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Differentiating Tangerine Peels from Other *Citrus reticulata* through GC-MS, UPLC-Q-Exactive Orbitrap-MS, and HPLC-PDA

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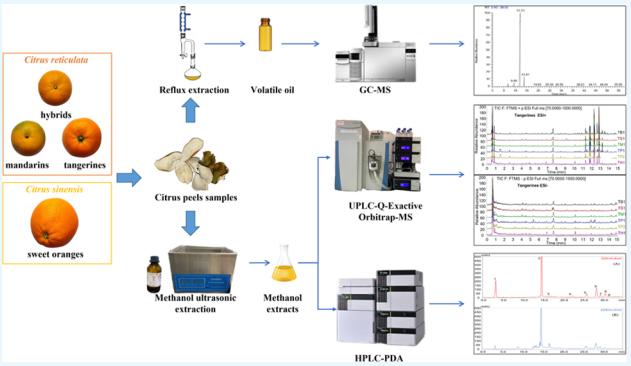
ABSTRACT: The nonvolatile and volatile compounds in the peels of 13 *Citrus reticulata* cultivars (4 mandarins, 5 tangerines, and 4 hybrids) and 5 *Citrus sinensis* (sweet oranges) cultivars were analyzed. Initially, 66 volatile compounds were detected using gas chromatography−mass spectrometry (GC-MS). Tangerines were distinguished from other citrus cultivars (mandarins, sweet oranges, hybrids) by having higher volatile oil extraction rates and higher relative contents of o-Cymene, α-Terpinene, D-α-Pinene, Terpinolene, γ-Terpinene, L-β-Pinene, and 3-Thujene. Additionally, 115 nonvolatile compounds were tentatively identified using ultra-performance liquid chromatography-Q-Exactive Orbitrap tandem mass spectrometry (UPLC-Q-Exactive Orbitrap-MS). *C. sinensis* contained fewer compounds than did *C. reticulata*. Pterostilbene was detected in all tangerines but not in mandarins and hybrids, suggesting its potential as a marker compound for differentiating tangerines from other *C. reticulata*. Lastly, a high-performance liquid chromatography-photodiode array (HPLC-PDA) was used to quantify 9 major nonvolatile components. Heat map and principal component analysis showed that the contents of tangerines differed from other cultivars (sweet oranges, mandarins, and hybrids). It may be caused by the higher content of synephrine, nobiletin, tangeretin, and 5-hydroxy-6,7,8,3′,4′-pentamethoxyflavone in tangerines. The study may obtain information for the application of different types of *C. reticulata* (tangerines, mandarins, or hybrids) and *C. sinensis* peels, thereby promoting their recycling.



1. INTRODUCTION

Citrus fruit crops rank among the world's most widely grown, mainly including loose-skin mandarin (*Citrus reticulata*), lemon (*Citrus limon*), pomelo (*Citrus grandis*), and sweet orange (*Citrus sinensis*). As one of the most popular fruits, citrus pulps are consumed in large quantities, while the peels are often discarded as garbage. Studies have revealed that citrus peels contain many important medicinal secondary metabolites, including essential oils,[1] flavonoids (such as hesperidin[2] and nobiletin[3]), coumarins (such as scoparone[4] and osthole[5]), limonoids (such as limonin[6] and nomilin[7]), and alkaloids (such as synephrine[8]), which have multiple biological activities such as antioxidant,[6] anticancer,[9] anti-inflammatory,[3] antibacterial,[10] hepatoprotective,[1] antidiabetic,[10] and neuroprotection.[11] Therefore, systematic analysis of citrus peel compounds has an important reference value for recycling and utilization.

Different citrus species peels have been examined qualitatively and quantitatively in several studies. Li et al. detected 52 volatile compounds in four *citrus* species using gas chromatography−mass spectrometry (GC-MS) and found that *C. sinensis* and *C. reticulata* could be clustered into one cluster, *Citrus paradisi* and *C. limon* could be clustered into another cluster.[12] By using UHPLC-Q-TOF-MS, Zhao et al. detected 92 compounds in 10 *C. reticulata* cultivars and 6 *C. sinensis* cultivars and found that *C. reticulata* had a higher number and content of compounds than *C. sinensis*.[13] In addition, several studies have investigated the differences in compounds between different cultivars of the same species. Zheng et al. detected 73 compounds in 10 cultivars of *C. reticulata* using UPLC-Q-TOF-MS and screened out 7 compounds to distinguish *C. reticulata* "Chachi" (named "Guangchenpi") from other *C. reticulate* (collectively called "Chenpi").[14] The potential of volatile compounds to differentiate *C. reticulata* "Chachi" from other *C. reticulata* has been explored by Lv et al. using GC-MS.[15]

In China, one of the most important origins of citrus, loose-skin mandarin (*C. reticulata*) includes mandarins, tangerines,

