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Qualitative and Quantitative Analyses of the Chemical Components of Peels from Different Pomelo Cultivars (*Citrus grandis* [L.] Osbeck) Based on Gas Chromatography−Mass Spectrometry, Ultraperformance Liquid Chromatography-Q-Exactive Orbitrap-MS, and High-Performance Liquid Chromatography-Photodiode Array Detection

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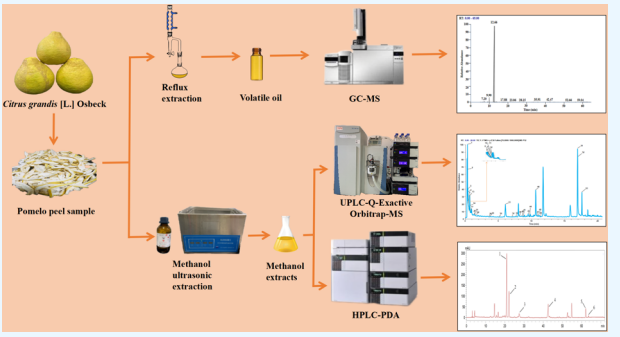
ABSTRACT: The volatile and nonvolatile phytochemicals in peels of 5 major pomelo cultivars (including *Citrus grandis* cv. Yuhuanyou, *C. grandis* cv. Liangpingyou, *C. grandis* cv. Guanximiyou, *C. grandis* cv. Duweiwendanyou, and *C. grandis* cv. Shatianyou) from 11 places in China were characterized. First, 194 volatile compounds in pomelo peels were identified by gas chromatography−mass spectrometry (GC−MS). Of these, 20 major volatile compounds were subjected to cluster analysis. The heatmap indicated that the volatile compounds in peels of *C. grandis* cv. Shatianyou and *C. grandis* cv. Liangpingyou were different from those in other varieties, while there was no difference among *C. grandis* cv. Guanximiyou, *C. grandis* cv. Yuhuanyou, and *C. grandis* cv. Duweiwendanyou from different origins. Second, 53 nonvolatile compounds were identified in pomelo peels by ultraperformance liquid chromatography-Q-exactive orbitrap tandem MS (UPLC-Q-exactive orbitrap-MS), of which 11 components were detected for the first time. Third, six major nonvolatile compounds were quantitatively analyzed with high-performance LC-photodiode array detection (HPLC-PDA). Combining the results of HPLC-PDA and the heatmap, 6 nonvolatile compounds in 12 batches of pomelo peel were well separated among varieties. Comprehensive analysis and identification of chemical components in pomelo peels are of great significance for their further development and utilization.



1. INTRODUCTION

Citrus grandis (L.) Osbeck, also known as pomelo, is distributed worldwide, including Asia and parts of Africa and Australia. China has abundant sources of pomelo,[1] and there are some famous varieties, including *Citrus grandis* cv. Yuhuanyou, *Citrus grandis* cv. Liangpingyou, *Citrus grandis* cv. Guanximiyou, *Citrus grandis* cv. Duweiwendanyou, and *Citrus grandis* cv. Shatianyou (Figure 1). Pomelo comprises two parts, i.e., peel and pulp, which can be easily separated from each other. Pomelo pulps are highly consumed as fresh products, while pomelo peels are often discarded as waste. However, studies have reported the anticancer,[2] anti-inflammatory,[3] anti-oxidant,[4] and hypoglycemic activities of pomelo peels,[5] which were related to essential oils, coumarins (such as auraptene,[2] bergamottin,[6] and D-limonene[7]), and flavonoids (such as naringin[8] and rhoifolin[3]). Therefore, pomelo peel can be used as a source of functional and nutritional compounds, which is not only economic but also has health-promoting effects.[9]

The qualitative and quantitative analyses of the compounds of citrus fruits, such as *Citrus limon* L.[10] and *Citrus reticulata* L.,[11] have received increasing attention. Until now, studies have reported variations in the types and contents of bioactive compounds among different citrus varieties.[12] Di Rauso Simeone et al. showed that the contents of major compounds in four *C. limon* cultivars varied, depending on varieties and

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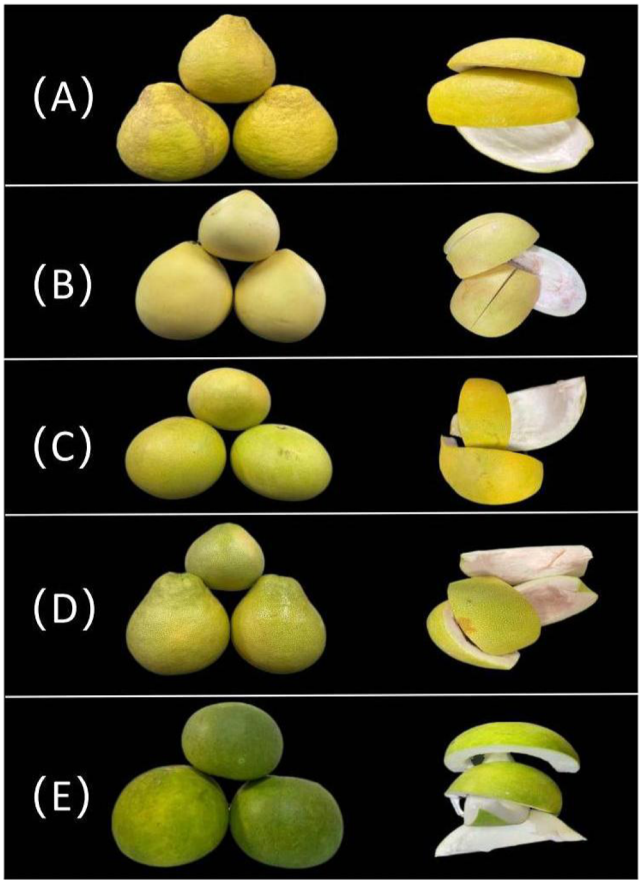

Figure 1. Appearances of pomelo fruits and pomelo peels. (A) *Citrus grandis* cv. Shatianyou, (B) *Citrus grandis* cv. Guanximiyou, (C) *Citrus grandis* cv. Yuhuanyou, (D) *Citrus grandis* cv. Duweiwendanyou, and (E) *Citrus grandis* cv. Liangpingyou.

sampling time.[13] Moreover, there were also studies on the phytochemicals in the peels of oranges and mandarins.[14] As a common citrus variety, pomelo has been applied for its beneficiary use in the modern medical era. Thus, it is significant to identify the compounds in pomelo peels with potential therapeutic activity. This information will advance the utilization of pomelo peels in functional and pharmaceutical industries.

Gas chromatography−mass spectrometry (GC−MS) is considered a common and reliable analytical platform for volatile compounds due to its superior selectivity, separation capability, and reproducibility.[15] Therefore, the chemical profile of the volatile constituents of pomelo peel samples from five cultivars was determined with GC−MS. Subsequently, the nonvolatile components of pomelo peel samples were identified with the sensitive and reliable ultraperformance liquid chromatography-Q-exactive orbitrap tandem MS (UPLC-Q-exactive orbitrap-MS) method. Furthermore, some important nonvolatile compounds in the peels of five pomelo cultivars were quantified with a novel, rapid, and sensitive high-performance LC-photodiode array detection (HPLC-PDA) method, including naringin, rhoifolin, meranzin hydrate, isomeranzin, auraptene, and bergamottin. The variations of volatile and nonvolatile compounds in the peels of different pomelo cultivars have been analyzed. This study may provide a reference for further research on pomelo peel from different varieties.

2. RESULTS AND DISCUSSION

2.1. GC−MS of Volatile Compounds in the Peels of Five Pomelo Cultivars. The qualitative and quantitative analyses of the volatile components in pomelo peels were performed with GC−MS. The representative total ion chromatogram of the volatile compounds in pomelo peel was displayed (Figure 2). As exhibited in Table 1, the extraction rate of volatile oil was calculated with the weight of the extracted volatile oil and the weight of samples. As shown in Table 1, the extraction rate of various volatile compounds was different in the peels of five pomelo varieties. The extraction rate of volatile oils was highest (3.27%) in *C. grandis* cv. Liangpingyou and lowest (0.27−0.35%) in *C. grandis* cv. Guanximiyou.

In this study, a total of 194 volatile compounds were observed in the peels of 5 pomelo cultivars, including 1 alkane, 100 alkenes, 10 ketones, 5 phenols, 52 alcohols, 7 aldehydes, 6 carboxylic acids, and 13 other compounds. Among them, 20 volatile compounds with high levels were considered the major species and analyzed using GC−MS (Table 2). Results indicated that the volatile oils in pomelo peels contained abundant D-limonene (relative content: 58.79−96.54%), which exhibited anti-inflammatory and anti-oxidative activities.[16]

The 20 major volatile compounds were subjected to multivariate statistics to determine and classify the peels from different pomelo cultivars. A heatmap was used to visualize the levels of the 20 major volatile components in the peels of 12 batches of pomelo (Figure 3). *C. grandis* cv. Shatianyou (S1, S2, and S3) and *C. grandis* cv. Liangpingyou (L1) were naturally classified as one group, whereas *C. grandis* cv. Guanximiyou (MH2 and MB1), *C. grandis* cv. Yuhuanyou (Y1 and Y2), and *C. grandis* cv. Duweiwendanyou (D1 and D2) were classified as one group. Moreover, *C. grandis* cv. Guanximiyou (MH1 and MB2) was classified as another group.

Combining the results of the aforementioned analyses, it was seen that the profiles of 20 major volatile compounds of *C. grandis* cv. Shatianyou (S1, S2, and S3) and *C. grandis* cv. Liangpingyou (L1) were different from those of other varieties, which may be related to their high content of D-limonene (95.25−96.54%). Similarities were observed in the volatile compounds of *C. grandis* cv. Guanximiyou (MH2 and MB1), *C. grandis* cv. Yuhuanyou (Y1 and Y2), and *C. grandis* cv. Duweiwendanyou (D1 and D2). Moreover, *C. grandis* cv. Guanximiyou samples from different origins (MH1, MH2, MB1, and MB2) were not completely clustered together based on the heatmap result (Figure 3). MB2 exhibited clear separation from MH1, MH2, and MB1. This finding might be attributed to the high contents of linalyl anthranilate (1.68%) and 4-terpinenol (0.23%) in MB2, whereas MH1, MH2, and MB1 presented relatively low contents of linalyl anthranilate (0.00−0.52%) and 4-terpinenol (0.06−0.07%). Additionally, the content of *n*-hexadecanoic acid in MH1 was 0.62%, which was different from that in MH2 and MB1 (0.00−0.22%). The content variations of volatile components among different samples might result from several factors, including the genetic source, geographical conditions, growth environment, and instability of extracted essential oils.

