC-MS (7890A-5975C, Agilent Technologies Inc., Santa Clara, CA, USA) equipped with a DB-5ms column (30 m × 0.25 mm × 0.25 µm, Agilent, Santa Clara, CA, USA). An amount of 1 µL of EO samples (diluted in n-hexane, cyclohexanone 2.147 mg/mL) was injected into the GC inlet at 240 °C with a spilt ratio of 20:1. Specially, the volatile compounds of hydrosol were extracted using HS-SPME as described by Liu et al. [23]. Amounts of 4 mL of SCC extracts spiked with 20 µL of cyclohexanone (0.947 mg/mL) were placed into a 15 mL vial. After being equilibrated at 40 °C for 10 min, a 1 cm-long SPME fiber (50/30 µm DVB/CAR/PDMS, Supelco, Bellefonte, PA, USA) was exposed to the vial headspace for 40 min to absorb volatile compounds. Thereafter, the fiber was inserted into the GC inlet and desorbed for 5 min. Each sample was replicated three times.

The oven temperature was Initially held at 40 °C for 2 min, and increased to 130 °C at 4 °C/min, then raised to 160 °C at 3 °C/min for 1 min and finally ramped up to 240 °C at 10 °C/min for 5 min. Helium (99.999%) was used as the carrier gas at a constant flow

rate of 1.5 mL/min. The MS was operated in the electronic impact (EI) ionization mode at 70 eV, and performed in full scan mode (range of m/z 45–350) with a scan frequency of 4.58 scans/s. The interface temperature, the ion source and the quadrupole temperature were 280 °C, 230 °C and 150 °C, respectively. The solvent delay was 2 min.

2.6. Gas Chromatography–Olfactometry (GC-O) Analysis

The identification of aroma-active compounds was performed by an ODP 3 olfactory detection port (Gerstel, GmbH & Co., KG, Düsseldorf, Germany), which was integrated into GC-MS. The column effluent was split (1:1) to MSD and ODP, and the GC-MS settings were consistent with Section 2.3. Both the olfactory port and the transfer line were maintained at 250 °C and the sniffing time was approximately 30 min; therefore, the moist air was pumped into the sniffing port to avoid noise dryness.

Two GC-O strategies, i.e., aroma extract dilution analysis (AEDA) and modified frequency (MF), were applied for evaluating the key aroma-active compounds. AEDA was performed by adjusting the GC injector split ratio which proved to be a reliable approach [24]. In the present experiment, the split ratio was set as 2^1:1, 2^2:1, 2^3:1, 2^4:1, . . ., and 2^n:1. Four well-trained and experienced panelists were asked to sniff at the olfactory port and record the retention time and descriptors of the odor. The aroma was considered to be present when it was sensed more than twice by different panelists. The flavor dilution (FD) factor corresponded to the maximum split ratio of the samples at which the odor of the compound could be perceived. The sniffing experiment was repeated in duplicate for each dilution.

MF is a hybrid technique based on the combination of aroma intensity and detection frequency, which has been proven to provide more reliable results [25]. Four panelists were asked to score the intensity of each odor using a 4-point scale (1 = weak, 2 = clear and moderate, 3 = strong, 4 = extremely strong). MF was calculated as follows [18,25]:

$$\mathrm{MF}(\%) = \sqrt{\mathrm{AI_{ave}}(\%) \times \mathrm{DF}(\%)}$$

where $\mathrm{AI_{ave}}$ (%) is the average aroma intensity divided by the maximum intensity ("4") and DF (%) is the detection frequency in a total of eight sniffing tests.

2.7. Qualitative and Quantitative Analysis of Volatile Compounds

Tentative identification of volatile compounds was performed by retrieving and matching the mass spectral library (NIST14.L). Then, the C_8-C_{20} n-alkanes mixture was analyzed under the identical conditions as samples to calculate RI values for further confirmation.

The quantification of volatile compounds was performed by the internal standard method, in which the concentration of each compound was normalized to that of cyclohexanone. The specific calculation was to divide the peak areas of the target compounds by the peak area of the cyclohexanone and multiply this ratio by the concentration of the cyclohexanone (expressed as mg/mL) [26].

2.8. Statistical Data Analysis

GC-MS data were presented as the mean ± SD (standard deviation). Principal component analysis (PCA) and discriminant factor analysis (DFA) of Heracles flash GC e-nose data were performed using Alphasoft 7.2.5 (Alpha M.O.S., Toulouse, France). FD and MF olfactograms of GC-O results were created by Microsoft Excel 2021. The Veen was plotted by TBtools v.2.039. Partial least square regression (PLSR) and orthogonal partial least squares discriminant analysis (OPLS-DA) were carried out by the Unscrambler X 10.4 and SIMCA 14.1 software, respectively.

3. Results and Discussion

3.1. Difference of Overall Flavor in Six Citrus EOs Based on Sensory Analysis and e-Nose Analysis

A sensory panel evaluated the flavor of six citrus EOs, which were described as fruity, sweet, floral, green, fatty and woody (Figure 1A,B). Notably, significant differences were observed between two varieties of citrus EOs, with HY EO presenting a stronger fatty, green and woody odor, while GF EO having a stronger fruity, sweet and floral aroma. The comparison of different extraction methods showed that the EOs extracted by CP and MADH had a more intense flavor than those extracted by SCC. This was explained by the fact that the first two were pure essential oils, whereas the latter was hydrolate containing little oil. Additionally, the characteristic aroma of the CP EO was more prominent among three methods. Therefore, for subsequent GC-O experiments, CP EOs were chosen for further analysis.

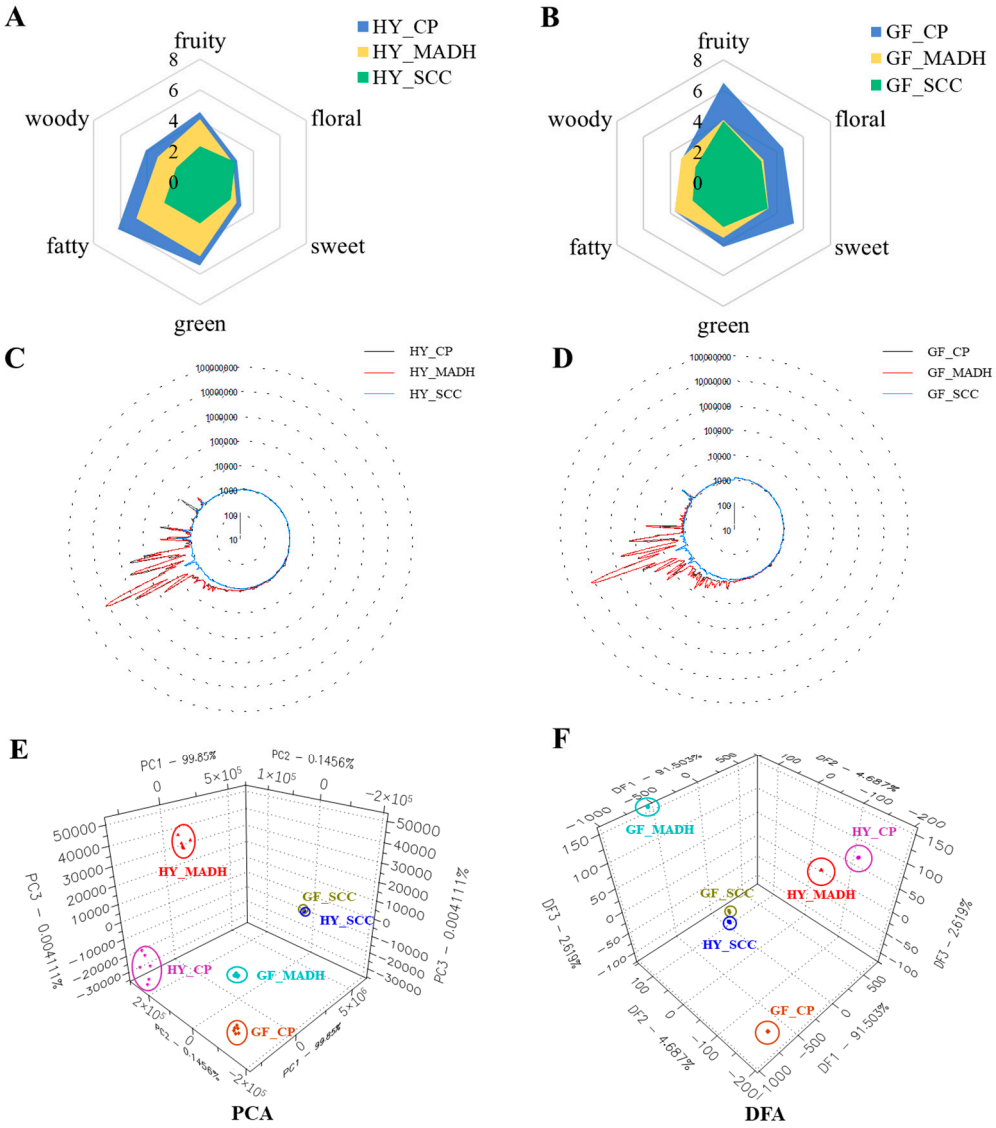

Figure 1. Difference of overall flavor in six citrus EOs. Sensory analysis of HY EOs (**A**) and GF EOs (**B**); the e-nose radar map of HY EOs (**C**) and GF EOs (**D**); PCA (**E**) and DFA (**F**) of six citrus EOs based on e-nose data. EOs, essential oils; HY, Huyou; GF, grapefruit; CP, cold pressing; MADH, microwave-assisted hydrodistillation; SCC, spinning cone column; PCA, principal component analysis; DFA, discriminant factor analysis.