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Table 1. Information of Citrus Peel Samples from 18 Cultivars

ID	cultivar	origin	collection date	extraction rate (%)
O	**Sweet Oranges** (*C. sinensis*)			
OB1	Bingtangcheng	Mayang Miao Autonomous County, Huaihua City, Hunan Province	2024/03/18	5.26
OB2	Bingtangcheng	Mayang Miao Autonomous County, Huaihua City, Hunan Province	2024/03/19	2.50
OJ1	Jincheng	Pengan County, Nanchong City, Sichuan Province	2024/03/19	3.18
ON1	Newhall Navel Orange	Zhijiang City, Yichang City, Hubei Province	2024/03/18	4.31
ON2	Newhall Navel Orange	Fengjie country, Chongqing City, Sichuan province	2024/03/19	3.68
OL1	Lane Late Navel Orange	Yidu City, Yichang City, Hubei Province	2024/03/18	3.50
OT1	Tarrocco Blood Orange	Zizhong County, Neijiang City, Sichuan Province	2024/03/19	2.58
OT2	Tarrocco Blood Orange	Mayang Miao Autonomous County, Huaihua City, Hunan Province	2024/03/19	5.94
M	**Mandarins** (*C. reticulata*)			
MG1	Gonggan	Xingning District, Nanning City, Guangxi Province	2024/03/28	5.43
MH1	Huangguogan	Hanyuan County, Yaan City, Sichuan Province	2024/03/18	5.62
MJ1	Jiaogan	Rongcheng District, Jieyang City, Guangdong Province	2024/03/18	4.57
MO1	Ougan	Longwan District, Wenzhou City, Zhejiang Province	2024/03/19	3.20
T	**Tangerines** (*C. reticulata*)			
TB1	Bendizao	Huangyan District, Taizhou City, Zhejiang Province	2024/03/20	3.00
TS1	Shatangju	Lingchuan County, Guilin City, Guangxi Province	2024/03/17	7.45
TM1	Mashuiju	Lingchuan County, Guilin City, Guangxi Province	2024/03/17	5.60
TP1	Ponkan	Changshan County, Quzhou City, Zhejiang Province	2024/03/18	7.50
TP2	Ponkan	Yongchun County, Quanzhou City, Fujian province	2024/03/18	7.41
TH1	Hongju	Wanzhou District, Chongqing City, Sichuan Province	2024/03/21	3.32
H	**Hybrids** (*C. reticulata*)			
HS1	Shiranui	Pengshan District, Meishan City, Sichuan Province	2024/04/10	1.66
HB1	Beni Madonna	Muyang County, Suqian City, Jiangsu Province	2024/04/08	5.00
HO1	Orah	Binchuan County, Dali Bai Autonomous Prefecture, Yunnan Province	2024/04/15	5.06
HC1	ChunJian	Pujiang County, Chengdu City, Sichuan Province	2024/04/10	5.89