In summary, the types of main volatile components were similar in pomelo peels, but their contents were different in peels from different pomelo cultivars. *C. grandis* cv. Shatianyou and *C. grandis* cv. Liangpingyou were different from other cultivars. Furthermore, no difference was found in the samples of *C.*

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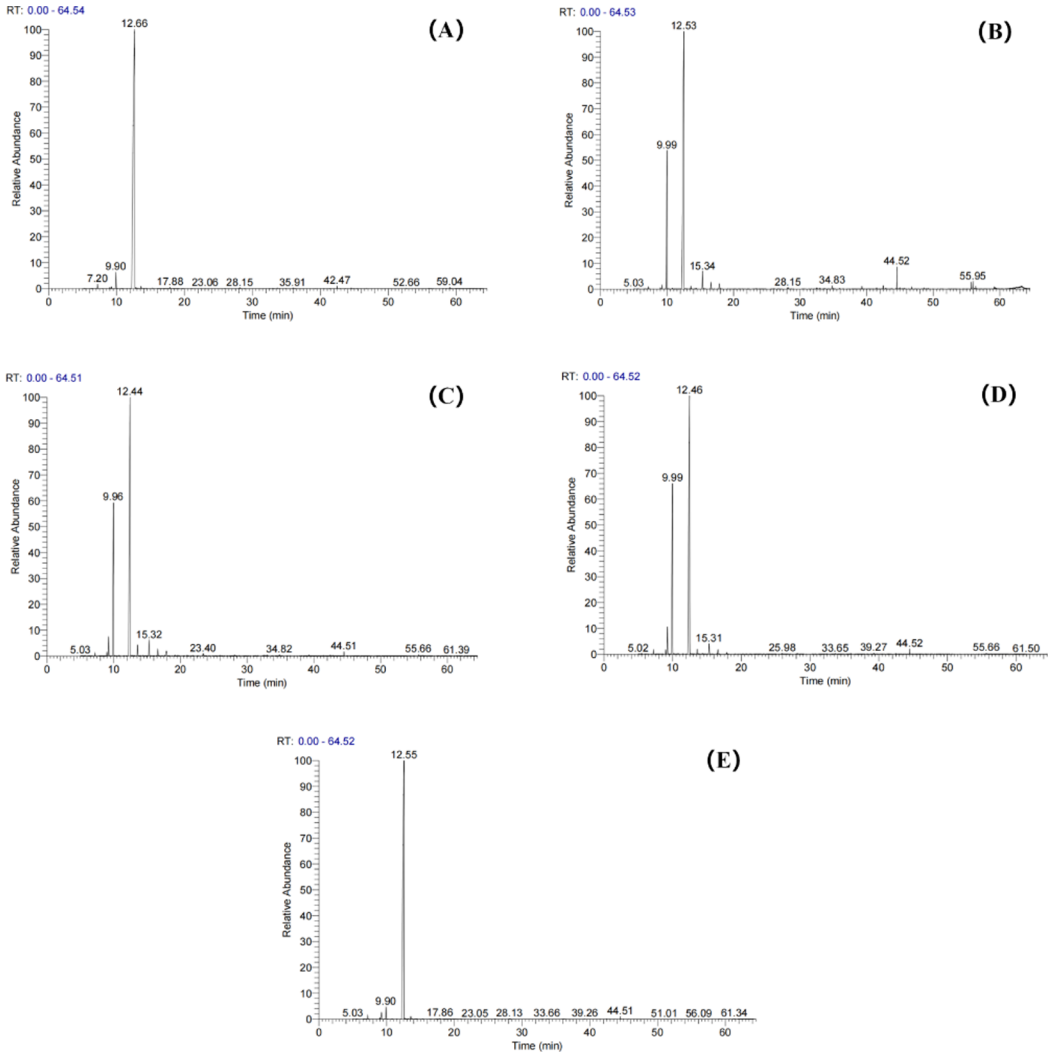

Figure 2. Representative total ion chromatograms of volatile compounds of pomelo peels for (A) *Citrus grandis* cv. Shatianyou, (B) *Citrus grandis* cv. Guanximiyou, (C) *Citrus grandis* cv. Yuhuanyou, (D) *Citrus grandis* cv. Duweiwendanyou, and (E) *Citrus grandis* cv. Liangpingyou.

Table 1. Information of Pomelo Peel Samples from Five Cultivars

no.	cultivars	sample source	collecting time	extraction rate (%) rate (%)
S1	*C. grandis* cv. Shatianyou	Bingcun, Meixian District, Meizhou City, Guangdong Province	9/9/2021	1.92
S2	*C. grandis* cv. Shatianyou	Rongxian District, Yulin City, Guangxi Province	7/9/2021	2.35
S3	*C. grandis* cv. Shatianyou	Songkou, Meixian District, Meizhou City, Guangdong Province	6/9/2021	1.34
MH1	*C. grandis* cv. Guanximiyou	Meizhou City, Guangdong Province	6/9/2021	0.27
MH2	*C. grandis* cv. Guanximiyou	Da Xi, Pinghe District, Zhangzhou City, Fujian Province	7/9/2021	0.34
MB1	*C. grandis* cv. Guanximiyou	Bingcun, Meixian District, Meizhou City, Guangdong Province	6/9/2021	0.35
MB2	*C. grandis* cv. Guanximiyou	Daxi, Pinghe District, Zhangzhou City, Fujian Province	9/9/2021	0.28
Y1	*C. grandis* cv. Yuhuanyou	Qinggang District, Yuhuan City, Zhejiang Province	8/9/2021	0.40
Y2	*C. grandis* cv. Yuhuanyou	Taishan Village, Qinggang District, Yuhuan City, Zhejiang Province	9/9/2021	0.42
D1	*C. grandis* cv. Duweiwendanyou	Licheng District, Putian City, Fujian Province	13/9/2021	1.69
D2	*C. grandis* cv. Duweiwendanyou	Xianyou District, Putian City, Fujian Province	10/9/2021	1.72
L1	*C. grandis* cv. Liangpingyou	Liangping District, Chongqing City	9/9/2021	3.27

grandis cv. Guanximiyou, *C. grandis* cv. Yuhuanyou, and *C. grandis* cv. Duweiwendanyou from different origins.

2.2. Analysis of the Nonvolatile Compounds in the Peels of Five Pomelo Cultivars. The representative total ion chromatogram of nonvolatile compounds in the pomelo peel in the positive mode is shown (Figure 4). According to the retention time and fragment ion information of constituents provided by UPLC-Q-exactive orbitrap-MS analysis, 53 components were determined from the methanol extracts from peels of 5 pomelo cultivars, including 21 flavonoids, 21 coumarins, 2 organic acids, and 9 other compounds. All the compounds were identified in comparison with the Orbitrap Traditional Chinese Medicine Library (OTCML), standard reference, and literature information. The molecular ions, related product ions, and retention time of 53 compounds observed in UPLC-Q-exactive orbitrap-MS are shown in Table

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ACS Omega 2023, 8, 6253−6267

Table 2. Summary of 20 Major Volatile Compounds in 5 Cultivars Identified by GC−MS

no.	Compound	RT (min)	S1	S2	S3	MH1	MH2	MB1	MB2	Y1	Y2	D1	D2	L1
						Alkene								
3	(1R)-α-pinene	7.22	0.39	0.44	0.34	0.21	0.26	0.25	0.33	0.31	0.27	0.45	0.41	0.45
7	β-phellandrene	8.99			0.09				0.24	0.46			0.53	
8	Sabenene	9.01	0.05	0.09		0.12	0.16	0.17			0.22	0.52		0.19
9	(−)-β-pinene	9.24	1.6	0.12	2.02	20.7	22.46	23.95	25.12	27.39	22.41	32.73	30.97	1.1
11	Myrcene	9.9		1.79			0.01	0.01	0.11			0.01	0.01	1.63
22	D-limonene	12.5	96.46	96.39	96.54	66.38	71.35	69.43	58.79	61.21	69.48	61.14	62.36	95.25
27	(Z)-ocimene	13.67	0.02	0.18	0.24	0.32	0.67	0.63	2.11	1.63	0.61	0.62	0.81	0.39
94	2-butyloctanol	39.27	0.02	0.04	0.02	0.06	0.19	0.14	0.14	0.11	0.19	0.1	0.13	0.01
108	caryophyllene	42.48	0.09	0.12	0.06	0.26	0.12	0.19	0.19	0.08	0.14	0.07	0.11	
115	1*H*-cubebene	44.52	0.02	0.04	0.02	1.46	0.89	1.05	0.87	0.42	1.34	0.41	0.47	0.22
153	cembrene	54.53				0.62	0.12	0.21	0.52	0.21	0.23	0.12	0.48	
						Alcohol								
40	linalool	17.98	0.02	0.13	0.11	0.74	0.39				0.56	0.24	0.21	0.1
62	1,5,7-octatrien-3-ol,2,6-dimethyl-	23.44				0.07	0.08	0.29	0.72	0.58	0.13		0.01	
65	4-terpinenol	26.02	0.03	0.03	0.02	0.08	0.06	0.07	0.23	0.13	0.07		0.1	0.04
69	(±)-α-terpineol	28.18	0.06	0.11	0.08	0.3	0.16	0.13	0.37	0.25	0.01	0.15	0.12	0.09
80	(Z)-nerol	32.53			0.02	0.59	0.09	0.07	0.65	0.37	0.47	0.09	0.04	
						Ester								
38	linalyl anthranilate	17.88	0.07	0.02	0.02			0.52	1.68				0.02	0.02
						n-Hexadecanoic Acid								
157	*n*-hexadecanoic acid	55.95				0.62		0.1	0.22	0.05	0.1			
						Other Compounds								
30	(Z)-linalool oxide	15.33	0.04	0.03	0.04	0.09	1	0.05	0.02	0.1	1.52	0.02	0.05	0.09
33	(E)-linalool oxide	16.61		0.01					1.13			0.72	0.66	

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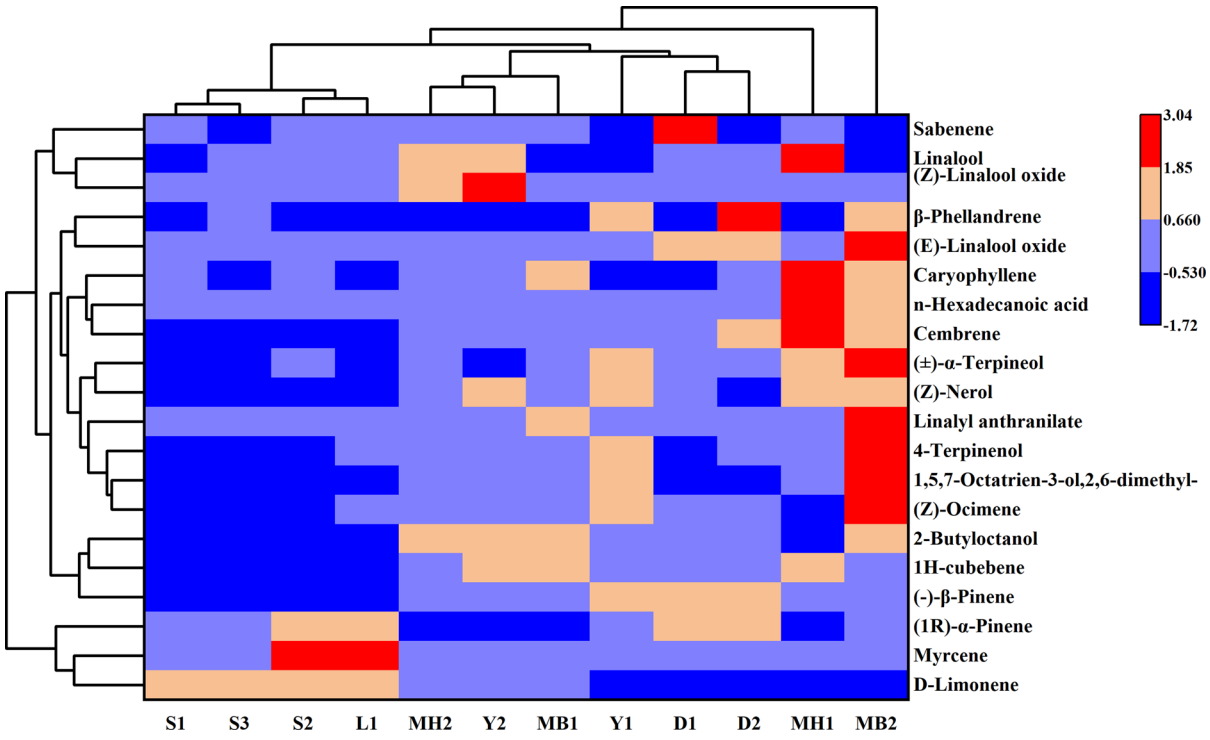

Figure 3. Heatmap and dendrogram of 20 main volatile compounds in pomelo peels from 5 cultivars.