Further, the Heracles Neo e-nose was also applied to distinguish the flavor profiles of six EOs. The radar map (Figure 1C,D) of e-nose analysis reflected the intensity and abundance of odor, which was plotted based on the peak area and retention time. The results showed that the chromatograms of CP and MADH EOs were similar, but differentiated from those of SCC extracts greatly. For a better visualization of the data, the three-dimensional plots of PCA (Figure 1E) and DFA (Figure 1F) were created to differentiate between different EO samples. PCA is an unsupervised method for reducing data dimensionality while retaining as much original information as possible [27]. The cumulative variance contribution rate of PC1, PC2 and PC3 exceeded 99.999%, indicating that the PCA model was strongly effective to explain the total variance. The samples distribution in each group were closely clustered, indicating good repeatability. The distance between the GF_SCC and HY_SCC groups was relatively close, suggesting the minor aroma difference between the two samples. Apart from that, the region division of other EO samples was relatively obvious.

In contrast to PCA, DFA is a supervised pattern recognition method, which increases the between-class variance and reduces the within-class variance, resulting in better discrimination in different sample groups [27]. The total variance was 98.809% (PC1 = 91.503%, PC2 = 4.687% and PC3 = 2.619%), revealing that the EOs from different citrus varieties and extraction methods clearly had distinct regional distribution characteristics. The DFA results were consistent with those of PCA, which further verified the reliability of PCA. In conclusion, the Heracles NEO e-nose can be an excellent discriminator to distinguish the flavor of citrus EOs.

3.2. Identification of Volatile Compounds in Six Citrus EOs

The volatile compounds of six citrus EOs were detected by GC-MS and the results are shown in Table 1. A total of 101 volatile compounds were identified in HY EOs and GF EOs, including 14 monoterpenes, 29 sesquiterpenes, 14 aldehydes, 24 alcohols, 6 esters, 3 ketones and 11 others.

Table 1. Concentration (mean ± standard deviation, mg/mL) of volatile compounds in six citrus essential oils (EOs) by GC-MS.

	Compounds	CAS	RI [1]	HYEO			GFEO		
				CP	MADH	SCC	CP	MADH	SCC
	Monoterpenes								
1	*α*-Thujene	2867-05-2	930	3.710 ± 0.065	2.460 ± 0.182	0.157 ± 0.021	0.040 ± 0.006	0.034 ± 0.006	0.004 ± 0.001
2	*α*-Pinene	80-56-8	937	14.982 ± 0.246	10.456 ± 0.634	0.360 ± 0.067	8.581 ± 0.132	7.419 ± 0.056	0.137 ± 0.046
3	Camphene	79-92-5	952	0.117 ± 0.012	0.098 ± 0.008	-	0.913 ± 0.035	0.042 ± 0.001	-
4	Sabinene	3387-41-5	975	2.803 ± 0.074	2.435 ± 0.240	0.065 ± 0.013	3.901 ± 0.052	4.418 ± 0.016	0.078 ± 0.026
5	*β*-Pinene	127-91-3	979	9.555 ± 0.254	7.227 ± 0.734	0.158 ± 0.028	0.357 ± 0.005	0.387 ± 0.009	0.010 ± 0.004
6	*β*-Myrcene	123-35-3	996	20.029 ± 0.348	18.050 ± 1.781	0.405 ± 0.031	22.459 ± 0.195	21.274 ± 0.145	0.497 ± 0.144
7	*α*-Phellandrene	99-83-2	1004	0.724 ± 0.007	0.686 ± 0.101	0.026 ± 0.004	1.329 ± 0.120	0.659 ± 0.007	0.023 ± 0.014
8	3-Carene	13466-78-9	1011	-	-	0.001 ± 0.000	-	-	-
9	*α*-Terpinene	99-86-5	1017	2.182 ± 0.032	1.712 ± 0.153	0.137 ± 0.020	-	-	-
10	*p*-Cymene	99-87-6	1025	1.685 ± 0.157	1.583 ± 0.137	0.504 ± 0.078	-	-	-
11	*D*-Limonene	5989-27-5	1046	906.587 ± 26.930	871.57 ± 83.199	24.767 ± 3.052	953.855 ± 16.627	950.59 ± 13.92	29.158 ± 6.430
12	(*E*)-*β*-ocimene	3779-61-1	1049	1.217 ± 0.024	1.438 ± 0.246	0.041 ± 0.009	1.410 ± 0.103	1.296 ± 0.058	0.041 ± 0.008
13	*γ*-Terpinene	99-85-4	1060	89.911 ± 2.797	70.723 ± 6.583	2.317 ± 0.302	0.094 ± 0.010	0.116 ± 0.011	0.005 ± 0.001
14	Terpinolene	586-62-9	1088	4.484 ± 0.172	4.164 ± 0.573	0.661 ± 0.038	0.517 ± 0.085	0.607 ± 0.020	0.009 ± 0.001

Table 1. *Cont.*

	Compounds	CAS	RI [1]	HYEO			GFEO		
				CP	MADH	SCC	CP	MADH	SCC
	Sesquiterpenes								
1	*δ*-Elemene	20307-84-0	1339	7.339 ± 0.37	3.396 ± 0.371	0.457 ± 0.065	-	-	-
2	*α*-Copaene	3856-25-5	1376	2.214 ± 0.061	1.358 ± 0.158	0.263 ± 0.049	2.489 ± 0.082	2.065 ± 0.018	0.324 ± 0.028
3	*β*-Cubebene	13744-15-5	1389	1.261 ± 0.096	0.660 ± 0.055	0.093 ± 0.020	1.545 ± 0.065	0.926 ± 0.021	0.109 ± 0.025
4	*β*-Elemene	515-13-9	1391	3.687 ± 0.257	2.164 ± 0.346	0.216 ± 0.035	-	-	0.033 ± 0.006
5	*β*-Ylangene	20479-06-5	1421	0.187 ± 0.019	0.122 ± 0.013	-	-	-	-
6	Caryophyllene	87-44-5	1418	1.394 ± 0.042	0.730 ± 0.076	0.133 ± 0.016	3.993 ± 0.103	2.455 ± 0.009	0.522 ± 0.039
7	*γ*-Elemene	29873-99-2	1433	1.831 ± 0.205	1.133 ± 0.250	0.209 ± 0.029	-	-	-
8	*β*-Copaene	18252-44-3	1432	-	-	0.017 ± 0.006	-	-	0.007 ± 0.001
9	*α*-Guaiene	3691-12-1	1440	0.271 ± 0.007	0.119 ± 0.012	0.012 ± 0.002	0.036 ± 0.004	-	0.010 ± 0.001
10	*cis*-*β*-Farnesene	28973-97-9	1444	2.829 ± 0.123	0.968 ± 0.105	0.199 ± 0.026	0.056 ± 0.002	-	0.009 ± 0.001
11	Humulene	6753-98-6	1454	0.998 ± 0.033	0.554 ± 0.052	0.098 ± 0.012	0.550 ± 0.012	0.367 ± 0.009	0.086 ± 0.005
12	*cis*-Muurola-4(15),5-diene	157477-72-0	1463	-	-	-	-	-	0.004 ± 0.001
13	Isocadinene	16729-00-3	1481	-	-	-	-	-	0.007 ± 0.002
14	*γ*-Muurolene	30021-74-0	1477	-	-	-	-	-	0.013 ± 0.003
15	Germacrene D	023986-74-5	1481	44.294 ± 1.579	19.866 ± 1.847	3.443 ± 0.814	1.053 ± 0.047	0.622 ± 0.001	0.124 ± 0.008
16	*β*-Selinene	17066-67-0	1486	-	-	-	-	-	0.007 ± 0.002
17	Valencene	4630-07-3	1492	-	0.294 ± 0.067	0.031 ± 0.003	-	-	0.008 ± 0.007
18	Bicylogermacrene	24703-35-3	1495	3.865 ± 0.134	2.000 ± 0.198	0.298 ± 0.028	0.432 ± 0.017	0.319 ± 0.005	0.068 ± 0.004
19	*α*-Muurolene	10208-80-7	1499	-	-	-	0.126 ± 0.002	0.082 ± 0.009	0.028 ± 0.005
20	*α*-Bulnesene	3691-11-0	1505	0.380 ± 0.034	-	-	0.114 ± 0.054	0.073 ± 0.008	0.014 ± 0.002
21	*α*-Farnesene	502-61-4	1508	-	0.526 ± 0.060	0.050 ± 0.005	-	-	-
22	*γ*-Cadinene	39029-41-9	1513	-	-	0.117 ± 0.025	-	-	0.009 ± 0.003
23	*β*-Cadinene	523-47-7	1518	1.743 ± 0.062	1.034 ± 0.192	0.276 ± 0.012	1.893 ± 0.108	1.200 ± 0.030	0.322 ± 0.061
24	Calamenene	483-77-2	1523	-	-	-	-	-	0.016 ± 0.005
25	*β*-Sesquiphellandrene	20307-83-9	1524	0.237 ± 0.021	0.103 ± 0.023	0.013 ± 0.002	-	-	-
26	1,4-Cadinadiene	16728-99-7	1532	-	-	0.227 ± 0.054	-	-	0.011 ± 0.003
27	*α*-Cadinene	24406-05-1	1538	-	-	0.007 ± 0.001	-	-	0.003 ± 0.001
28	*α*-Calacorene	21391-99-1	1542	-	-	-	-	-	0.004 ± 0.002
29	Germacrene B	15423-57-1	1556	7.904 ± 0.271	4.344 ± 0.371	0.596 ± 0.061	-	-	-
	Aldehydes								
1	3-Hexenal	4440-65-7		-	-	0.003 ± 0.000	-	-	-
2	Hexanal	66-25-1	801	0.056 ± 0.006	0.024 ± 0.003	0.003 ± 0.000	-	0.023 ± 0.003	-
3	(*E*)-2-hexenal	6728-26-3	859	0.031 ± 0.004	-	0.003 ± 0.000	0.022 ± 0.003	0.064 ± 0.006	-
4	Octanal	124-13-0	1010	-	-	0.006 ± 0.001	2.126 ± 0.027	2.149 ± 0.035	0.045 ± 0.008
5	Nonanal	124-19-6	1111	0.110 ± 0.008	0.072 ± 0.003	0.013 ± 0.004	0.295 ± 0.010	0.428 ± 0.024	0.012 ± 0.001
6	Citronellal	106-23-0	1158	0.386 ± 0.015	0.227 ± 0.025	0.009 ± 0.002	0.271 ± 0.008	0.409 ± 0.010	0.016 ± 0.004
7	Decanal	112-31-2	1219	0.991 ± 0.053	0.711 ± 0.082	0.029 ± 0.010	2.950 ± 0.056	3.780 ± 0.009	0.175 ± 0.033
8	Neral	106-26-3	1261	-	-	0.003 ± 0.001	0.399 ± 0.014	0.620 ± 0.006	0.017 ± 0.001
9	Perilla aldehyde	2111-75-3	1273	-	0.057 ± 0.006	0.015 ± 0.003	0.162 ± 0.017	0.300 ± 0.021	0.013 ± 0.001
10	Undecanal	112-44-7	1308	0.228 ± 0.018	0.166 ± 0.015	0.016 ± 0.003	0.050 ± 0.003	0.085 ± 0.001	0.007 ± 0.001
11	Geranial	141-27-5	1280	-	-	0.005 ± 0.001	0.580 ± 0.044	0.818 ± 0.008	0.033 ± 0.002
12	Dodecanal	112-54-9	1409	0.568 ± 0.077	0.399 ± 0.046	0.060 ± 0.013	0.390 ± 0.011	0.395 ± 0.005	0.057 ± 0.007
13	*β*-Sinensal	60066-88-8	1695	0.747 ± 0.034	0.549 ± 0.047	0.062 ± 0.003	-	-	0.002 ± 0.001
14	(*E*,*E*)-Farnesal	502-67-0	1733	0.148 ± 0.016	-	-	0.058 ± 0.009	0.035 ± 0.004	0.005 ± 0.002