3, and their chemical structures are illustrated in Figure 5. A total of 11 identified compounds were first reported in pomelo peels, including 7 flavonoids, 2 coumarins, and 2 other compounds. These compounds were determined as vitexin, apigenin 7-glucoside, isorhamnetin, pinocembrin, tectorigenin, iristetrigenin B, 7-methoxy-4-methylcoumarin, columbianetin acetate, *N*,*N*-dimethyl-L-proline, and curdione.

In addition, as shown in Table 4, some compounds were detected in all 12 batches of samples, such as rhoifolin, naringenin, naringin, nobiletin, tangeretin, chrysosplenetin B, isomeranzin, meranzin hydrate, bergapten, osthole, 7-hydroxycoumarin, auraptene, and *N*,*N*-dimethyl-L-proline. These compounds may be closely associated with the biological activity of pomelo peels, such as anti-inflammatory and antioxidant properties.[17,18]

Furthermore, the content difference of different components was observed among five different varieties. For instance, *C. grandis* cv. Shatianyou contained the greatest number of coumarins (18), whereas *C. grandis* cv. Shatianyou contained the least number of flavonoids (12). Some compounds, including vicenin and isorhamnetin, were not found in *C. grandis* cv. Shatianyou. Except for *C. grandis* cv. Shatianyou, all varieties contained no artemetin, which might be one of the distinctive compounds to differentiate *C. grandis* cv. Shatianyou from other cultivars. Moreover, 14 flavonoids were observed in *C. grandis* cv. Yuhuanyou and *C. grandis* cv. Duweiwendanyou, which were the most abundant. Additionally, *C. grandis* cv. Shatianyou and *C. grandis* cv. Liangpingyou had the most and least types of nonvolatile compounds, respectively.

2.3. Simultaneous Quantification of Six Bioactive Compounds by HPLC-PDA. In this study, six major nonvolatile compounds in pomelo peels were determined with HPLC-PDA using external standard methods based on corresponding calibration curves. As exhibited in Table 5, the calibration equations and correlation coefficients (R^2) of naringin, rhoifolin, meranzin hydrate, isomeranzin, auraptene, and bergamottin were provided. There was good linearity across the tested concentration ranges, and all R^2 values were above 0.999. The limits of detection (LOD) and limits of quantification (LOQ) were designated signal-to-noise (S/N) ratios of 3 and 10, respectively. Moreover, the method showed good reliability and feasibility. The mean extraction recovery was within the range of 90.13−105.87%. In addition, the relative standard deviations of repeatability (0.72−2.84%), precision (0.12−0.60%), stability (0.37−2.13%), and recovery (1.07−2.81%) were below 3.00% for all standards.

The developed analytical method was subsequently applied to analyze the peels of five pomelo cultivars. The quantitative analysis results of six major nonvolatile compounds are displayed in Table 6. As displayed in Table 6, the content of naringin was the highest in all 12 batches of samples, varying from 84.44 to 288.40 mg·g^{-1}. It was followed by meranzin hydrate (0.01−37.97 mg·g^{-1}), auraptene (0.52−23.05 mg·g^{-1}), rhoifolin (1.42−19.67 mg·g^{-1}), isomeranzin (0.49−10.89 mg·g^{-1}), and bergamottin (0.35−3.88 mg·g^{-1}). Studies revealed that these *Citrus* flavonoids and coumarins exhibited diverse biological activities.[19] Other researchers have reported that naringin exhibited bone regeneration,[20] anti-inflammatory,[21] and anticancer effects.[22] Moreover, some pharmacological research demonstrated that meranzin hydrate possessed anti-anxiety and anti-depressant effects.[23]

The contents of the above-mentioned 6 nonvolatile compounds were different in 12 batches of pomelo peels, suggesting differences among cultivars and origins. The quantification results were compared and displayed with a heatmap (Figure 6). Samples were divided into two main clusters (clusters A and B). Cluster A included *C. grandis* cv. Shatianyou (S1, S2, and S3) and *C. grandis* cv. Liangpingyou (L1), and this classification might be related to the low content of rhoifolin and the high content of meranzin hydrate. Cluster B

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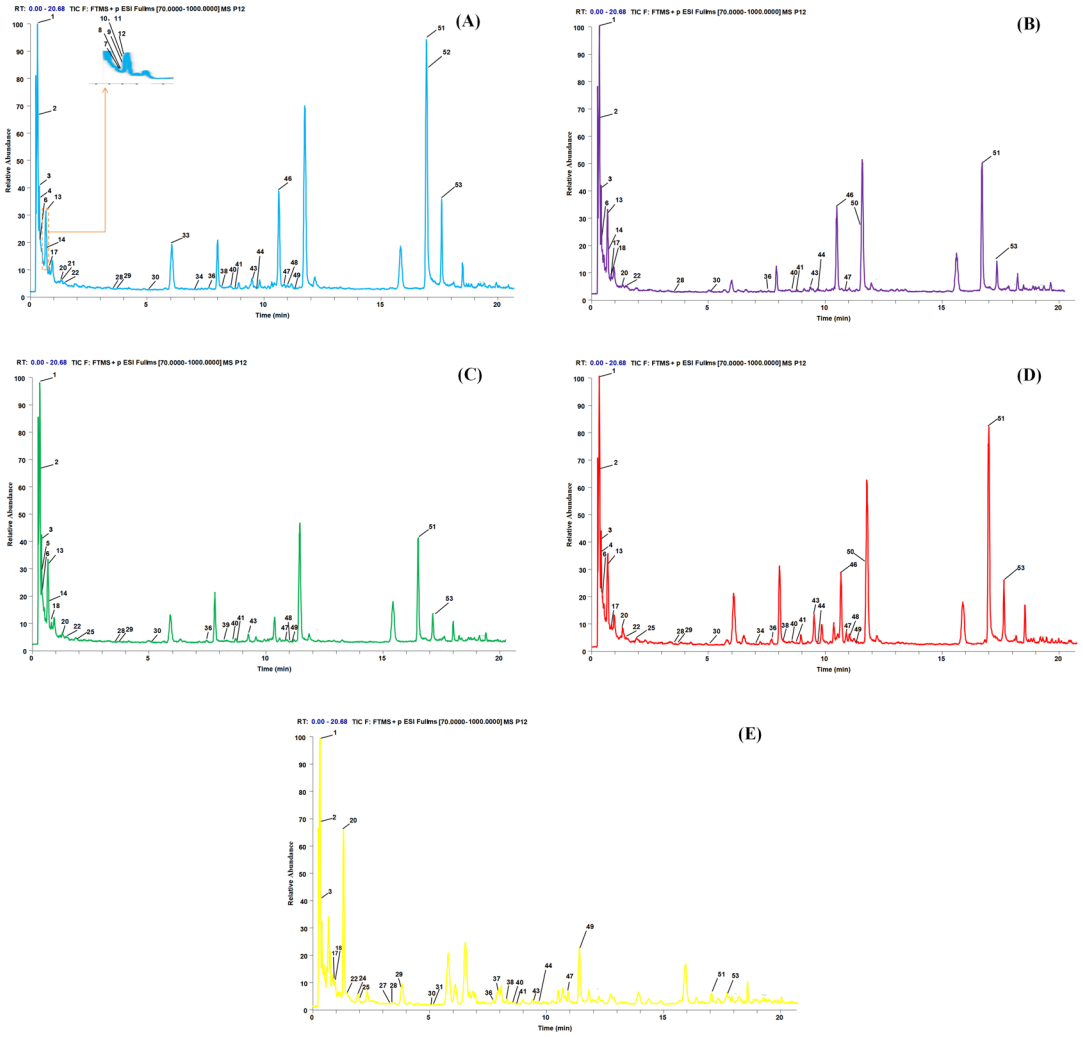

Figure 4. Representative total ion chromatograms of nonvolatile compounds of pomelo peels for (A) *Citrus grandis* cv. Shatianyou, (B) *Citrus grandis* cv. Guanximiyou, (C) *Citrus grandis* cv. Yuhuanyou, (D) *Citrus grandis* cv. Duweiwendanyou, and (E) *Citrus grandis* cv. Liangpingyou.

comprised *C. grandis* cv. Guanximiyou (MH1, MH2, MB1, and MB2), *C. grandis* cv. Yuhuanyou (Y1 and Y2), and *C. grandis* cv. Duweiwendanyou (D1 and D2). This classification might be because these cultivars contained a high content of naringin and a low content of meranzin hydrate. In short, *C. grandis* cv. Shatianyou and *C. grandis* cv. Liangpingyou contained similar levels of six major nonvolatile compounds, whereas *C. grandis* cv. Guanximiyou, *C. grandis* cv. Yuhuanyou, and *C. grandis* cv. Duweiwendanyou had relatively consistent levels of the six chemicals.

3. CONCLUSIONS

The volatile and nonvolatile compounds were determined and analyzed in 12 batches of pomelo peel samples from 5 different cultivars obtained from 11 major origins around China. A total of 194 volatile compounds were observed with GC−MS. The main volatile compounds in all pomelo peel samples were similar, but their contents were different. The contents of volatile compounds in *C. grandis* cv. Shatianyou and *C. grandis* cv. Liangpingyou were different from those in other cultivars. No difference was found in samples of *C. grandis* cv. Guanximiyou, *C. grandis* cv. Yuhuanyou, and *C. grandis* cv. Duweiwendanyou from different origins. For nonvolatile compounds, 53 chemicals were identified with UPLC-Q-exactive orbitrap-MS. Additionally, the quantification of six major nonvolatile compounds based on HPLC-PDA and the heatmap revealed good separation between different cultivars and batches of pomelo. Notably, *C. grandis* cv. Shatianyou and *C. grandis* cv. Liangpingyou exhibited similarity in both volatile and nonvolatile compounds based on the results of GC−MS and HPLC-PDA. This study may provide some information for the development and application of peels from different pomelo cultivars in functional and medicinal industries, thus facilitating their recycling.

4. MATERIALS AND METHODS

4.1. Plant Materials. A total of 12 batches of pomelo peel samples from 5 different cultivars were obtained from 11 major origins around China in September 2021 (Table 1). All these samples were selected as experimental materials for subsequent analysis. Samples were identified and authenticated by Prof. Guodong Zheng of the Laboratory of Pharmacognosy, Guangzhou Medical University in Guangdong Province, China.

4.2. Chemical Materials. Eighteen (18) commercial standards (≥98% purity) were obtained from Weikeqi (Sichuan, China). These standards were naringin, naringenin, rhoifolin, hesperidin, nobiletin, tangeretin, fraxetin, meranzin hydrate, isomeranzin, bergapten, isoscopoletin, auraptene, bergamottin, scopoletin, 7-hydroxycoumarin, ferulic acid, *N*,*N*-dimethyl-L-

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Table 3. Non-Volatile Compounds Identified in Five Cultivars by UPLC-Q-Exactive Orbitrap-MS

peak no.	RT (min)	experimental $[M + H]^+$ (m/z)	major secondary fragment ions (m/z)	molecular formula	identification	reference
			Flavonoids			
3	0.39	595.1663	577.1547, 541.1345, 511.1235, 481.1129, 457.1133, 427.1026, 409.0917, 391.0814, 379.0813, 349.0707, 337.0708, 325.0707, 295.0603, 283.0601, 271.0603, 257.0441, 229.0498, 219.0291, 203.0343, 195.0288, 145.0285, 121.0287, 103.0390, 85.0291, 57.0339	$C_{27}H_{30}O_{15}$	vicenin	24
6	0.5	433.1132	415.1024, 397.0919, 379.0812, 367.0813, 349.0705, 337.0707, 323.0913, 313.0707, 297.0757, 283.0602, 271.0602, 271.0602, 256.0728, 243.0284, 213.0548, 183.0291, 165.0184, 145.0285, 121.0286, 109.0284, 79.0185	$C_{21}H_{20}O_{10}$	vitexin	25
9	0.64	579.1710	433.1128, 344.1442, 313.0717, 283.0602, 271.0601, 243.0653, 203.0698, 171.0288, 153.0183, 119.0494, 85.0290	$C_{27}H_{30}O_{14}$	rhoifolin	[a]
10	0.65	271.0599	253.0490, 243.0650, 225.0547, 203.0699, 187.0394, 171.0288, 163.0387, 153.0184, 145.0286, 129.0181, 119.0494, 109.0284, 91.0548, 67.0186	$C_{15}H_{10}O_5$	apigenin	26
11	0.65	433.1132	332.4244, 313.0716, 283.0602, 271.0603, 261.1127, 243.0661, 225.0536, 189.0553, 171.0291, 153.0184, 119.0494, 91.0547, 67.0187	$C_{21}H_{20}O_{10}$	apigenin 7-glucoside	27
12	0.66	273.0756	255.0648, 231.0651, 207.0654, 189.0545, 179.0337, 171.0287, 157.0650, 153.0182, 147.0440, 129.0182, 123.0441, 119.0493, 107.0494, 91.0547, 83.0134, 67.0186, 55.0186	$C_{15}H_{12}O_5$	naringenin	[a]
13	0.68	581.1873	527.1444, 443.1302, 417.1174, 401.1236, 383.1126, 365.1016, 339.0863, 315.0862, 285.0755, 273.0757, 263.0549, 219.0289, 195.0289, 171.0289, 153.0183, 129.0547, 119.0493, 91.0547, 85.0290, 71.0499	$C_{27}H_{32}O_{14}$	naringin	[a]
14	0.71	609.1819	463.1243, 361.0900, 301.0708, 286.0473, 258.0524, 229.0494, 201.0560, 153.0184, 129.0546, 85.0290	$C_{28}H_{32}O_{15}$	diosmin	28
15	0.72	303.0865	285.0755, 261.0773, 244.1052, 219.0647, 201.0548, 189.0548, 177.0549, 171.0290, 163.0392, 153.0185, 149.0600, 137.0598, 123.0444, 117.0339, 111.0080, 89.0392, 83.0134, 67.0187, 55.0186	$C_{16}H_{14}O_6$	hesperitin	29
16	0.72	611.1978	449.1441, 413.1235, 395.1128, 369.0969, 345.0969, 315.0864, 303.0865, 281.0660, 263.0551, 219.0292, 195.0291, 177.0548, 153.0184, 129.0548, 111.0444, 85.0291, 71.0499	$C_{28}H_{34}O_{15}$	hesperidin	[a]
22	1.44	317.0660	302.0424, 285.0400, 273.0391, 257.0449, 229.0499, 217.0500, 203.0341, 189.0548, 165.0184, 153.0185, 139.0390, 121.0285, 105.0340, 92.0263, 68.9980	$C_{14}H_{12}O_7$	isorhamnetin	30
25	1.94	257.0812	239.0706, 215.0707, 191.0703, 179.0348, 173.0291, 171.0291, 153.0186, 145.0656, 131.0495, 123.0444, 107.0496, 103.0548, 97.0290, 91.0549, 79.0550, 67.0187, 57.1876	$C_{15}H_{12}O_4$	pinocembrin	31
27	3.48	301.0709	286.0473, 258.0523, 240.0417, 229.0500, 213.0548, 195.0444, 184.0514, 168.0051, 147.0440, 121.0285, 91.0552, 68.9974	$C_{16}H_{12}O_6$	tectorigenin	32
31	5.16	331.0815	316.0580, 301.0346, 288.0636, 273.0396, 245.0444, 231.0498, 203.0342, 189.0550, 173.0600, 159.0442, 147.0443, 139.0028, 121.0287, 107.0492, 91.0548, 68.9977	$C_{17}H_{14}O_7$	iristetrigenin B	33
35	7.63	343.1180	328.0945, 313.0709, 299.0899, 285.0759, 270.0529, 257.0811, 243.0659, 211.0764, 199.0240, 181.0132, 171.0288, 153.0184, 144.1716, 133.0651, 125.0236, 108.8341, 90.3219, 69.0341	$C_{19}H_{18}O_6$	5,7,8,4′-tetramethoxyflavone	[b]
38	8.21	361.0921	346.0677, 331.0439, 317.0650, 303.0493, 287.0538, 275.0546, 257.0442, 229.0489, 197.0443, 181.0130, 164.0466, 137.0596, 121.0283, 93.0337, 65.0392	$C_{18}H_{16}O_8$	5,7,3′-trihydroxy-6,4′,5′-trimethoxyflavone	[b]
39	8.55	345.0972	330.0736, 315.0502, 301.0704, 287.0553, 272.0319, 259.0601, 231.0652, 213.0549, 189.0548, 181.0136, 153.0184, 131.0494, 121.0287, 107.0496, 85.0291, 65.0393	$C_{18}H_{16}O_7$	lysionotin	34
40	8.6	403.1391	388.1156, 373.0921, 358.0683, 345.0971, 327.0863, 313.0710, 301.0709, 287.0539, 274.0840, 258.0524, 229.0342, 211.0239, 193.0136, 183.0291, 165.0549, 148.0517, 127.0393, 99.0443, 69.0344	$C_{21}H_{22}O_8$	nobiletin	[a]
41	8.77	375.1077	360.0841, 345.0607, 330.0370, 302.0418, 274.0474, 257.0449, 229.0502, 201.0545, 181.0136, 151.0392, 137.0234, 109.0288, 74.4930	$C_{19}H_{18}O_8$	chrysosplenetin B	35
43	9.54	373.1284	358.1048, 343.0814, 328.0579, 315.0857, 297.0759, 283.0603, 271.0602, 244.0733, 229.0321, 211.0240, 193.0139, 183.0290, 168.0052, 145.0647, 135.0442, 127.0393, 99.0441, 69.0341	$C_{20}H_{20}O_7$	tangeretin	[a]
45	10.55	389.1236	373.0987, 374.0998, 343.0455, 331.0815, 301.0354, 275.0919, 257.0815, 242.0583, 217.0495, 181.0133, 165.0549, 153.0185, 137.0236, 109.0287, 79.0546	$C_{20}H_{20}O_8$	artemetin	36
			Coumarins			
5	0.48	191.0704	173.1327, 163.0755, 158.1095, 149.0599, 145.1010, 133.1013, 123.0813, 119.0495, 115.0547, 105.0703, 93.0704, 91.0548, 86.0242, 79.0549, 69.0342, 55.0551	$C_{11}H_{10}O_3$	7-methoxy-4-methylcoumarin	[b]
8	0.61	247.0965	229.0860, 211.0756, 201.0547, 187.0753, 175.0391, 163.0390, 159.0441, 147.0441, 142.0776, 131.0493, 119.0494, 103.0546, 91.0547, 85.0651, 65.0393	$CH_{14}H_{14}O_4$	marmesin	37

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ACS Omega 2023, 8, 6253−6267

Table 3. continued

peak no.	RT (min)	experimental $[M + H]^+$ (m/z)	major secondary fragment ions (m/z)	molecular formula	identification	reference
			Coumarins			
17	0.78	193.0498	178.0262, 161.0598, 150.0314, 154.0782, 143.0598, 137.0598, 133.0286, 122.0365, 115.0546, 105.0703, 95.0496, 89.0391, 79.0548, 66.0472, 55.0187	$C_{10}H_8O_4$	isoscopoletin	[a]
19	0.91	409.1492	391.1861, 261.1112, 247.0965, 229.0859, 211.0751, 201.0547, 187.0390, 159.0441, 131.0492, 97.0289, 85.0290, 69.0343	$C_{20}H_{24}O_9$	nodakenin	38
20	1.31	261.1123	261.1123, 243.1017, 231.1023, 217.0860, 201.0548, 189.0548, 177.0547, 159.0442, 145.0648, 131.0493, 128.0625, 115.0544, 103.0547, 95.0491, 85.0655, 67.0550	$C_{15}H_{16}O_4$	isomeranzin	[a]
21	1.32	279.1228	261.1121, 243.1064, 231.1020, 217.0861, 201.0548, 189.0547, 177.0547, 159.0441, 145.0647, 131.0492, 117.0702, 103.0546, 95.0497, 77.0388, 67.0550	$C_{15}H_{18}O_5$	meranzin hydrate	[a]
24	1.92	305.1024	203.0342, 189.0550, 175.0393, 159.0443, 147.0443, 131.0494, 119.0493, 91.0549, 67.0549, 59.0500	$C_{16}H_{16}O_6$	oxypeucedanin hydrate	39
26	2.18	289.1073	261.1127, 248.1003, 243.1019, 228.0784, 213.0549, 189.0549, 185.0603, 173.0600, 159.0443, 155.0856, 145.0650, 131.0494, 127.0397, 117.0702, 103.0547, 91.0547, 81.0705, 69.0707	$C_{16}H_{16}O_5$	columbianetin acetate	40
28	3.57	179.0342	169.9784, 161.0600, 151.0388, 138.0549, 135.0443, 128.9511, 123.0441, 119.0494, 111.0443, 107.0495, 95.0495, 91.0548, 83.0496, 68.9979, 55.9353	$C_9H_6O_4$	esculetin	41
29	3.71	209.0448	194.0214, 191.0711, 181.0499, 166.0265, 163.0390, 153.0550, 149.0236, 138.0313, 131.0493, 121.0288, 110.0367, 92.0259, 82.0419, 68.9980, 55.0188	$C_{10}H_8O_5$	fraxetin	[a]
30	5.09	217.0499	217.0499, 202.0264, 189.0543, 178.0263, 174.0314, 161.0600, 146.0364, 131.0494, 118.0419, 115.0546, 105.0702, 91.0547, 74.0971, 55.0185	$C_{12}H_8O_4$	bergapten	[a]
32	5.41	247.0603	235.0233, 232.0368, 229.0856, 217.0134, 203.0692, 189.0185, 175.0392, 164.5641, 161.0235, 147.0442, 133.0288, 119.0860, 104.1075, 95.0132, 60.0816	$C_{13}H_{10}O_5$	isopimpinellin	42
34	7.06	231.1014	223.9490, 189.0543, 175.0393, 147.0443, 131.0491, 119.0491, 112.0470, 103.0547, 91.0548, 65.0392	$C_{14}H_{14}O_3$	7-demethylsuberosin	[b]
37	7.91	287.0918	203.0343, 175.0392, 159.0444, 147.0443, 131.0494, 119.0497, 91.0548, 85.0655, 67.0549, 59.0501	$C_{16}H_{14}O_5$	oxypeucedanin	39
42	9.48	187.0393	163.9645, 159.0443, 143.0494, 135.9704, 131.0494, 118.9676, 115.0547, 103.0547, 95.0497, 81.0705, 55.9351	$C_{11}H_6O_3$	isopsoralen	43
44	9.72	193.0498	178.0258, 175.0384, 165.0545, 161.0229, 150.0310, 137.0595, 133.0283, 122.0362, 117.0335, 105.0335, 94.0416, 77.03912, 66.04706	$C_{10}H_8O_4$	scopoletin	[a]
47	10.85	245.1176	226.2699, 203.0706, 189.0549, 183.6220, 174.0308, 159.0443, 147.0438, 131.0494, 121.9895, 115.0547, 103.0547, 95.0498, 77.0392, 53.0394	$C_{15}H_{16}O_3$	osthole	44
49	11.31	271.0970	241.9205, 209.7384, 203.0342, 175.0392, 159.0443, 147.0443, 131.0494, 119.0495, 103.0548, 91.0548, 84.4042, 69.0707, 65.0393	$C_{16}H_{14}O_4$	isoimperatorin	39
51	16.93	163.0391	135.0442, 121.2155, 119.0495, 114.8570, 107.0496, 105.0451, 95.0498, 91.0548, 79.0548, 72.2852, 65.0394, 53.0394	$C_{14}H_{14}O_3$	7-hydroxycoumarin	[a]
52	16.95	299.1644	256.9647, 231.1012, 203.0700, 189.0551, 175.0392, 163.0392, 159.0449, 137.1326, 119.0495, 107.0496, 95.0861, 91.0548, 81.0706, 69.0707	$C_{19}H_{22}O_3$	auraptene	[a]
53	17.6	339.1594	203.0342, 175.0392, 159.0444, 147.0443, 131.0494, 119.0495, 91.0548, 81.0706, 69.0707	$C_{21}H_{22}O_4$	bergamottin	[a]
			Organic acids			
7	0.6	225.0758	207.0651, 201.7923, 192.0414, 189.0544, 181.0854, 175.0389, 164.0465, 151.1117, 147.0440, 132.0207, 123.0441, 119.0493, 113.9640, 105.0701, 95.0496, 91.0547, 81.0703, 69.0340, 65.0391, 61.0403	$C_{11}H_{12}O_5$	sinapic acid	45
18	0.78	195.0655	186.0556, 180.0312, 177.0545, 163.0386, 154.0585, 149.0598, 145.0283, 135.0441, 125.0598, 117.0337, 111.0443, 107.0494, 95.0499, 91.0545, 89.0390, 79.0548, 65.0392, 57.0706	$C_{10}H_{10}O_4$	ferulic acid	[a]
			Other Compounds			
1	0.31	144.1019	128.0712, 116.1075, 102.0552, 84.0813, 81.0343, 70.0658, 58.0659, 55.0550	$C_7H_{13}NO_2$	N,*N*-dimethyl-L-proline	[a]
2	0.33	127.0393	122.4651, 109.0286, 99.0807, 97.0289, 88.7007, 85.0652, 81.0340, 79.0548, 71.0497, 67.0549, 57.0342, 53.0393	$C_6H_6O_3$	5-hydroxymethylfurfural	46
4	0.42	237.1851	222.1535, 219.1746, 212.4210, 201.1639, 191.1796, 177.0538, 173.1326, 151.1119, 145.1013, 137.0964, 133.1014, 123.1170, 119.0859, 109.1016, 105.0704, 95.0861, 93.0705, 83.0496, 81.0706, 71.0499, 55.0551	$C_{15}H_{24}O_2$	curdione	47
23	1.7	134.0602	132.2048, 116.0498, 106.0654, 99.0060, 95.0495, 89.0391, 79.0548, 76.5491, 71.5103, 64.7083	C_8H_7NO	6-hydroxyindole	[b]
33	6.06	153.1275	141.7384, 135.1169, 115.9385, 111.0806, 107.0859, 105.0705, 97.0652, 95.0860, 93.0704, 91.0546, 83.0860, 81.0705, 79.0549, 73.0656, 71.0499, 69.0706, 67.0549, 59.0500, 55.0551	$C_{10}H_{16}O$	camphor	[b]