Table 1. *Cont.*

	Compounds	CAS	RI [1]	HYEO			GFEO		
				CP	MADH	SCC	CP	MADH	SCC
	Alcohols								
1	*cis*-3-Hexenol	928-96-1	861	-	0.034 ± 0.003	0.002 ± 0.000	-	-	-
2	(*E*)-2-Hexen-1-ol	928-95-0	862	-	-	-	-	0.018 ± 0.005	-
3	1-Hexanol	111-27-3	870	-	0.095 ± 0.005	-	-	0.054 ± 0.009	-
4	1-Octanol	111-87-5	1071	-	0.174 ± 0.003	0.007 ± 0.001	0.599 ± 0.036	3.700 ± 0.042	0.144 ± 0.026
5	Linalool	78-70-6	1107	-	0.327 ± 0.040	0.028 ± 0.004	0.918 ± 0.038	2.403 ± 0.020	0.198 ± 0.015
6	(*E*)-p-2,8-1-menthadienol	7212-40-0	1123	-	0.107 ± 0.009	-	0.136 ± 0.056	0.187 ± 0.029	-
7	1-Nonanol	143-08-8	1165	-	-	0.008 ± 0.002	-	0.149 ± 0.004	0.014 ± 0.002
8	Terpinen-4-ol	562-74-3	1177	-	0.235 ± 0.042	0.011 ± 0.002	-	0.250 ± 0.017	0.024 ± 0.003
9	*α*-Terpineol	98-55-5	1189	-	0.192 ± 0.024	0.013 ± 0.002	0.494 ± 0.010	1.515 ± 0.019	0.124 ± 0.015
10	*cis*-Carveol	1197-06-4	1229	-	0.137 ± 0.024	0.003 ± 0.000	0.060 ± 0.018	0.461 ± 0.009	0.053 ± 0.011
11	Nerol	106-25-2	1228	-	0.095 ± 0.007	0.011 ± 0.002	0.042 ± 0.006	0.352 ± 0.018	0.024 ± 0.004
12	Citronellol	106-22-9	1241	-	0.257 ± 0.032	0.020 ± 0.003	-	0.335 ± 0.019	0.033 ± 0.003
13	Geraniol	106-24-1	1255	-	0.044 ± 0.004	0.002 ± 0.000	0.066 ± 0.009	0.506 ± 0.016	0.038 ± 0.003
14	1-Decanol	112-30-1	1273	-	0.126 ± 0.010	0.018 ± 0.002	-	0.503 ± 0.015	0.068 ± 0.005
15	Elemol	639-99-6	1549	0.264 ± 0.017	0.290 ± 0.045	0.037 ± 0.002	0.241 ± 0.014	0.313 ± 0.015	0.030 ± 0.007
16	*trans*-Nerolidol	40716-66-3	1563	-	-	-	0.083 ± 0.013	0.070 ± 0.001	0.015 ± 0.004
17	Germacrene D-4-ol	198991-79-6	1574	0.513 ± 0.023	0.190 ± 0.018	-	0.128 ± 0.015	0.084 ± 0.007	0.007 ± 0.001
18	Isospathulenol	88395-46-4	1638	0.144 ± 0.015	0.291 ± 0.031	0.018 ± 0.000	-	-	-
19	*T*-Muurolol	19912-62-0	1642	-	0.117 ± 0.057	0.020 ± 0.003	-	-	0.003 ± 0.001
20	*α*-Cadinol	481-34-5	1653	0.094 ± 0.026	0.372 ± 0.048	0.043 ± 0.012	-	-	-
21	*β*-Eudesmol	473-15-4	1649	-	-	-	-	-	0.011 ± 0.005
22	Intermedeol	6168-59-8	1667	0.274 ± 0.021	0.460 ± 0.087	0.027 ± 0.004	-	-	-
23	Shyobunol	35727-45-8	1699	-	-	-	0.047 ± 0.006	0.047 ± 0.007	0.005 ± 0.001
24	(2*E*,6*E*)-Farnesol	4602-84-0	1713	-	-	-	0.118 ± 0.016	0.131 ± 0.006	0.008 ± 0.004
	Esters								
1	Octyl acetate	112-14-1	1211	0.309 ± 0.002	0.246 ± 0.027	0.023 ± 0.009	0.361 ± 0.012	0.495 ± 0.003	0.037 ± 0.008
2	Bornyl acetate	76-49-3	1285	-	-	0.011 ± 0.003	-	-	-
3	*cis*-Carvyl acetate	1134-95-8	1337	0.074 ± 0.006	0.324 ± 0.054	0.008 ± 0.003	-	-	0.005 ± 0.001
4	*α*-Terpinyl acetate	80-26-2	1350	-	-	-	0.044 ± 0.011	0.076 ± 0.008	0.005 ± 0.001
5	Citronellyl acetate	150-84-5	1354	0.612 ± 0.030	0.725 ± 0.070	0.076 ± 0.015	0.135 ± 0.008	0.143 ± 0.004	0.069 ± 0.009
6	Neryl acetate	141-12-8	1365	2.353 ± 0.076	2.561 ± 0.249	0.339 ± 0.071	0.088 ± 0.004	0.116 ± 0.011	0.026 ± 0.003
	Ketones								
1	(-)-Carvone	6485-40-1	1264	-	0.122 ± 0.007	0.009 ± 0.001	0.028 ± 0.002	0.159 ± 0.001	0.009 ± 0.003
2	Solavetivone	54878-25-0		0.108 ± 0.012	0.078 ± 0.026	-	-	-	-
3	Nootkatone	4674-50-4	1808	10.115 ± 0.553	6.432 ± 0.546	0.336 ± 0.035	0.086 ± 0.004	0.087 ± 0.010	0.004 ± 0.002
	Others								
1	(*E*)-limonene oxide	4959-35-7	1139	0.191 ± 0.008	0.772 ± 0.155	0.016 ± 0.003	0.127 ± 0.015	0.576 ± 0.020	0.017 ± 0.007
2	Caryophyllene oxide	1139-30-6	1581	-	-	0.002 ± 0.000	-	-	0.006 ± 0.003
3	7-Methoxycoumarin	531-59-9	1732	-	-	-	0.035 ± 0.017	-	-
4	7-Hydroxycoumarin	93-35-6	1836	-	-	-	0.943 ± 0.241	-	-
5	Hexadecanoic acid, methyl ester	112-39-0	1932	-	0.042 ± 0.007	-	-	-	-
6	n-Hexadecanoic acid	57-10-3	1968	0.108 ± 0.029	0.099 ± 0.026	-	0.120 ± 0.017	0.099 ± 0.039	-

Table 1. *Cont.*

	Compounds	CAS	RI [1]	HYEO			GFEO		
				CP	MADH	SCC	CP	MADH	SCC
7	Bergaptene	484-20-8	2062	-	-	-	0.095 ± 0.010	-	-
8	Osthole	484-12-8	2143	0.203 ± 0.053	-	-	0.800 ± 0.110	-	-
9	Isoauraptene	1088-17-1	2246	0.153 ± 0.035	-	-	1.431 ± 0.263	-	-
10	Meranzin	23971-42-8	2258	0.129 ± 0.028	-	-	1.759 ± 0.976	-	-
11	Auraptenol	51559-35-4	2298	-	-	-	0.213 ± 0.036	-	-

[1] The RI values were obtained from AromChemBase (Alphasoft, Alpha M.O.S., Toulouse, France); "-" means not detected by GC-MS; CP, cold pressing; MADH, microwave-assisted hydrodistillation; SCC, spinning cone column.

Terpenes (>90%) were the most abundant volatile compounds in citrus EOs, including monoterpenes and sesquiterpenes. The top five volatiles in HY EOs were *D*-limonene (77.25–83.10%), γ-terpinene (6.74–8.48%), germacrene D (1.14–3.83%), β-myrcene (1.24–1.73%) and α-pinene (0.33–1.29%), while in GF EOs they were D-limonene (88.21–94.01%), β-myrcene (1.51–2.20%), caryophyllene (0.22–1.58%), α-pinene (0.42–0.84%) and α-copaene (0.19–0.98%). Most of these have also been identified as characteristic flavor compounds of citrus in previous studies [28–30]; however, the concentration of germacrene D in HY was significantly higher than that in most citrus [13]. Compared to GF EOs, multiple terpenes were unique in HY EOs; i.e., α-terpinene, *p*-cymene, δ-elemene, β-ylangene, γ-elemene, β-sesquiphellandrene and germacrene B. On the other hand, α-muurolene, *cis*-muurola-4(15),5-diene, isocadinene, γ-muurolene, β-selinene, calamenene and α-calacorene were unique terpenoids that only existed in GF EOs.