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ACS Omega 2023, 8, 6253−6267

Table 3. continued

peak no.	RT (min)	experimental $[M + H]^+$ (m/z)	major secondary fragment ions (m/z)	molecular formula	identification	reference
Other Compounds						
36	7.67	471.2021	453.1918, 435.1810, 425.1965, 409.2016, 391.1884, 367.1910, 349.1818, 339.1961, 321.1861, 279.1387, 251.1058, 227.1071, 213.0914, 205.0499, 175.0756, 161.0600, 145.0651, 133.0651, 119.0860, 105.0704, 95.0134, 79.0549, 69.0707	$C_{26}H_{30}O_8$	limonin	[a]
46	10.62	175.0390	147.0443, 134.0603, 131.0496, 121.0291, 119.0495, 105.0335, 103.0549, 95.0499, 91.0549, 79.0549, 70.0659, 65.00394, 58.3657	$C_{10}H_6O_3$	lawsone	48
48	11	151.1121	133.1011, 131.0858, 123.1168, 121.1012, 113.9638, 109.0649, 107.0857, 105.0700, 98.5123, 97.0650, 95.0494, 93.0702, 91.0546, 87.0045, 83.0495, 81.0704, 79.0547, 71.0497, 69.0341, 67.0548, 65.0392, 57.0342, 55.0550	$C_{10}H_{14}O$	perillene	[b]
50	11.62	219.1745	201.1635, 191.1790, 177.127, 163.1115, 159.1168, 149.0959, 145.0959, 135.0803, 131.0856, 123.1168, 119.0855, 111.0805, 107.0856, 97.0651, 95.0858, 93.0702, 91.0546, 81.0704, 19.0547, 69.0705, 67.0549, 57.0704, 55.0550	$C_5H_{22}O$	α-cyperone	49

[a]Confirmation in comparison with standard substances. [b]Refers to the database.

proline, and limonin. Methanol and acetonitrile of HPLC grade were obtained from Merck (Germany). Hexane and formic acid were provided by Honeywell (USA) and Thermo Fisher Scientific (China), respectively. Water was purified using the Milli-Q system from Millipore (Milford, MA, USA).

4.3. Preparation of Standard Solutions. Appropriate amounts of 18 reference substances were separately dissolved in methanol to obtain solutions with a concentration of 100 $\mu g \cdot mL^{-1}$. The resulting solutions were used as standard stock solutions for UPLC-Q-exactive orbitrap-MS analysis. The contents of six main compounds (including naringin, rhoifolin, meranzin hydrate, isomeranzin, auraptene, and bergamottin) in pomelo peel were calculated using external standard methods based on corresponding calibration curves. The standard substances of these six main compounds were separately prepared in methanol and diluted to appropriate concentrations for HPLC-PDA.

4.4. Sample Preparation. Samples of pomelo were cleaned using distilled water and peeled. Pomelo peels were collected, sliced, and air-dried to constant weight for about 2 weeks for further analysis. Then, dry pomelo peels were comminuted and passed through a 40-mesh sieve to obtain a fine powder for the following UPLC-Q-exactive orbitrap-MS and HPLC-PDA detection.

The volatile compounds in pomelo peels were extracted with water and then determined with GC−MS. 100 g of the powdered sample was added in approximately 1.5 L of water and transferred into a 2 L round-bottomed distillation flask. The mixture was heated to reflux for 1 h and filtered to obtain the extract. Then, 50 μL of the extract was transferred into a test tube containing 950 μL of hexane. The mixture was filtered with a 0.22 μm PTFE membrane before performing GC−MS.

The nonvolatile compounds in pomelo peels were extracted with methanol and then analyzed with UPLC-Q-exactive orbitrap-MS. 0.5 g of the dried and powdered sample was mixed with 50 mL of methanol in a conical flask. The flask with samples was immersed into an ultrasonic bath (KQ-800KDE instrument, Kunshan Ultrasonic Instruments Co. Ltd., China) for 30 min at 320 W (40 kHz) for the extraction of nonvolatile compounds. Afterward, the sample was filtered and analyzed with the UPLC-Q-exactive orbitrap-MS system.

The main nonvolatile compounds were quantified with HPLC-PDA. 0.5 g of the powdered sample was mixed with 10 mL of methanol. The mixture was treated in an ultrasonic bath for 30 min. Afterward, the supernatant was collected in a conical flask and passed through a 0.22 μm PTFE membrane to obtain the methanol extract for further experiments.

4.5. Qualitative and Quantitative GC−MS System for the Analysis of Volatile Compounds in Pomelo Peels. The GC system consisted of a TRACE DSQ GC instrument (Thermo Finnigan, USA), and a TG-5SILMS GC capillary column (0.25 mm × 30 m, 0.25 μm) was used to analyze the volatile compounds of pomelo peels. GC−MS operating conditions were as follows: the initial temperature was set at 60 °C, increased to 80 °C at a rate of 1 $°C \cdot min^{-1}$, further increased to 250 °C at a rate of 5 $°C \cdot min^{-1}$, and finally increased to 260 °C at a rate of 20 $°C \cdot min^{-1}$ and held for 1 min. Temperatures at the injection port and detector were maintained at 270 °C. The solvent delay was fixed at 4 min. High-purity helium (1 $mL \cdot min^{-1}$) was used as carrier gas at a split ratio of 30:1. Moreover, the injection volume for samples was 1 μL. Detection was performed in the full-scan mode ranging from m/z 35 to m/z 300, with a scan speed of 0.2 amu·

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ACS Omega 2023, 8, 6253−6267

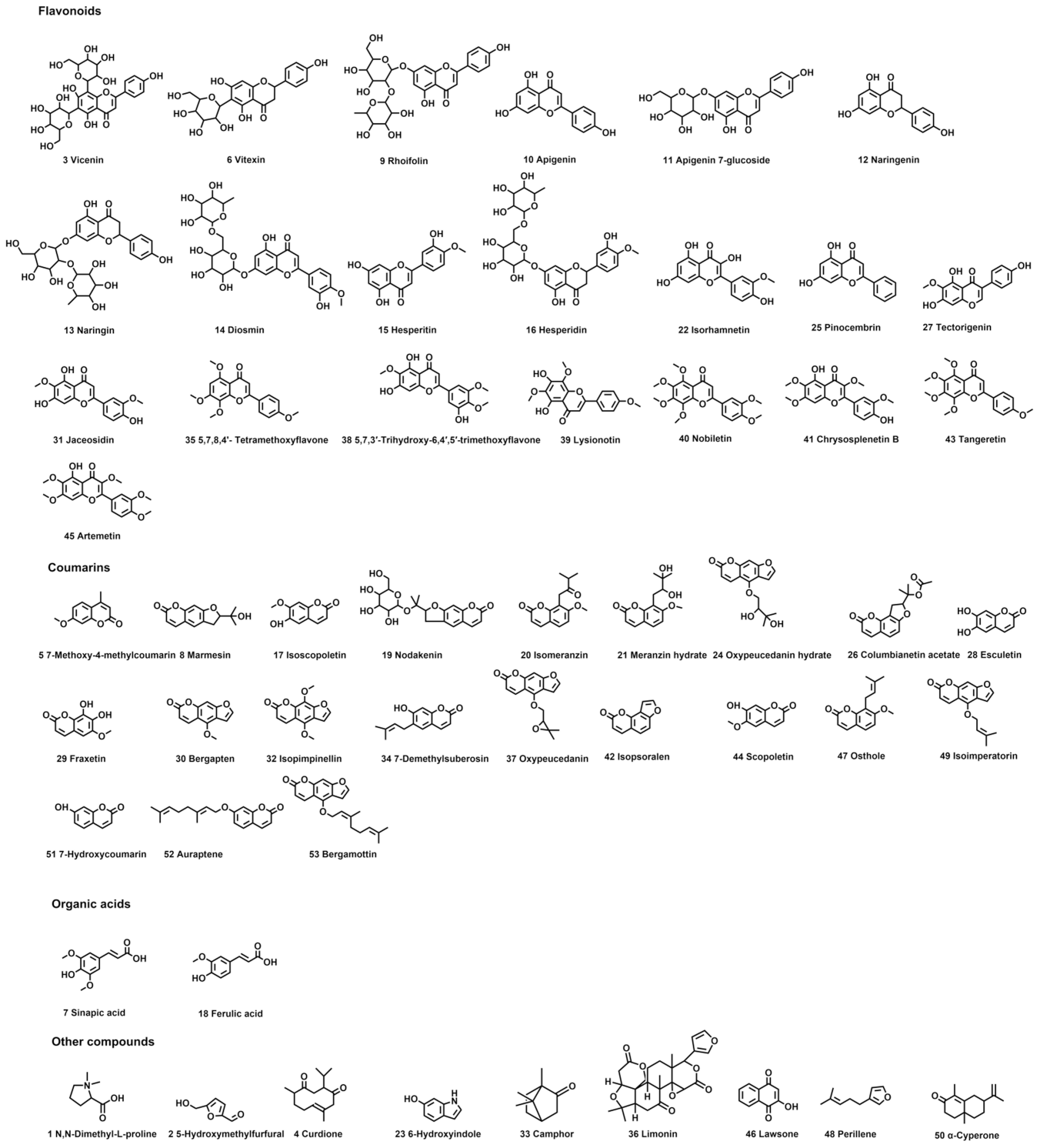

Figure 5. Chemical structures of the 53 components that were tentatively identified.

s^{-1}, and the electron impact (EI^+) mode (70 eV) was used. All volatile compounds were identified by comparing their recorded mass spectra with the NIST08.L database.

4.6. Qualitative UPLC-Q-Exactive Orbitrap-MS System for the Analysis of Methanol Extracts of Pomelo Peels. Samples were first separated on a ZORBAX Eclipse Plus C_{18} column with a stable flow rate of 0.4 mL·min^{-1} and a column temperature of 40 °C. The mobile phase included 0.1% formic acid aqueous solution (A) and acetonitrile (B). The nonvolatile compounds of pomelo peels were eluted under the following conditions: 25% B at 0−4 min, 25−50% B at 4−10 min, 50% B at 10−14 min, 50−85% B at 14−18 min, and 85−100% B at 18−25 min. The injection volume was 2 μL for both the sample and standard solutions.

The full-scan mode was applied for data acquisition in positive ionization modes ranging from m/z 70 to m/z 1000 with a resolution of 70,000. The spray voltage was 35 kV, the capillary temperature was 320 °C, and the auxiliary gas heating temperature was 300 °C. The flow rate of the sheath, sweep, and auxiliary gases was 10.0, 1.7, and 3.3 L·min^{-1}, respectively.

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Table 4. Non-Volatile Compound Differences among Pomelo Peels from Five Cultivars

peak no.	identification	RT (min)	molecular formula	S1	S2	S3	MH1	MH2	MB1	MB2	Y1	Y2	D1	D2	L1
			Flavonoids												
3	vicenin	0.39	$C_{27}H_{30}O_{15}$				√	√	√	√	√	√	√	√	√
6	vitexin	0.50	$C_{21}H_{20}O_{10}$				√	√	√	√	√	√	√	√	
9	rhoifolin	0.64	$C_{27}H_{30}O_{14}$	√	√	√	√	√	√	√	√	√	√	√	√
10	apigenin	0.65	$C_{15}H_{10}O_5$				√	√	√	√	√	√	√	√	
11	apigenin 7-glucoside	0.65	$C_{21}H_{20}O_{10}$					√							
12	naringenin	0.66	$C_{15}H_{12}O_5$	√	√	√	√	√	√	√	√	√	√	√	√
13	naringin	0.68	$C_{27}H_{32}O_{14}$	√	√	√	√	√	√	√	√	√	√	√	√
14	diosmin	0.71	$C_{28}H_{32}O_{15}$							√	√	√		√	
15	hesperitin	0.72	$C_{16}H_{14}O_6$				√								
16	hesperidin	0.72	$C_{28}H_{34}O_{15}$				√								
22	isorhamnetin	1.44	$C_{14}H_{12}O_7$				√	√	√	√	√	√	√	√	√
25	pinocembrin	1.94	$C_{15}H_{12}O_4$	√	√	√	√	√	√		√	√	√		√
27	tectorigenin	3.48	$C_{16}H_{12}O_6$												√
31	iristetrigenin B	5.16	$C_{17}H_{14}O_7$	√	√	√									√
35	5,7,8,4′-tetramethoxyflavone	7.63	$C_{19}H_{18}O_6$			√									
38	5,7,3′-trihydroxy-6,4′,5′-trimethoxyflavone	8.21	$C_{18}H_{16}O_8$	√	√	√							√	√	√
39	lysionotin	8.55	$C_{18}H_{16}O_7$	√	√	√					√	√			
40	nobiletin	8.6	$C_{21}H_{22}O_8$	√	√	√	√	√	√	√	√	√	√	√	√
41	chrysosplenetin B	8.77	$C_{19}H_{18}O_8$	√	√	√	√	√	√	√	√	√	√	√	√
43	tangeretin	9.54	$C_{20}H_{20}O_7$	√	√	√	√	√	√	√	√	√	√	√	√
45	artemetin	10.55	$C_{20}H_{20}O_8$	√	√	√									
			Coumarins												
5	7-methoxy-4-methylcoumarin	0.48	$C_{11}H_{10}O_3$	√	√		√		√		√				
8	marmesin	0.61	$CH_{14}H_{14}O_4$	√	√	√				√	√	√	√	√	√
17	isoscopoletin	0.78	$C_{10}H_8O_4$	√			√			√			√	√	√
19	nodakenin	0.91	$C_{20}H_{24}O_9$		√										
20	isomeranzin	1.31	$C_{15}H_{16}O_4$	√	√	√	√	√	√	√	√	√	√	√	√
21	meranzin hydrate	1.32	$C_{15}H_{18}O_5$	√	√	√	√	√	√	√	√	√	√	√	√
24	oxypeucedanin hydrate	1.92	$C_{16}H_{16}O_6$	√	√	√									√
26	columbianetin acetate	2.18	$C_{16}H_{16}O_5$	√		√									
28	esculetin	3.57	$C_9H_6O_4$	√			√	√	√	√	√	√	√	√	√
29	fraxetin	3.71	$C_{10}H_8O_5$	√	√	√					√	√	√	√	√
30	bergapten	5.09	$C_{12}H_8O_4$	√	√	√	√	√	√	√	√	√	√	√	√
32	isopimpinellin	5.41	$C_{13}H_{10}O_5$				√	√							
34	7-demethylsuberosin	7.06	$C_{14}H_{14}O_3$	√	√	√	√	√	√		√	√	√	√	
37	oxypeucedanin	7.91	$C_{16}H_{14}O_5$		√	√									√
42	isopsoralen	9.48	$C_{11}H_6O_3$			√							√		
44	scopoletin	9.72	$C_{10}H_8O_4$	√			√			√			√	√	√
47	osthole	10.85	$C_{15}H_{16}O_3$	√	√	√	√	√	√	√	√	√	√	√	√
49	isoimperatorin	11.31	$C_{16}H_{14}O_4$	√	√	√	√	√	√		√		√	√	√
51	7-hydroxycoumarin	16.93	$C_{14}H_{14}O_3$	√	√	√	√	√	√	√	√	√	√	√	√
52	auraptene	16.95	$C_{19}H_{22}O_3$	√	√	√	√	√	√	√	√	√	√	√	√
53	bergamottin	17.60	$C_{11}H_6O_3$				√	√	√	√	√	√	√	√	√
			Organic Acids												
7	sinapic acid	0.60	$C_{11}H_{12}O_5$				√		√	√	√	√	√	√	
18	ferulic acid	0.78	$C_{10}H_{10}O_4$			√	√		√	√	√				√
			Other Compounds												
1	N,*N*-dimethyl-L-proline	0.31	$C_7H_{13}NO_2$	√	√	√	√	√	√	√	√	√	√	√	√
2	5-hydroxymethylfurfural	0.33	$C_6H_6O_3$	√	√	√	√	√		√	√	√	√	√	√
4	curdione	0.42	$C_{15}H_{24}O_2$	√				√							√
	curcumol						√								
23	6-hydroxyindole	1.70	C_8H_7NO			√	√								
33	camphor	6.06	$C_{10}H_{16}O$	√	√		√	√				√		√	
36	Limonin	7.67	$C_{26}H_{30}O_8$	√	√	√			√	√	√	√	√	√	√
46	Lawsone	10.62	$C_{10}H_6O_3$							√			√	√	
48	Perillene	11.00	$C_{10}H_{14}O$			√					√		√	√	
50	*α*-cyperone	11.62	$C_5H_{22}O$	√				√		√		√	√		

4.7. Quantitative HPLC-PDA System for the Analysis of Methanol Extracts of Six Main Nonvolatile Compounds. Chromatographic separation was carried out at 30 °C on a Diamonsil C_{18} column (250 mm × 4.6 mm, 5 μm). The mobile

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ACS Omega 2023, 8, 6253−6267

Table 5. Linear Correlation, Repeatability, Precision, Stability, and Recovery Investigation of Seven Chemical Compounds

compounds	calibration curves	correlation coefficients (R^2)	linear range (μg/mL)	LOD (μg/mL)	LOQ (μg/mL)	repeatability RSD (%), n = 6	precision RSD (%), n = 6	stability RSD (%), n = 6	extraction recovery (%), n = 6	recovery RSD (%), n = 6
naringin	$y = 20053x + 4000000$	0.999	500.00−3133.08	0.007	0.024	1.91	0.45	2.13	91.07	1.30
rhoifolin	$y = 31529x + 24430$	1.000	22.19−412.00	0.007	0.024	2.76	0.34	0.37	92.38	1.65
meranzin hydrate	$y = 51450x + 64334$	1.000	0.78−386.00	0.003	0.009	1.54	0.60	1.62	90.13	1.07
isomeranzin	$y = 53693x - 11226$	1.000	4.00−112.00	0.005	0.018	2.84	0.60	1.20	105.87	2.78
auraptene	$y = 49794x + 43015$	0.999	6.25−246.00	0.003	0.009	2.50	0.28	0.68	94.72	2.67
bergamottin	$y = 26185x - 4122$	0.999	2.54−42.20	0.013	0.043	0.72	0.58	1.37	96.71	2.81

Table 6. Results of Determination of Non-Volatile Constituents in Five Cultivars by HPLC-PDA[a]