Aldehydes play an important role in citrus aroma due to their low threshold values [31]. In both HY and GF EOs, decanal was the predominant aldehyde, with citronellal, undecanal, nonanal and dodecanal being the other common aldehydes. However, octanal, neral and geranial were representative aldehydes with a high level in GF EOs but only detected in trace amounts in HY EOs. β-sinensal was the second most abundant aldehyde in HY EOs but almost absent in GFEOs. Previous studies [32] have demonstrated that fatty acid degradation aldehydes (e.g., nonanal and decanal) impart a fatty and green scent, while others (e.g., citral and citronellal) exhibit fresh, pleasant and citrus notes. It was suggested that the differences in aldehydes composition may account for the aroma difference between HY and GF.

Alcohols are also one of the primary contributors to citrus aromas. Elemol was found to be the only alcohol compound present in all six EOs, and it was found in close proximity in HY EOs and GF EOs. However, most alcohols were present in higher levels in GF EOs compared to HY EOs, including octanol, linalool, nonanol, α-terpineol, *cis*-carveol, nerol, citronellol, geraniol and decanol, which may be another reason for the aroma difference between HY and GF. One exception to this trend was germacrene D-4-ol, which was the most concentrated alcohol in HYEOs and found to be significantly higher than in GF EOs. Moreover, isospathulenol, α-cadinol and intermedeol only existed in HY EOs, whereas (*E*)-2-hexen-1-ol, *trans*-nerolidol, shyobunol and (2*E*,6*E*)-farnesol were only present in GF EOs.

Esters also constitute an important part of citrus aroma, which are primarily formed through the esterification of acids and alcohols. There were three aliphatic esters (i.e., hexyl acetate, octyl acetate and bornyl acetate) and four monoterpene esters (i.e., *cis*-carvyl acetate, α-terpinyl acetate, citronellyl acetate and neryl acetate) detected in six EOs. Of these, citronellyl acetate, neryl acetate and *cis*-carvyl acetate were significantly richer in HY EOs than in GF EOs, hexyl acetate and bornyl acetate were only present in trace amounts in HY SCC extracts and α-terpinyl acetate was only present in GF EOs.

Carvone and nootkatone were the two ketones detected in six citrus EOs. Carvone was probably generated by the oxidation of limonene during oxidative storage and thermal treatment [33]. Surprisingly, we found that the content of nootkatone in HY EO was nearly one hundred times higher than that in GF EO, despite previous studies predominantly

associating nootkatone as a characteristic aroma compound of GF [34]. Therefore, HY peels may be a viable and sustainable source of natural nootkatone production, which is highly demanded by the flavor, fragrance and cosmetic industries [35].

Other compounds detected in six EOs included two terpene oxides (i.e., (*E*)-limonene oxide, caryophyllene oxide), n-hexadecanoic acid and multiple coumarins (i.e., 7-methoxycoumarin, 7-hydroxycoumarin, bergaptene, osthole, isoauraptene, meranzin and auraptenol). All the coumarins detected were significantly more abundant in GF EO than in HY EO.

3.3. Differences of Volatiles in Citrus EOs with Different Extraction Methods

There were 54, 66, 71, 60, 58 and 70 volatile compounds detected in HY_CP, HY_MADH, HY_SCC, GF_CP, GF_MADH and GF_SCC, respectively. The result showed that both the citrus varieties and extraction methods would affect the composition and content of volatile compounds in citrus EOs. As shown in Figure 2A, the SCC extracts contained extremely low levels of volatile compounds, which was in agreement with the results of the sensory and e-nose analysis. However, compared to CP and MADH, SCC extracts contained some unique volatiles, which were mainly sesquiterpenes such as γ-cadinene, calamenene, 1,4-cadinadiene and α-cadinene.

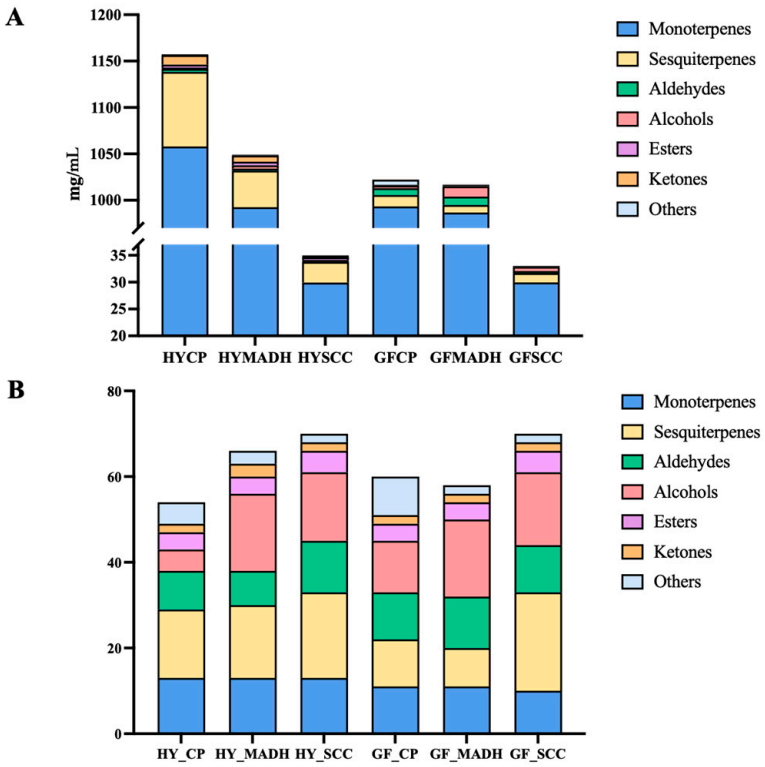

Figure 2. Comparison of the concentration and number of volatile compounds among six EOs. Bar chart for the concentration of volatile compounds in six EOs (**A**); bar chart for the number of volatile compounds in six EOs (**B**). EOs, essential oils; HY, Huyou; GF, grapefruit; CP, cold pressing; MADH, microwave-assisted hydrodistillation; SCC, spinning cone column.

Compared to CP, the EOs extracted by MADH and SCC had an expanded number of volatile compounds, as shown in Figure 2B. This was attributed to the fact that the two methods involved a heating process in which some heat sensitive compounds were degraded as well as the hydrolysis of readily hydrolysable compounds, resulting in the generation of a range of new compounds. Most notably, there was a significant increase in the number and content of alcohols. Most alcohol compounds were significantly more abundant in MADH EOs than in CP EOs. The similar phenomenon was observed in heat-

sterilized orange juice compared to untreated juice [36]. In addition, six alcohol compounds were only present in MADH and SCC extracts, i.e., (*E*)-2-hexen-1-ol, hexanol, terpinen-4-ol, nonanol, decanol and T-muurolol. Among these, terpinen-4-ol was generally identified as an off-flavor marker in heat-treated orange juice, which was produced by the degradation of linalool [32,37].

The content of terpenes was also strongly affected by the extraction method, particularly terpenes, most of which were significantly lower in MADH EOs than in CP EOs, such as *β*-cubebene, caryophyllene, *cis*-*β*-farnesene and germacrene D. Terpenes were prone to oxidation and degradation reactions when exposed to oxygen and heat, converting them into terpene alcohols or oxides [38]. Thus, we observed an increase in limonene oxide and terpenic alcohols in MADH EOs at the same time. However, such molecular change produces unpleasant odors like medicinal, woody and waxy that negatively affect the sensory quality [39].

The effect of the extraction method on aldehyde content was different in the two citrus. In HY EOs, most aldehydes were lower in MADH EO than in CP EO, such as hexanal, nonanal, citronellal, decanal and undecanal. Previous studies have confirmed that thermal treatments easily lead to the loss of aldehydes [30], but some direct chain aldehydes such as (*E*)-2-hexenal may increase due to the degradation of polyunsaturated fatty acids [36]. However, in GF EOs, we observed that the contents of most aldehyde compounds were increased in MADH EO compared to CP EO.

Carvone was also significantly abundant in MADH EOs and it was also an off-flavor compound, resulting from the oxidative production of *D*-limonene during heating processing [40]. Additionally, some nonvolatile compounds such as coumarins (i.e., 7-hydroxycoumarin, bergaptene, osthole, isoauraptene, meranzin and auraptenol) only existed in CP EOs. In conclusion, the appropriate extraction method can be selected according to the individual needs in practice.

3.4. Analysis of Aroma-Active Compounds Based on AEDA and MF

CP EOs with better sensory properties were selected for GC-O analysis. As shown in Table 2, a total of 59 aroma-active compounds were identified by GC-MS-O in two EOs, including 14 terpenes, 12 alcohols, 11 aldehydes, 2 esters, 2 ketones and 15 unidentified compounds. Moreover, 49 and 41 compounds were detected in HY EO and GF EO, respectively. In terms of the number of aroma compounds, HY EO exhibited a richer aromatic profile.

Table 2. Analysis of aroma-active compounds in HY EO and GF EO based on MF and AEDA.