	HPLC-PDA ($mg \cdot g^{-1}$)					
sample	naringin	rhoifolin	meranzin hydrate	isomeranzin	auraptene	bergamottin
S1	161.95 ± 0.93^{a}	1.42 ± 0.04^{e}	20.39 ± 0.07^{c}	4.27 ± 0.01^{A}	1.75 ± 0.03^{a}	0.00 ± 0.00^{a}
S2	163.16 ± 1.04^{a}	2.94 ± 0.03^{A}	18.33 ± 0.06^{d}	4.50 ± 0.04^{B}	1.62 ± 0.07^{b}	0.00 ± 0.00^{a}
S3	84.44 ± 1.26^{c}	4.47 ± 0.04^{a}	17.22 ± 0.05^{e}	4.06 ± 0.01^{C}	1.90 ± 0.01^{c}	0.00 ± 0.00^{a}
MH1	242.17 ± 0.77^{b}	17.45 ± 0.22^{b}	3.94 ± 0.02^{A}	0.73 ± 0.02^{a}	2.91 ± 0.01^{d}	0.87 ± 0.00^{c}
MH2	218.07 ± 0.21^{d}	9.20 ± 0.03^{B}	3.25 ± 0.00^{B}	$0.65 \pm 0.02^{a,b}$	1.83 ± 0.00^{e}	0.35 ± 0.01^{b}
MB1	261.16 ± 0.26^{e}	15.60 ± 0.16^{C}	2.76 ± 0.03^{C}	0.49 ± 0.01^{b}	1.73 ± 0.00^{a}	0.38 ± 0.00^{b}
MB2	244.95 ± 1.03^{A}	17.34 ± 0.19^{b}	0.10 ± 0.00^{a}	0.82 ± 0.00^{a}	7.76 ± 0.01^{f}	1.51 ± 0.00^{d}
Y1	256.16 ± 1.55^{B}	17.83 ± 0.23^{c}	0.16 ± 0.00^{a}	1.67 ± 0.10^{c}	6.40 ± 0.02^{A}	1.45 ± 0.03^{A}
Y2	240.21 ± 0.75^{b}	$17.81 \pm 0.16^{c,d}$	0.14 ± 0.00^{a}	1.79 ± 0.05^{c}	8.85 ± 0.03^{B}	1.86 ± 0.00^{B}
D1	288.40 ± 0.70^{C}	19.67 ± 0.04^{D}	0.01 ± 0.00^{b}	3.65 ± 0.30^{d}	16.32 ± 0.04^{C}	2.75 ± 0.03^{C}
D2	253.71 ± 0.77^{D}	$17.52 \pm 0.23^{b,d}$	0.01 ± 0.00^{b}	3.60 ± 0.01^{d}	23.05 ± 0.04^{D}	3.88 ± 0.04^{D}
L1	263.41 ± 1.42^{E}	4.24 ± 0.02^{a}	11.38 ± 0.01^{D}	2.73 ± 0.03^{D}	0.52 ± 0.01^{E}	0.00 ± 0.00^{a}

[a]Different letters indicate significant differences ($P < 0.05$). Data are presented as mean ± standard deviation ($n = 3$).

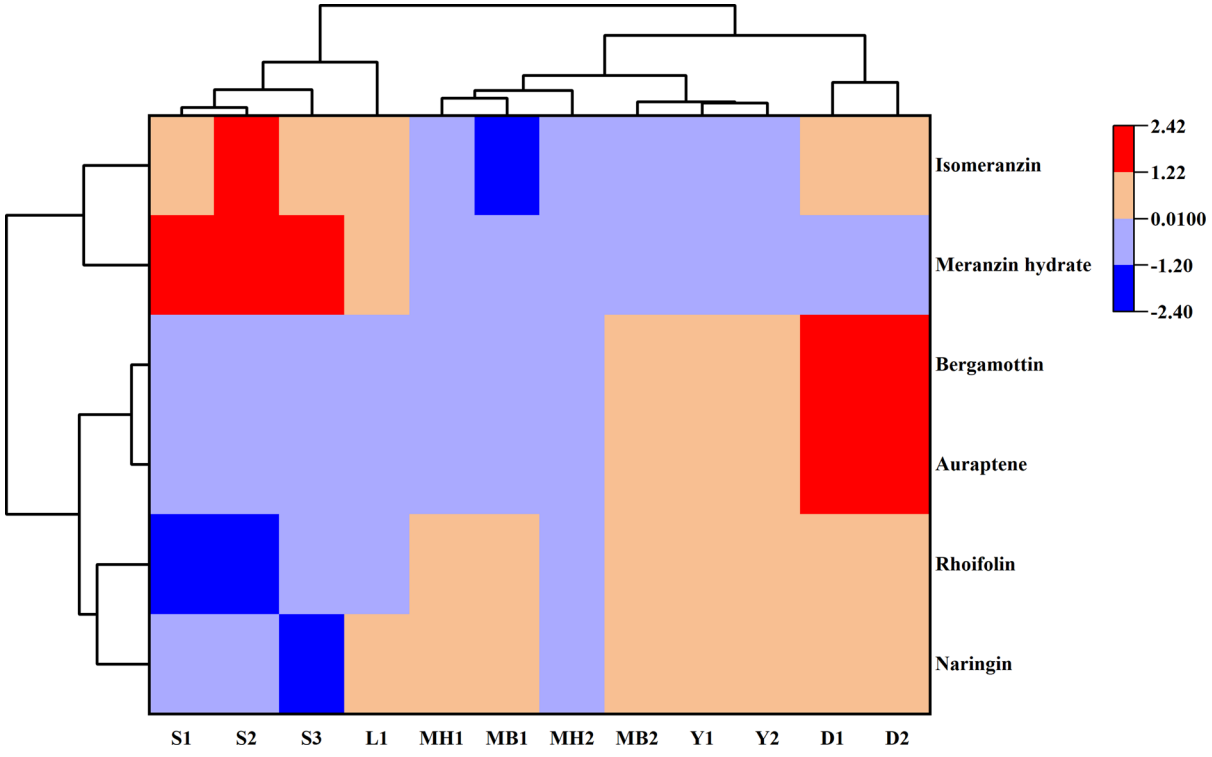

Figure 6. Heatmap and dendrogram of six major nonvolatile compounds in pomelo peels from five cultivars.

phase included 0.1% (v/v) phosphoric acid water solution (pH 3.70, A) and 30% methanol +70% acetonitrile (B). The nonvolatile compounds of pomelo peels were eluted by using a gradient program: 0−20% B at 0−5 min, 20−40% B at 5−15 min, 40% B at 15−30 min, 40−60% B at 30−40 min, 60−70% B at 40−45 min, 70−80% B at 45−50 min, 80% B at 50−55 min, and 80−100% B at 55−60 min. The flow rate was 1 $mL \cdot min^{-1}$. A 15 μL aliquot was used for injection and analysis. Furthermore, naringin (283 nm), rhoifolin (325 nm), meranzin hydrate (325

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nm), isomeranzin (325 nm), auraptene (325 nm), and bergamottin (325 nm) were monitored using a PDA detector.

4.8. Statistical Analysis. Data were statistically analyzed using Statistical Product and Service Solutions version 25 (SPSS Inc, Chicago, USA). Analysis of variance was performed to determine any significant difference in measurement, and $P < 0.05$ indicated statistical significance. Clustering analysis allowed the grouping of different cultivars based on their similarities.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

GC−MS, gas chromatography−mass spectrometry; HPLC-PDA, high-performance liquid chromatography-photodiode array detection; OCTML, Orbitrap Chinese Traditional Medicine Library; TIC, total ion chromatogram; UPLC-Q-Exactive Orbitrap/MS, ultraperformance liquid chromatography-Q-Exactive Orbitrap tandem mass spectrometry

REFERENCES

(1) Xi, W.; Fang, B.; Zhao, Q.; Jiao, B.; Zhou, Z. Flavonoid composition and antioxidant activities of Chinese local pummelo (Citrus grandis Osbeck.) varieties. *Food Chem.* **2014**, *161*, 230−238.

(2) Gyawali, R.; Jeon, D. H.; Moon, J.; Kim, H.; Song, Y. W.; Hyun, H. B.; Jeong, D.; Cho, S. K. Chemical Composition and Antiproliferative Activity of Supercritical Extract ofCitrus grandis(L.) Osbeck Fruits from Korea. *J. Essent. Oil-Bear. Plants* **2012**, *15*, 915−925.

(3) Kuo, P.-C.; Liao, Y.-R.; Hung, H.-Y.; Chuang, C.-W.; Hwang, T.-L.; Huang, S.-C.; Shiao, Y.-J.; Kuo, D.-H.; Wu, T.-S. Anti-Inflammatory and Neuroprotective Constituents from the Peels of Citrus grandis. *Molecules* **2017**, *22*, 967.

(4) Chowdhury, M. R. H.; Sagor, M. A. T.; Tabassum, N.; Potol, M. A.; Hossain, H.; Alam, M. A. Supplementation of Citrus maxima Peel Powder Prevented Oxidative Stress, Fibrosis, and Hepatic Damage in Carbon Tetrachloride (CCl4) Treated Rats. *Evidence-Based Complementary Altern. Med.* **2015**, *2015*, 598179.

(5) Oboh, G.; Ademosun, A. O. Shaddock peels (Citrus maxima) phenolic extracts inhibit α-amylase, α-glucosidase and angiotensin I-converting enzyme activities: A nutraceutical approach to diabetes management. *Diabetes, Metab. Syndrome* **2011**, *5*, 148−152.

(6) Jung, Y. Y.; Ko, J.-H.; Um, J.-Y.; Sethi, G.; Ahn, K. S. A Novel Role of Bergamottin in Attenuating Cancer Associated Cachexia by Diverse Molecular Mechanisms. *Cancers* **2021**, *13*, 1347.

(7) Yu, L.; Yan, J.; Sun, Z. D-limonene exhibits anti-inflammatory and antioxidant properties in an ulcerative colitis rat model via regulation of iNOS, COX-2, PGE2 and ERK signaling pathways. *Mol. Med. Rep.* **2017**, *15*, 2339−2346.

(8) Chen, R.; Qi, Q. L.; Wang, M. T.; Li, Q. Y. Therapeutic potential of naringin: an overview. *Pharm. Biol.* **2016**, *54*, 3203−3210.

(9) Guo, X.; Zhao, W.; Liao, X.; Hu, X.; Wu, J.; Wang, X. Extraction of pectin from the peels of pomelo by high-speed shearing homogenization and its characteristics. *LWT–Food Sci. Technol.* **2017**, *79*, 640−646.

https://doi.org/10.1021/acsomega.2c05514
ACS Omega 2023, 8, 6253−6267

(10) Ben Hsouna, A.; Ben Halima, N.; Smaoui, S.; Hamdi, N. Citrus lemon essential oil: chemical composition, antioxidant and antimicrobial activities with its preservative effect against Listeria monocytogenes inoculated in minced beef meat. *Lipids Health Dis.* **2017**, *16*, 146.
(11) Lin, X.; Cao, S.; Sun, J.; Lu, D.; Zhong, B.; Chun, J. The Chemical Compositions, and Antibacterial and Antioxidant Activities of Four Types of Citrus Essential Oils. *Molecules* **2021**, *26*, 3412.
(12) Wang, F.; Chen, L.; Chen, H.; Chen, S.; Liu, Y. Analysis of Flavonoid Metabolites in Citrus Peels ("Dahongpao") Using UPLC-ESI-MS/MS. *Molecules* **2019**, *24*, 2680.
(13) Di Rauso Simeone, G.; Di Matteo, A.; Rao, M. A.; Di Vaio, C. Variations of peel essential oils during fruit ripening in four lemon (Citrus limon (L.) Burm. F.) cultivars. *J. Sci. Food Agric.* **2020**, *100*, 193−200.
(14) Zhao, X. J.; Xing, T. T.; Li, Y. F.; Jiao, B. N. Analysis of phytochemical contributors to antioxidant capacity of the peel of Chinese mandarin and orange varieties. *Int. J. Food Sci. Nutr.* **2019**, *70*, 825−833.
(15) Lebanov, L.; Ghiasvand, A.; Paull, B. Data handling and data analysis in metabolomic studies of essential oils using GC-MS. *J. Chromatogr. A* **2021**, *1640*, 461896.
(16) Anandakumar, P.; Kamaraj, S.; Vanitha, M. K. D-limonene: A multifunctional compound with potent therapeutic effects. *J. Food Biochem.* **2021**, *45*, No. e13566.
(17) Yan, J.; Ni, B.; Sheng, G.; Zhang, Y.; Xiao, Y.; Ma, Y.; Li, H.; Wu, H.; Tu, C. Rhoifolin Ameliorates Osteoarthritis via Regulating Autophagy. *Front. Pharmacol.* **2021**, *12*, 661072.
(18) Den Hartogh, D. J.; Tsiani, E. Antidiabetic Properties of Naringenin: A Citrus Fruit Polyphenol. *Biomolecules* **2019**, *9*, 99.
(19) Tocmo, R.; Pena-Fronteras, J.; Calumba, K. F.; Mendoza, M.; Johnson, J. J. Valorization of pomelo (Citrus grandis Osbeck) peel: A review of current utilization, phytochemistry, bioactivities, and mechanisms of action. *Compr. Rev. Food Sci. Food Saf.* **2020**, *19*, 1969−2012.
(20) Shao, Y.; You, D.; Lou, Y.; Li, J.; Ying, B.; Cheng, K.; Weng, W.; Wang, H.; Yu, M.; Dong, L. Controlled Release of Naringin in GelMA-Incorporated Rutile Nanorod Films to Regulate Osteogenic Differentiation of Mesenchymal Stem Cells. *ACS Omega* **2019**, *4*, 19350−19357.
(21) Aihaiti, Y.; Song Cai, Y.; Tuerhong, X.; Ni Yang, Y.; Ma, Y.; Shi Zheng, H.; Xu, K.; Xu, P. Therapeutic Effects of Naringin in Rheumatoid Arthritis: Network Pharmacology and Experimental Validation. *Front. Pharmacol.* **2021**, *12*, 672054.
(22) Zhou, J.; Xia, L.; Zhang, Y. Naringin inhibits thyroid cancer cell proliferation and induces cell apoptosis through repressing PI3K/AKT pathway. *Pathol. Res. Pract.* **2019**, *215*, 152707.
(23) Liu, X.; Zhou, J.; Zhang, T.; Chen, K.; Xu, M.; Wu, L.; Liu, J.; Huang, Y.; Nie, B.; Shen, X.; Ren, P.; Huang, X. Meranzin hydrate elicits antidepressant effects and restores reward circuitry. *Behav. Brain Res.* **2021**, *398*, 112898.
(24) Alves, G. d. A. D.; Fernandes da Silva, D.; Venteu Teixeira, T.; de Souza, R. O.; Rogez, H.; Fonseca, M. J. V. Obtainment of an enriched fraction of Inga edulis: identification using UPLC-DAD-MS/MS and photochemopreventive screening. *Prep. Biochem. Biotechnol.* **2020**, *50*, 28−36.
(25) Lin, Y.-T.; Mao, Y.-W.; Imtiyaz, Z.; Chiou, W.-F.; Lee, M.-H. Comprehensive LC-MS/MS-based phytochemical perspectives and osteogenic effects of Uraria crinita. *Food Funct.* **2020**, *11*, 5420−5431.
(26) Cheruvu, H. S.; Yadav, N. K.; Valicherla, G. R.; Arya, R. K.; Hussain, Z.; Sharma, C.; Arya, K. R.; Singh, R. K.; Datta, D.; Gayen, J. R. LC-MS/MS method for the simultaneous quantification of luteolin, wedelolactone and apigenin in mice plasma using hansen solubility parameters for liquid-liquid extraction: Application to pharmacokinetics of Eclipta alba chloroform fraction. *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.* **2018**, *1081−1082*, 76−86.
(27) Gharari, Z.; Bagheri, K.; Sharafi, A. Fractional analysis of dichloromethane extract of Scutellaria araxensis Grossh root and shoot by HPLC-PDA-ESI-MSn. *Nat. Prod. Res.* **2022**, *36*, 4031−4035.
(28) Chen, X.; Xu, L.; Guo, S.; Wang, Z.; Jiang, L.; Wang, F.; Zhang, J.; Liu, B. Profiling and comparison of the metabolites of diosmetin and diosmin in rat urine, plasma and feces using UHPLC-LTQ-Orbitrap MSn. *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.* **2019**, *1124*, 58−71.
(29) Ye, X.; Cao, D.; Zhao, X.; Song, F.; Huang, Q.; Fan, G.; Wu, F. Chemical fingerprint and metabolic profile analysis of Citrus reticulate 'Chachi' decoction by HPLC-PDA-IT-MSn and HPLC-Quadrupole-Orbitrap-MS method. *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.* **2014**, *970*, 108−120.
(30) Yin, N.-W.; Wang, S.-X.; Jia, L.-D.; Zhu, M.-C.; Yang, J.; Zhou, B.-J.; Yin, J.-M.; Lu, K.; Wang, R.; Li, J.-N.; Qu, C.-M. Identification and Characterization of Major Constituents in Different-Colored Rapeseed Petals by UPLC-HESI-MS/MS. *J. Agric. Food Chem.* **2019**, *67*, 11053−11065.
(31) Fu, Y.; Yang, J.; Chen, S.; Sun, X.; Zhao, P.; Xie, Z. Screening, and identification of the binding position, of xanthine oxidase inhibitors in the roots of Lindera reflexa Hemsl using ultrafiltration LC-MS combined with enzyme blocking. *Biomed. Chromatogr.* **2019**, *33*, No. e4577.
(32) Li, L.; Zhang, X.; Bu, F.; Chen, N.; Zhang, H.; Gu, J. Simultaneous determination of eight constituents in rat plasma by HPLC-MS/MS and its application to a pharmacokinetic study after oral administration of Shejin-liyan Granule. *Biomed. Chromatogr.* **2019**, *33*, No. e4648.
(33) Sait, S.; Hamri-Zeghichi, S.; Boulekbache-Makhlouf, L.; Madani, K.; Rigou, P.; Brighenti, V.; Pio Prencipe, F.; Benvenuti, S.; Pellati, F. HPLC-UV/DAD and ESI-MSn analysis of flavonoids and antioxidant activity of an Algerian medicinal plant: Paronychia argentea Lam. *J. Pharm. Biomed. Anal.* **2015**, *111*, 231−240.
(34) Niu, C.; Sun, J.; Zheng, Y.; Wang, L.; Zhang, J.; Chen, R.; Ye, W. Determination of isosinensetin in rat plasma by UHPLC-MS/MS: Application to oral and intravenous pharmacokinetic study in healthy rats. *J. Pharm. Biomed. Anal.* **2020**, *184*, 113210.
(35) Malongane, F.; McGaw, L. J.; Nyoni, H.; Mudau, F. N. Metabolic profiling of four South African herbal teas using high resolution liquid chromatography-mass spectrometry and nuclear magnetic resonance. *Food Chem.* **2018**, *257*, 90.
(36) Han, X.; He, J.; Chen, Q.; Sun, Y.; Zhang, X.; Gu, Z.; Sha, X. Development of an LC-MS/MS-based assay to determine artemitin in rat plasma and its application in a pharmacokinetic study. *Biomed. Chromatogr.* **2018**, *32*, No. e4356.
(37) Ahn, M.-J.; Lee, M. K.; Kim, Y. C.; Sung, S. H. The simultaneous determination of coumarins in Angelica gigas root by high performance liquid chromatography-diode array detector coupled with electrospray ionization/mass spectrometry. *J. Pharm. Biomed. Anal.* **2008**, *46*, 258−266.
(38) Lee, S. Y.; Jeong, J. H.; Kim, B. N.; Park, S. J.; Park, Y.-C.; Lee, G. Y. LC-MS/MS analysis of puerarin and 18β-glycyrrhetinic acid in human plasma after oral administration of Samso-eum and its application to pharmacokinetic study. *Biomed. Chromatogr.* **2020**, *34*, No. e4774.
(39) Vogl, S.; Zehl, M.; Picker, P.; Urban, E.; Wawrosch, C.; Reznicek, G.; Saukel, J.; Kopp, B. Identification and quantification of coumarins in Peucedanum ostruthium (L.) Koch by HPLC-DAD and HPLC-DAD-MS. *J. Agric. Food Chem.* **2011**, *59*, 4371−4377.
(40) Yang, Y.-F.; Zhang, L.; Yang, X.-W. Distribution Assessments of Coumarins from Angelicae Pubescentis Radix in Rat Cerebrospinal Fluid and Brain by Liquid Chromatography Tandem Mass Spectrometry Analysis. *Molecules* **2018**, *23*, 225.
(41) Yang, L.; Meng, X.; Yu, X.; Kuang, H. Simultaneous determination of anemoside B4, phellodendrine, berberine, palmatine, obakunone, esculin, esculetin in rat plasma by UPLC-ESI-MS/MS and its application to a comparative pharmacokinetic study in normal and ulcerative colitis rats. *J. Pharm. Biomed. Anal.* **2017**, *134*, 43−52.
(42) Leng, Z.; Zhong, B.; Wu, H.; Liu, Z.; Rauf, A.; Bawazeer, S.; Suleria, H. A. R. Identification of Phenolic Compounds in Australian-Grown Bell Peppers by Liquid Chromatography Coupled with Electrospray Ionization-Quadrupole-Time-of-Flight-Mass Spectrome-

https://doi.org/10.1021/acsomega.2c05514
ACS Omega 2023, 8, 6253−6267

try and Estimation of Their Antioxidant Potential. *ACS Omega* **2022**, *7*, 4563−4576.
(43) Luan, L.; Shen, X.; Liu, X.; Wu, Y.; Tan, M. Qualitative analysis of Psoraleae Fructus by HPLC-DAD/TOF-MS fingerprint and quantitative analysis of multiple components by single marker. *Biomed. Chromatogr.* **2018**, *32*, No. e4059.
(44) Lu, Y.; Li, N.; Deng, Y.; Zhao, L.; Guo, X.; Li, F.; Xiong, Z. Simultaneous determination of icariin, naringin and osthole in rat plasma by UPLC-MS/MS and its application for pharmacokinetic study after oral administration of Gushudan capsules. *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.* **2015**, *993*−*994*, 75−80.
(45) Olszewska, M. A.; Granica, S.; Kolodziejczyk-Czepas, J.; Magiera, A.; Czerwińska, M. E.; Nowak, P.; Rutkowska, M.; Wasiński, P.; Owczarek, A. Variability of sinapic acid derivatives during germination and their contribution to antioxidant and anti-inflammatory effects of broccoli sprouts on human plasma and human peripheral blood mononuclear cells. *Food Funct.* **2020**, *11*, 7231−7244.
(46) González-Gómez, L.; Morante-Zarcero, S.; Pérez-Quintanilla, D.; Sierra, I. Simultaneous Determination of Furanic Compounds and Acrylamide in Insect-Based Foods by HPLC-QqQ-MS/MS Employing a Functionalized Mesostructured Silica as Sorbent in Solid-Phase Extraction. *Foods* **2021**, *10*, 1557.
(47) Meng, X.; Zhang, T.; Li, Y.; Pan, Q.; Jiang, J.; Luo, Y.; Chong, L.; Yang, Y.; Xu, S.; Zhou, L.; et al. Development and application of an analytical method for curdione quantification in pregnant Sprague-Dawley rats by LC-MS/MS. *Biomed. Chromatogr.* **2015**, *29*, 1499−1505.
(48) Sreekanth, R.; Menachery, S. P. M.; Aravind, U. K.; Marignier, J.-L.; Belloni, J.; Aravindakumar, C. T. Oxidation reactions of hydroxy naphthoquinones: mechanistic investigation by LC-Q-TOF-MS analysis. *Int. J. Radiat. Biol.* **2014**, *90*, 495−502.
(49) Liu, P.; Shang, E.-X.; Zhu, Y.; Qian, D.-W.; Duan, J.-A. Volatile component interaction effects on compatibility of Cyperi Rhizoma and Angelicae Sinensis Radix or Chuanxiong Rhizoma by UPLC-MS/MS and response surface analysis. *J. Pharm. Biomed. Anal.* **2018**, *160*, 135−143.

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ACS Omega 2023, 8, 6253−6267