No. *	Compounds	Identification	Odor Characteristic	HY EO		GF EO	
				MF%	FD	MF%	FD
1	*β*-Myrcene	MS, RI, O	balsamic, fruity	78.06	128	81.97	128
2	Octanal	MS, RI, O	fatty, citrus	-	-	84.52	256
3	*D*-Limonene	MS, RI, O	citrus, lemon	79.06	256	80.18	256
4	*γ*-Terpinene	MS, RI, O	engine oil	73.95	1	-	-
5	1-Octanol	MS, RI, O	pungent	-	-	49.74	1
6	Terpinolene	MS, RI, O	pine	35.36	8	63.74	8
7	Unknown1	O	sweet, fruity	55.90	2	-	-
8	Linalool	MS, RI, O	floral	83.45	8	85.70	256
9	Nonanal	MS, RI, O	aldehydic	-	-	43.30	2
10	*cis*-Limonene oxide	MS, RI, O	herbaceous	37.50	1	-	-
11	Unknown 2	O	tallowy, pungent	72.89	2	39.53	4
12	Citronellal	MS, RI, O	floral, lemon	87.50	4	83.85	8
13	Unknown 3	O	herbaceous	-	-	35.36	1
14	Unknown 4	O	metallic	70.71	32	66.14	64
15	*α*-Terpineol	MS, RI, O	fruity, floral	68.47	32	77.06	64
16	Decanal	MS, RI, O	aldehydic, citrus	84.78	4	84.78	64
17	Citronellol	MS, RI, O	floral, fresh	59.51	1	53.03	16
18	Neral	MS, RI, O	citrus, floral, fresh	-	-	67.31	4
19	(-)-Carvone	MS, RI, O	minty, fresh	-	-	43.30	8

Table 2. *Cont.*

No. *	Compounds	Identification	Odor Characteristic	HY EO		GF EO	
				MF%	FD	MF%	FD
20	Geraniol	MS, RI, O	citrus, floral	63.74	32	71.44	512
21	Geranial	MS, RI, O	citrus, lemon, floral	-	-	60.38	4
22	1-Decanol	MS, RI, O	fatty	43.30	1	50.00	2
23	Thymol	MS, RI, O	woody, grass	45.07	1	-	-
24	Carvacrol	MS, RI, O	chicken shit	41.46	1	60.92	2
25	Unknown 5	O	fatty	68.47	64	76.03	1
26	Undecanal	MS, RI, O	aldehydic	39.53	1	33.07	1
27	(*E*,*E*)-2,4-Decadienal	MS, RI, O	tallowy, peanut	46.77	8	58.63	32
28	Carvyl acetate	MS, RI, O	woody, fruity	82.92	128	53.03	2
29	Unknown 6	O	stinky	61.24	1	-	-
30	Citronellyl acetate	MS, RI, O	fruity	50.00	8	-	-
31	Unknown 7	O	grass	46.77	8	-	-
32	Ylangene	MS, RI, O	fatty	-	-	58.09	4
33	Copaene	MS, RI, O	raw soybean	81.97	32	81.28	32
34	*β*-Elemene	MS, RI, O	herbaceous	55.90	16	-	-
35	Unknown 8	O	metallic	39.53	1	-	-
36	Dodecanal	MS, RI, O	aldehydic, tallowy	79.06	16	76.03	4
37	*α*-Guaiene	MS, RI, O	floral, woody	50.00	4	43.30	1
38	*cis*-*β*-Farnesene	MS, RI, O	poker	81.97	16	70.99	2
39	*trans*-2-Dodecenal	MS, RI, O	soapy, aldehydic	76.03	32	-	-
40	*γ*-Muurolene	MS, RI, O	herbaceous	-	-	25.00	1
41	Germacrene D	MS, RI, O	paper, ink	70.71	128	-	-
42	Valencene	MS, RI, O	sweet, herbaceous	37.50	8	-	-
43	Bicylogermacrene	MS, RI, O	green	33.07	1	-	-
44	Unknown 9	O	fruity, floral	30.62	1	63.74	1
45	Elemol	MS, RI, O	spicy, stinky	41.46	8	75.00	16
46	Germacrene B	MS, RI, O	woody	76.03	128	-	-
47	Unknown 10	O	stinky	55.90	4	-	-
48	Unknown 11	O	stinky	73.95	8	-	-
49	Unknown 12	O	grass, fruity	86.60	128	35.36	2
50	*T*-Muurolol	MS, RI, O	stinky, fishy	89.64	64	43.30	2
51	*α*-Cadinol	MS, RI, O	pungent	87.50	256	59.95	16
52	*β*-Eudesmol	MS, RI, O	sweet	43.30	64	-	-
53	*β*-Sinensal	MS, RI, O	fishy	100.00	32	-	-
54	Unknown 13	O	woody	59.95	2	48.41	8
55	7-Methoxycoumarin	MS, RI, O	sweet, cream	81.01	16	81.97	32
56	Nootkatone	MS, RI, O	woody, grapefruit	99.22	256	45.07	2
57	7-Hydroxycoumarin	MS, RI, O	scorched	57.28	1	54.01	8
58	Unknown 14	O	sweet	-	-	35.36	16
59	Unknown 15	O	sweet	54.49	16	50.00	2

* Odorants are numbered consecutively according to their elution order from the DB-5ms column; "-" means not smelled; Unknown compounds are not identified by MS but smelled by sniffers. EOs, essential oils; HY, Huyou; GF, grapefruit; AEDA, aroma extract dilution analysis; MF, modified frequency.

3.4.1. AEDA Results

FD factor was employed to screen out key aroma-active compounds and a large FD indicates the compound contributes significantly to the overall aroma. In the present study, aroma-active compounds with FD > 16 were considered as key odorants and a total of 29 compounds were selected. The FD factor olfactograms of them are shown in Figure 3A.

β-Myrcene (FD = 128, balsamic/fruity) and *D*-limonene (FD = 256, citrus/lemon/minty) were key aroma-active compounds with a high FD in both HY EO and GF EO. This suggested their crucial role in the formation of citrus flavor, which was consistent with previous research [1,21]. Specifically, among the compounds with FD > 100, *α*-cadinol (FD = 256, pungent), nootkatone (FD = 256, woody), carvyl acetate (FD = 128, woody/fruity), germacrene D (FD = 128, paper/ink), germacrene B (FD = 128, woody) and unknown 12 (FD = 128, grass/fruity) were found to be unique key odorants in HY EO. This explained why HY EO exhibited a strongly woody and green scent distinctly different from GF EO. On the other hand, geraniol (FD = 512, citrus/floral), octanal (FD = 256, fatty/citrus) and linalool (FD = 256, floral) were identified as unique key odorants in GF EO, which contributed to a stronger fruity and floral aroma in GF EO as compared to HY EO. In

addition, other key aroma-active compounds with FD > 16 specific to HY EO included *T*-muurolol (FD = 64, stinky/fishy), *β*-eudesmol (FD = 64, sweet), unknown 5 (FD = 64, fatty), *β*-sinensal (FD = 32, fishy), *trans*-2-dodecenal (FD = 32, soapy/aldehydic), dodecanal (FD = 16, aldehydic/tallowy), *cis*-*β*-farnesene (FD = 16, poker) and unknown 15 (FD = 16, sweet), while decanal (FD = 64, aldehydic/tallowy/citrus), (*E*,*E*)-2,4-decadienal (FD = 32, tallowy/peanut), citronellol (FD = 16, floral/fresh) and elemol (FD = 16, spicy/stinky) were specific to GFEO.

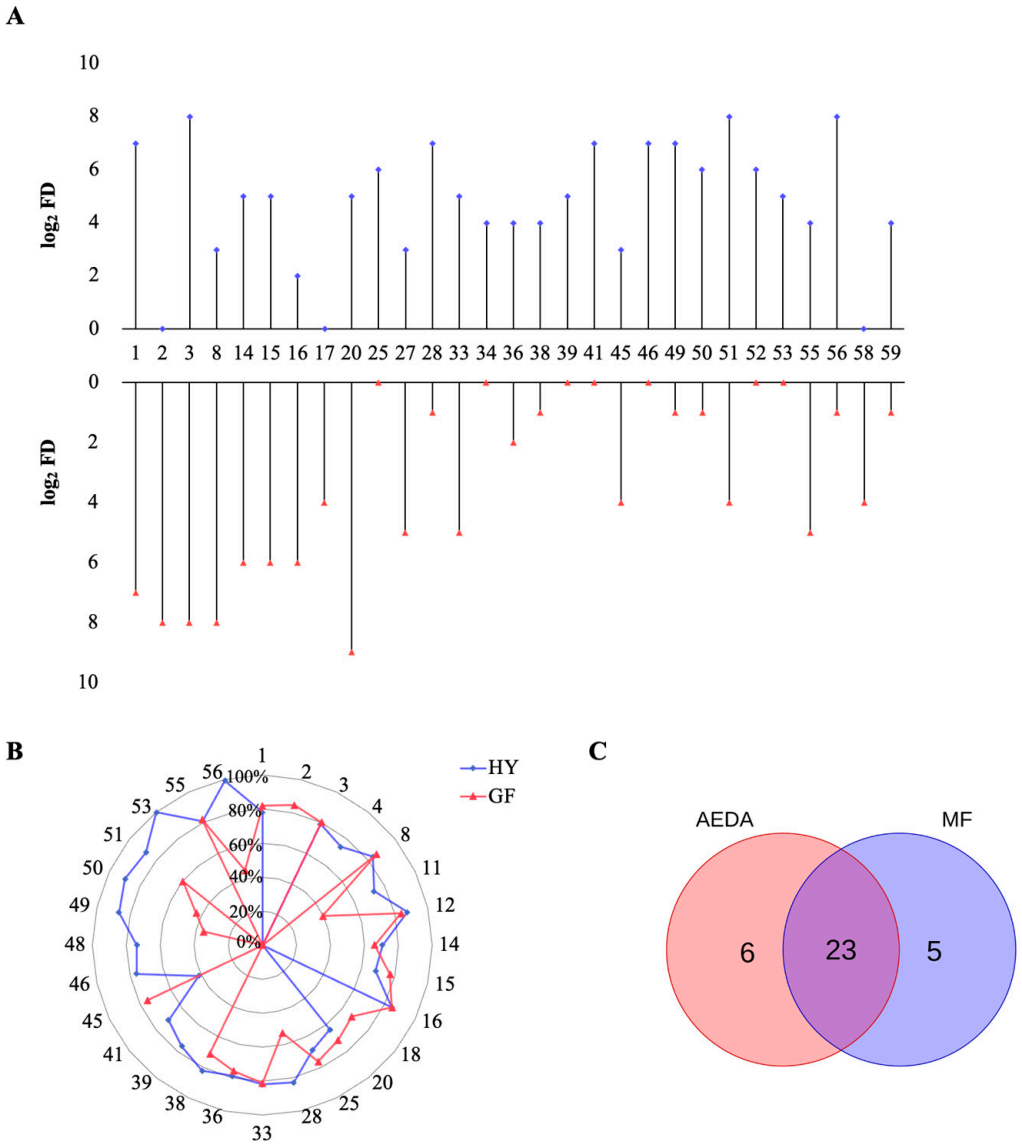

Figure 3. Comparison of key aroma-active compounds in HY EO and GF EO. FD factors (FD > 16) olfactograms of key odorants (**A**); MF (MF > 65%) olfactograms of key odorants (**B**). The Venn of AEDA and MF results (**C**). Numbers of compounds correspond to Table 2. HY, Huyou; GF, grapefruit; AEDA, aroma extract dilution analysis; MF, modified frequency.

Interestingly, 7-methoxycoumarin (FD = 16 and 32) was detected in the GC-O experiment and showed a pleasant sweet and cream aroma, although it is a nonvolatile compound usually identified by HPLC. In any case, *α*-copaene (FD = 32) featured a raw soybean scent, which had not been reported previously.

3.4.2. Modified Frequency (MF) Method Results

A total of 28 aroma-active compounds with MF > 65% were selected as key odorants and plotted into olfactograms based on MF (Figure 3B). As expected, the MF olfactogram obtained from HY EO was more complex and intense than that of GF EO. Moreover, among these compounds, β-sinensal was detected at full frequency and aroma intensity (i.e., MF = 100%) in HY EO, presenting a strong fishy note, but was not detected in GF EO. Contrarily, in AEDA results, β-sinensal had a small FD indicating little contribution to the overall aroma. This highlights differences between two olfactory analysis methods, which was also the case in the previous study [41]. Consequently, combining two strategies allowed us to screen out key odorants more accurately from multiple perspectives. Nootkatone (MF = 99.22%) was another key odorant with a second high MF of 99.22% in HY EO, characterized by a strong woody flavor with a significant contribution to HY EO, which was consistent with AEDA. However, compared to AEDA results, γ-terpinene (MF = 73.95%, engine oil), unknown 2 (MF = 72.89%, bedbug/tallowy/pungent) and unknown 11 (MF = 73.95%, stinky) were newly identified key odorants in HY EO, with neral (MF = 67.31%, citrus/floral/fresh) and elemol (MF = 75.00%, spicy/stinky) in GF EO. In addition, the common key aroma-active compounds in HY EO and GF EO were linalool (MF = 83.45%, 85.70%), citronellal (MF = 87.50%, 83.85%), decanal (MF = 84.78%, 84.78%), 7-methoxycoumarin (MF = 81.01%, 81.97%), dodecanal (MF = 79.06%, 76.03%), α-terpineol (MF = 68.47%, 77.06%), unknown 4 (MF = 70.71%, 66.14%) and unknown 5 (MF = 68.47%, 76.03%).

The Venn diagram (Figure 3C) showed that 23 key aroma-active compounds were determined jointly by both AEDA and MF, i.e., β-myrcene, octanal, *D*-limonene, linalool, unknown 4, α-terpineol, decanal, geraniol, unknown 5, carvyl acetate, copaene, dodecanal, *cis*-β-farnesene, *trans*-2-dodecenal, germacrene D, elemol, germacrene B, unknown 12, *T*-muurolol, α-cadinol, β-sinensal, 7-methoxycoumarin and nootkatone.

3.5. Correlation between Aroma-Active Compounds and Sensory Attributes

Based on the GC-O analysis, aldehydes, alcohols and terpenes were the main contributors to the flavor of HY EO and GF EO. The aroma attributes of key aroma-active compounds in HY EO and GF EO were mainly classified into six categories (sweet/fruity, floral, fatty, woody, green and unpleasant) and a flavor wheel was drawn on this basis (Figure 4A). Specifically, the aldehydes, such as octanal, citronellal, decanal, etc., mostly provided fatty and citrus scents which formed the characteristic citrus flavor. Some alcohols such as linalool, α-terpineol and geraniol offered floral and fruity odors, whereas elemol, *T*-muurolol and α-cadinol contributed off-flavors like pungent and spicy. The scents of terpenes were various and complex, with β-myrcene and *D*-limonene contributing to fruity notes, *cis*-β-Farnesene, germacrene D and germacrene B to woody notes, copaene and β-elemene to green and herbal notes and γ-terpinene presenting an engine oil note.

Additionally, PLSR was applied to assess the correlation between aroma-active compounds (X) and sensory evaluation data (Y). As shown in Figure 4B, the PLSR model included two principal components: factor 1 and factor 2, explaining 94% of the X-variance and 3% of the Y-variance, respectively. All the sensory attributes and aroma-active compounds were situated between the inner and outer ellipses, indicating that they were adequately explained by the PLSR model. The sensory variables of sweet, fruity and floral were concentrated on the positive X-axis, exhibiting positive correlations with β-myrcene, linalool, geraniol, geranial, and so on. Conversely, fatty, woody and green were located on the negative X-axis, and they were positively correlated with undecanal, β-elemene, nootkatone and others. These results were generally consistent with the olfactory description in the GC-O experiment.

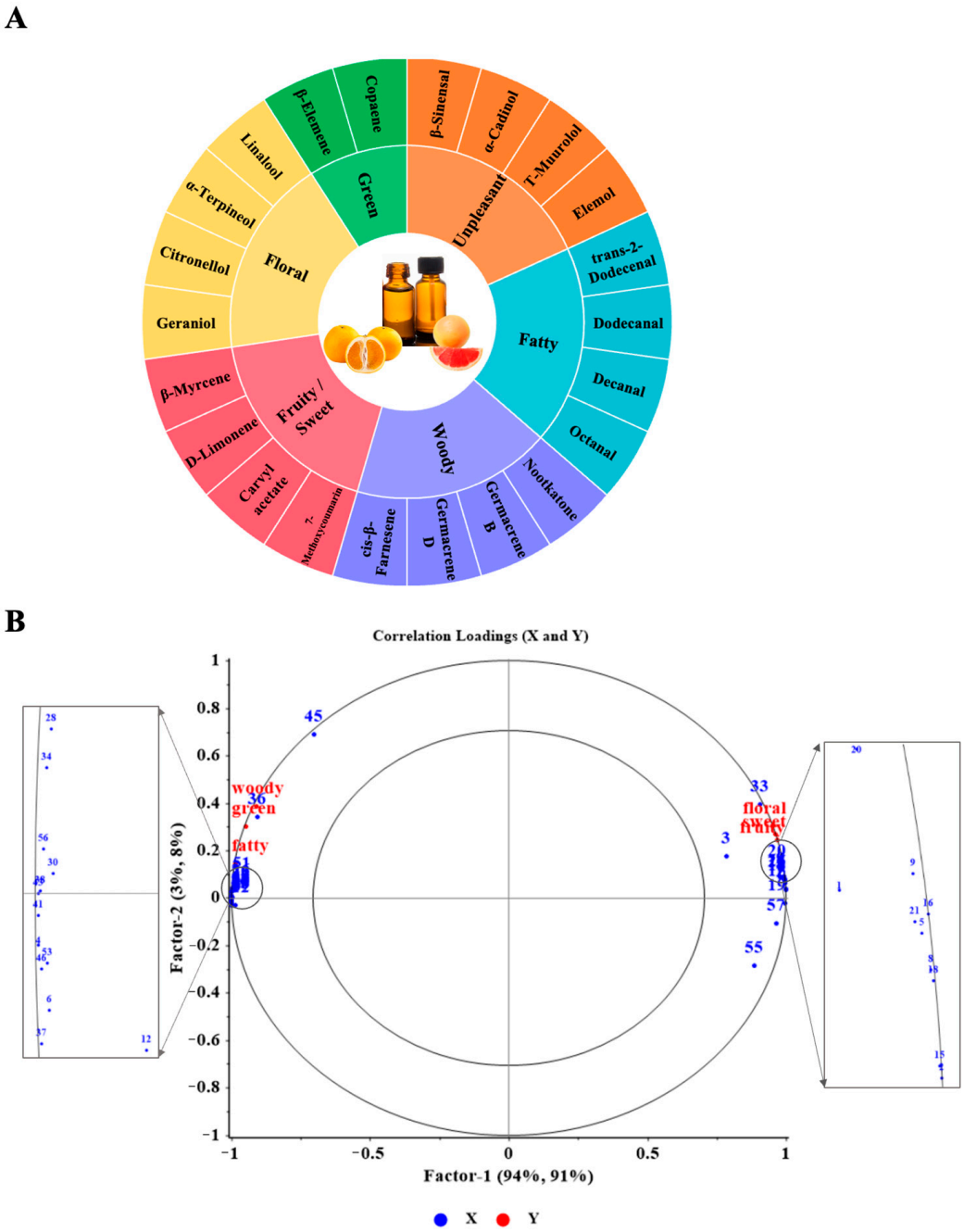

Figure 4. Correlation between aroma-active compounds and sensory attributes. Sensory flavor wheel based on GC-O analysis (**A**); PLSR model (**B**). Numbers of compounds correspond to Table 2.

3.6. Aroma Markers Differentiating between HY EO and GF EO

To screen differential aroma compounds between HY EO and GF EO, we performed OPLS-DA, a supervised classification method [42], based on the content of 59 aroma-active compounds in two CP EOs. As shown in Figure 5A, there was a clear separation between HY EO and GF EO, with HY samples distributed in the left area (second and third quadrants) and GF EO in the right (first and fourth quadrants). The OPLS-DA model was established with good fitting parameters (R^2Y = 1, Q^2 = 0.999), indicating strong explanatory validity and predictive ability. For the permutation test (n = 200), the intercepts of R^2 and Q^2 were −0.02 and −1.36, respectively, which further demonstrated the OPLS-DA model was statistically significance and not over-fitting (Figure 5C).

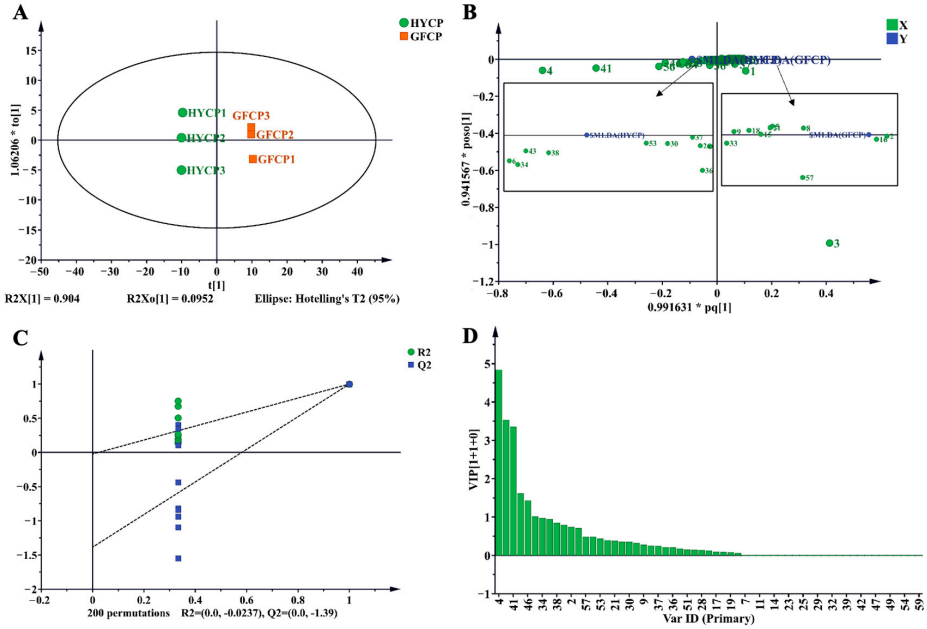

Figure 5. OPLS-DA model of aroma-active compounds in HYEO and GFEO. The score plot (**A**), loading plot (**B**), permutation test at 200 times (**C**) and VIP values (**D**). Numbers of compounds correspond to Table 2. HY, Huyou; GF, grapefruit; CP, cold pressing.

The OPLS-DA loading plot and VIP (variable importance in the projection) values were used to screen out aroma markers that showed great contribution in differentiating HY EO from GF EO. The loading plot (Figure 5B) revealed that γ-terpinene, germacrene D, germacrene B and nootkatone were mainly related to HY EO, while *D*-limonene, β-myrcene, octanal and decanal were mainly related to GF EO. Among them, germacrene D and *D*-limonene, which are located furthest from the center of the loading plot, contributed most greatly to the model. Furthermore, the VIP values measure the importance of each variable in the model, with larger VIP values indicating a greater contribution to the differentiation of samples [43]. Typically, VIP > 1 suggests the variables play an essential role in distinguishing different samples [43]. As shown in Figure 5D, six aroma-active compounds with VIP > 1 were filtered as aroma markers for differentiating HY EO and GF EO, including γ-terpinene (VIP = 4.84), *D*-limonene (VIP = 3.54), germacrene D (VIP = 3.36), nootkatone (VIP = 1.62), germacrene B (VIP = 1.44) and terpinolene (VIP = 1.02).

4. Conclusions

In this study, we comprehensively analyzed the aroma profiles of HY and GF EOs using sensory analysis, e-nose, GC-MS and GC-O combined with chemometric approaches. The extraction method had an effect on the flavor of HY and GF EOs, with CP EOs showing a stronger citrus characteristic aroma, MADH EOs richer in alcohols, ketones and oxides and SCC extracts containing a greater variety of volatiles. This may have implications for the production of citrus EOs. Additionally, HY EO exhibited a stronger woody, green and fatty aroma compared to GF EO, with a weaker floral, fruity and sweet aroma. The GC-O results demonstrated that this was due to the fact that the represented odorants in HY EO were germacrene D, germacrene B and nootkatone, whereas geraniol, linalool and octanal contributed significantly to GF EO. In addition, γ-terpinene, *D*-limonene, germacrene D, nootkatone, germacrene B and terpinolene were identified as aroma markers using VIP > 1 to distinguish HY from GF. Our study provides data support for establishing the correlation between the bioactivity and flavor compounds of HY in subsequent research.

Author Contributions: H.C.: Writing—review and editing, project administration. F.L.: Writing—original draft, formal analysis. Y.Z.: Conceptualization, validation. Z.Y.: Formal analysis, data curation. J.C.:

Methodology, validation. S.C.: Methodology, investigation. X.Y.: Methodology, funding acquisition, project administration. All authors have read and agreed to the published version of the manuscript.

Funding: This research was financially supported by the National Natural Science Foundation of China (Grant No. 32172334), the Science and Technology Cooperation Program of Zhejiang Province (2022SNJF083) and the Fundamental Research Funds for the Central Universities (226-2023-00098).

Institutional Review Board Statement: Not applicable.

Data Availability Statement: The dataset is available from the first author on reasonable request.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Xiao, Z.; Zhang, S.; Zhu, J.; Niu, Y.; Xiong, W.; Chen, F. Identification of Key Aromas of Grapefruit Juice and Study of Their Contributions to the Enhancement of Sweetness Perception. *Eur. Food Res. Technol.* **2023**, *249*, 537–551. [CrossRef]
2. Singh, B.; Singh, J.P.; Kaur, A.; Yadav, M.P. Insights into the Chemical Composition and Bioactivities of Citrus Peel Essential Oils. *Food Res. Int.* **2021**, *143*, 110231. [CrossRef] [PubMed]
3. Liu, N.; Li, X.; Zhao, P.; Zhang, X.; Qiao, O.; Huang, L.; Guo, L.; Gao, W. A Review of Chemical Constituents and Health-Promoting Effects of Citrus Peels. *Food Chem.* **2021**, *365*, 130585. [CrossRef] [PubMed]
4. Jeyaratnam, N.; Nour, A.H.; Kanthasamy, R.; Nour, A.H.; Yuvaraj, A.R.; Akindoyo, J.O. Essential Oil from Cinnamomum Cassia Bark through Hydrodistillation and Advanced Microwave Assisted Hydrodistillation. *Ind. Crops Prod.* **2016**, *92*, 57–66. [CrossRef]
5. Bustamante, J.; van Stempvoort, S.; García-Gallarreta, M.; Houghton, J.A.; Briers, H.K.; Budarin, V.L.; Matharu, A.S.; Clark, J.H. Microwave Assisted Hydro-Distillation of Essential Oils from Wet Citrus Peel Waste. *J. Clean. Prod.* **2016**, *137*, 598–605. [CrossRef]
6. Sandhu, H.K.; Sinha, P.; Emanuel, N.; Kumar, N.; Sami, R.; Khojah, E.; Al-Mushhin, A.A.M. Effect of Ultrasound-Assisted Pretreatment on Extraction Efficiency of Essential Oil and Bioactive Compounds from Citrus Waste By-Products. *Separations* **2021**, *8*, 244. [CrossRef]
7. Golmakani, M.-T.; Rezaei, K. Comparison of Microwave-Assisted Hydrodistillation Withthe Traditional Hydrodistillation Method in the Extractionof Essential Oils from *Thymus vulgaris* L. *Food Chem.* **2008**, *109*, 925–930. [CrossRef]
8. Luo, Y.; Wang, K.; Zhuang, H.; Li, D.; Meng, X.; Shi, M.; Yao, L.; Song, S.; Sun, M.; Wang, H.; et al. Elucidation of Aroma Compounds in Passion Fruit (*Passiflora Alata* Ait) Using a Molecular Sensory Approach. *J. Food Biochem.* **2022**, *46*, e14224. [CrossRef]
9. Riley, P.C.; Sykes, S.J. Industrial Applications of Spinning Cone Column Technology: A Review. *Distill. Absorpt.* **2002**, *1*, 1–15.
10. Sam, F.E.; Ma, T.-Z.; Salifu, R.; Wang, J.; Jiang, Y.-M.; Zhang, B.; Han, S.-Y. Techniques for Dealcoholization of Wines: Their Impact on Wine Phenolic Composition, Volatile Composition, and Sensory Characteristics. *Foods* **2021**, *10*, 2498. [CrossRef]
11. Graber, M.F.; Pérez-Correa, J.R.; Verdugo, G.; Valle, J.M.D.; Agosin, E. Spinning Cone Column Isolation of Rosemary Essential Oil. *Food Control* **2010**, *21*, 615–619. [CrossRef]
12. Xu, C.J.; Bao, L.; Zhang, B.; Bei, Z.M.; Ye, X.Y.; Zhang, S.L.; Chen, K.S. Parentage Analysis of Huyou (Citrus Changshanensis) Based on Internal Transcribed Spacer Sequences. *Plant Breed.* **2006**, *125*, 519–522. [CrossRef]
13. Guo, J.; Gao, Z.; Xia, J.; Ritenour, M.A.; Li, G.; Shan, Y. Comparative Analysis of Chemical Composition, Antimicrobial and Antioxidant Activity of Citrus Essential Oils from the Main Cultivated Varieties in China. *LWT-Food Sci. Technol.* **2018**, *97*, 825–839. [CrossRef]
14. Hu, Y.-H.; Liu, J.; Li, H.; Tang, W.; Li, X.-W.; Guo, Y.-W. Chemical Constituents from Citrus Changshan-Huyou and Their Anti-Inflammatory Activities. *Chem. Biodivers* **2020**, *17*, e2000503. [CrossRef]
15. Jiang, J.; Yan, L.; Shi, Z.; Wang, L.; Shan, L.; Efferth, T. Hepatoprotective and Anti-Inflammatory Effects of Total Flavonoids of Qu Zhi Ke (Peel of Citrus Changshan-Huyou) on Non-Alcoholic Fatty Liver Disease in Rats via Modulation of NF-κB and MAPKs. *Phytomedicine* **2019**, *64*, 153082. [CrossRef]
16. He, Y.; Zhu, Y.; Lv, J.; Gu, Y.; Wang, T.; Chen, J. Effects of Lactic Acid Bacteria Fermentation on the Bioactive Composition, Volatile Compounds and Antioxidant Activity of Huyou (Citrus Aurantium 'Changshan-Huyou') Peel and Pomace. *Food Qual. Saf.* **2023**, *7*, fyad003. [CrossRef]
17. Pérez-Jiménez, M.; Sherman, E.; Pozo-Bayón, M.A.; Pinu, F.R. Application of Untargeted Volatile Profiling and Data Driven Approaches in Wine Flavoromics Research. *Food Res. Int.* **2021**, *145*, 110392. [CrossRef]
18. Gu, Z.; Jin, Z.; Schwarz, P.; Rao, J.; Chen, B. Uncovering Aroma Boundary Compositions of Barley Malts by Untargeted and Targeted Flavoromics with HS-SPME-GC-MS/Olfactometry. *Food Chem.* **2022**, *394*, 133541. [CrossRef]
19. Ou, M.-C.; Liu, Y.-H.; Sun, Y.-W.; Chan, C.-F. The Composition, Antioxidant and Antibacterial Activities of Cold-Pressed and Distilled Essential Oils of *Citrus paradisi* and *Citrus grandis* (L.) Osbeck. *J. Evid.-Based Complement. Altern. Med.* **2015**, *2015*, e804091. [CrossRef]
20. Xiao, Z.; Wu, Q.; Niu, Y.; Wu, M.; Zhu, J.; Zhou, X.; Chen, X.; Wang, H.; Li, J.; Kong, J. Characterization of the Key Aroma Compounds in Five Varieties of Mandarins by Gas Chromatography–Olfactometry, Odor Activity Values, Aroma Recombination, and Omission Analysis. *J. Agric. Food Chem.* **2017**, *65*, 8392–8401. [CrossRef]

21. Shui, M.; Feng, T.; Tong, Y.; Zhuang, H.; Lo, C.; Sun, H.; Chen, L.; Song, S. Characterization of Key Aroma Compounds and Construction of Flavor Base Module of Chinese Sweet Oranges. *Molecules* **2019**, *24*, 2384. [CrossRef] [PubMed]
22. Chen, J.; Liu, F.; Ismail, B.B.; Wang, W.; Xu, E.; Pan, H.; Ye, X.; Liu, D.; Cheng, H. Effects of Ethephon and Low-Temperature Treatments on Blood Oranges (*Citrus sinensis* L. Osbeck): Anthocyanin Accumulation and Volatile Profile Changes during Storage. *Food Chem.* **2022**, *393*, 133381. [CrossRef] [PubMed]
23. Liu, F.; Chen, Y.; Chen, J.; Xu, E.; Pan, H.; Chen, S.; Ye, X.; Cheng, H. Characteristic Aroma Improvement Mechanisms of Heat-Sterilized Bayberry Juice Regulated by Exogenous Polyphenols. *Food Chem.* **2023**, *427*, 136644. [CrossRef] [PubMed]
24. Feng, Y.; Cai, Y.; Sun-Waterhouse, D.; Cui, C.; Su, G.; Lin, L.; Zhao, M. Approaches of Aroma Extraction Dilution Analysis (AEDA) for Headspace Solid Phase Microextraction and Gas Chromatography–Olfactometry (HS-SPME–GC–O): Altering Sample Amount, Diluting the Sample or Adjusting Split Ratio? *Food Chem.* **2015**, *187*, 44–52. [CrossRef]
25. Ríos-Reina, R.; Segura-Borrego, M.P.; Morales, M.L.; Callejón, R.M. Characterization of the Aroma Profile and Key Odorants of the Spanish PDO Wine Vinegars. *Food Chem.* **2020**, *311*, 126012. [CrossRef] [PubMed]
26. Fang, H.; Chen, J.; Tian, Y.; Liu, Y.; Li, H.; Cheng, H. Chemometrics Characterization of Volatile Changes in Processed Bayberry Juice versus Intact Fruit during Storage by Headspace Solid-Phase Micro-Extraction Combined with GC–MS. *J. Food Process. Preserv.* **2020**, *44*, e14444. [CrossRef]
27. Yu, D.; Zhang, X.; Guo, S.; Yan, H.; Wang, J.; Zhou, J.; Yang, J.; Duan, J.-A. Headspace GC/MS and Fast GC e-Nose Combined with Chemometric Analysis to Identify the Varieties and Geographical Origins of Ginger (*Zingiber officinale* Roscoe). *Food Chem.* **2022**, *396*, 133672. [CrossRef]
28. Gök, A.; İsmail Kirbaşlar, Ş.; Gülay Kirbaşlar, F. Comparison of Lemon Oil Composition after Using Different Extraction Methods. *J. Essent. Oil Res.* **2015**, *27*, 17–22. [CrossRef]
29. Zhang, H.; Xie, Y.; Liu, C.; Chen, S.; Hu, S.; Xie, Z.; Deng, X.; Xu, J. Comprehensive Comparative Analysis of Volatile Compounds in Citrus Fruits of Different Species. *Food Chem.* **2017**, *230*, 316–326. [CrossRef]
30. Sun, H.; Ni, H.; Chen, F.; Jiang, Z.; Huang, G.; Yang, Y. Effect of Oxygen and Heating on Aromas of Pummelo (Citrus Maxima) Essential Oil. *J. Essent. Oil Res.* **2018**, *30*, 92–104. [CrossRef]
31. Li, L.J.; Hong, P.; Chen, F.; Sun, H.; Yang, Y.F.; Yu, X.; Huang, G.L.; Wu, L.M.; Ni, H. Characterization of the Aldehydes and Their Transformations Induced by UV Irradiation and Air Exposure of White Guanxi Honey Pummelo (*Citrus grandis* (L.) Osbeck) Essential Oil. *J. Agric. Food Chem.* **2016**, *64*, 5000–5010. [CrossRef] [PubMed]
32. Jia, X.; Ren, J.; Fan, G.; Reineccius, G.A.; Li, X.; Zhang, N.; An, Q.; Wang, Q.; Pan, S. Citrus Juice Off-Flavor during Different Processing and Storage: Review of Odorants, Formation Pathways, and Analytical Techniques. *Crit. Rev. Food Sci. Nutr.* **2022**, 1–26. [CrossRef] [PubMed]
33. Perez-Cacho, P.R.; Rouseff, R. Processing and Storage Effects on Orange Juice Aroma: A Review. *J. Agric. Food Chem.* **2008**, *56*, 9785–9796. [CrossRef] [PubMed]
34. Da Cruz, M.A.; Plotto, A.; Ferrarezi, R.S.; Leite Junior, R.P.; Bai, J. Effect of Huanglongbing on the Volatile Organic Compound Profile of Fruit Juice and Peel Oil in 'Ray Ruby' Grapefruit. *Foods* **2023**, *12*, 713. [CrossRef] [PubMed]
35. Cheong, M.W.; Liu, S.Q.; Zhou, W.; Curran, P.; Yu, B. Chemical Composition and Sensory Profile of Pomelo (*Citrus grandis* (L.) Osbeck) Juice. *Food Chem.* **2012**, *135*, 2505–2513. [CrossRef] [PubMed]
36. Cheng, C.; Jia, M.; Gui, Y.; Ma, Y. Comparison of the Effects of Novel Processing Technologies and Conventional Thermal Pasteurisation on the Nutritional Quality and Aroma of Mandarin (*Citrus unshiu*) Juice. *Innov. Food Sci. Emerg. Technol.* **2020**, *64*, 102425. [CrossRef]
37. Zhang, J.; Liu, H.; Sun, R.; Zhao, Y.; Xing, R.; Yu, N.; Deng, T.; Ni, X.; Chen, Y. Volatolomics Approach for Authentication of Not-from-Concentrate (NFC) Orange Juice Based on Characteristic Volatile Markers Using Headspace Solid Phase Microextraction (HS-SPME) Combined with GC-MS. *Food Control* **2022**, *136*, 108856. [CrossRef]
38. Wang, Y.; Wang, S.; Fabroni, S.; Feng, S.; Rapisarda, P.; Rouseff, R. Chapter 22—Chemistry of Citrus Flavor. In *The Genus Citrus*; Talon, M., Caruso, M., Gmitter, F.G., Eds.; Woodhead Publishing: Sawston, UK, 2020; pp. 447–470. [CrossRef]
39. Nguyen, H.; Campi, E.M.; Roy Jackson, W.; Patti, A.F. Effect of Oxidative Deterioration on Flavour and Aroma Components of Lemon Oil. *Food Chem.* **2009**, *112*, 388–393. [CrossRef]
40. Xu, L.; Xu, Z.; Kelly, S.; Liao, X. Integrating Untargeted Metabolomics and Targeted Analysis for Not from Concentrate and from Concentrate Orange Juices Discrimination and Authentication. *Food Chem.* **2020**, *329*, 127130. [CrossRef]
41. Culleré, L.; Ferreira, V.; Chevret, B.; Venturini, M.E.; Sánchez-Gimeno, A.C.; Blanco, D. Characterisation of Aroma Active Compounds in Black Truffles (*Tuber melanosporum*) and Summer Truffles (*Tuber aestivum*) by Gas Chromatography–Olfactometry. *Food Chem.* **2010**, *122*, 300–306. [CrossRef]
42. Ai, J.; Wu, Q.; Battino, M.; Bai, W.; Tian, L. Using Untargeted Metabolomics to Profile the Changes in Roselle (*Hibiscus sabdariffa* L.) Anthocyanins during Wine Fermentation. *Food Chem.* **2021**, *364*, 130425. [CrossRef] [PubMed]
43. Song, X.; Canellas, E.; Nerin, C. Screening of Volatile Decay Markers of Minced Pork by Headspace-Solid Phase Microextraction–Gas Chromatography–Mass Spectrometry and Chemometrics. *Food Chem.* **2021**, *342*, 128341. [CrossRef] [PubMed]