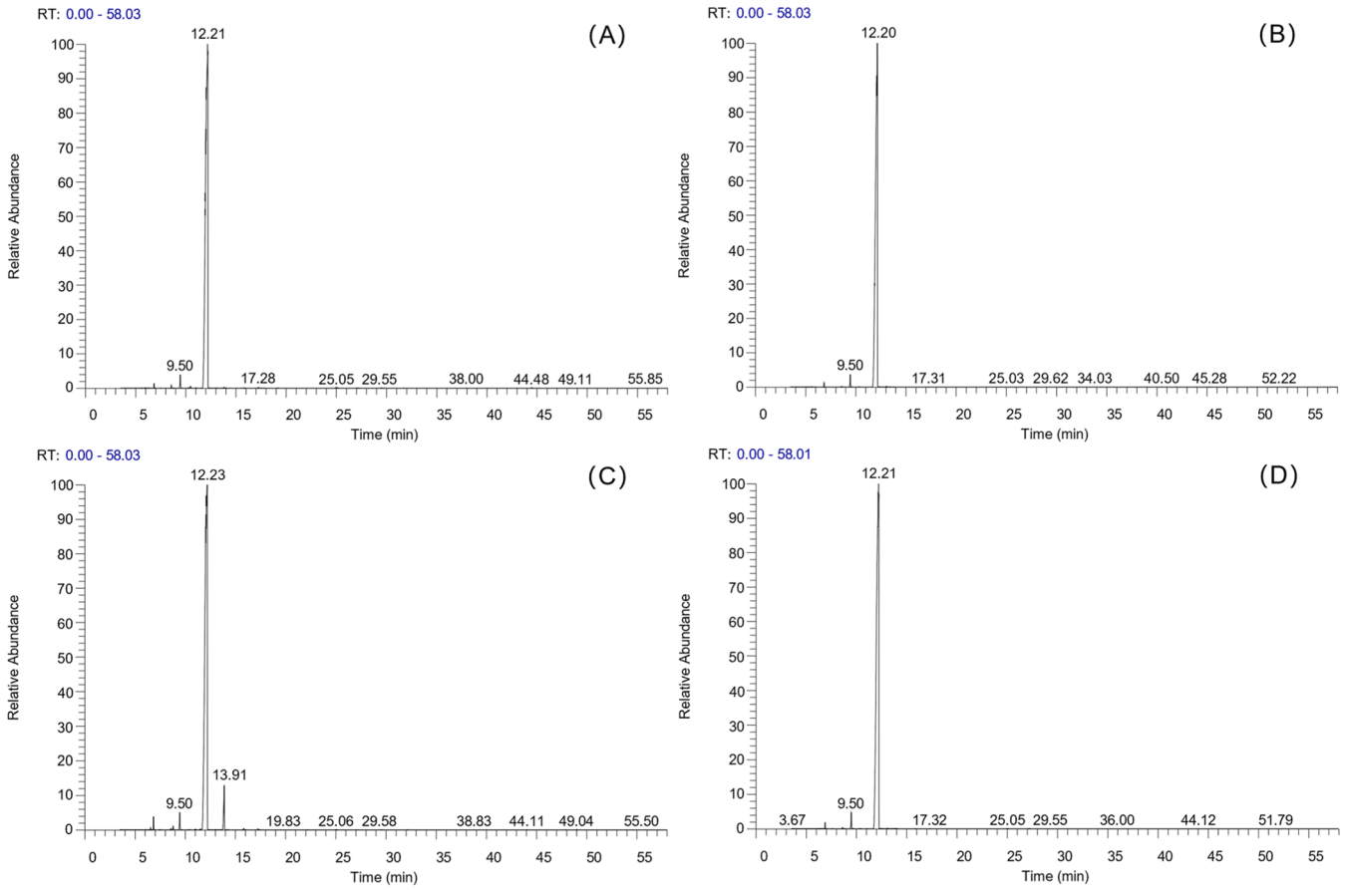

Figure 1. Representative total ion chromatograms of volatile compounds of Citrus peels for (A) sweet oranges (*C. sinensis* 'Newhall Navel Orange'), (B) Mandarins (*C. reticulata* 'Gonggan'), (C) Tangerines (*C. reticulata* 'Ponkan'), and (D) Hybrids (*C. reticulata* 'ChunJian')

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Table 2. Summary of 18 Main Volatile Components in 18 Cultivars Tentatively Identified by GC-MS

					sweet oranges (*C. sinensis*)								mandarins (*C. reticulata*)			
no.	RT (min)	component	CAS no.	type	OB1	OB2	OJ1	ON1	ON2	OL1	OT1	OT2	MG1	MH1	MJ1	MO1
5	6.55	3-thujene	2867-05-2	alkenes	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6	6.85	D-α-pinene	7785-70-8	alkenes	0.59	0.21	0.18	0.25	0.23	0.26	0.26	0.28	0.28	0.20	0.27	0.20
7	8.58	sabinene	3387-41-5	alkenes	0.23	0.06	0.05	0.19	0.17	0.20	0.03	0.05	0.06	0.04	0.02	0.03
8	8.82	L-β-pinene	18172-67-3	alkenes	0.03	0.01	0.01	0.02	0.02	0.02	0.01	0.01	0.01	0.01	0.00	0.00
9	9.50	β-pinene	127-91-3	alkenes	2.50	0.78	0.76	1.03	0.99	0.99	0.95	1.02	1.01	0.93	0.97	0.84
12	10.40	α-phellandrene	99-83-2	alkenes	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.02	0.02	0.02	0.02
13	10.51	3-carene	13466-78-9	alkenes	0.49	0.27	0.08	0.22	0.22	0.29	0.04	0.08	0.00	0.00	0.00	0.00
14	11.05	α-terpinene	99-86-5	alkenes	0.04	0.02	0.01	0.05	0.03	0.04	0.01	0.01	0.00	0.00	0.00	0.00
16	11.56	o-cymene	527-84-4	alkanes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
17	12.20	D-limonene	5989-27-5	alkenes	94.49	98.23	98.51	97.56	97.84	97.75	98.18	98.14	98.45	98.43	98.37	98.56
19	13.11	β-*cis*-ocimene	3338-55-4	alkenes	0.00	0.01	0.01	0.02	0.02	0.02	0.01	0.01	0.06	0.19	0.09	0.18
21	13.84	γ-terpinene	99-85-4	alkenes	0.07	0.04	0.03	0.10	0.06	0.06	0.02	0.07	0.01	0.01	0.02	0.01
24	15.92	terpinolene	586-62-9	alkenes	0.09	0.05	0.02	0.05	0.04	0.05	0.01	0.02	0.00	0.00	0.00	0.00
26	17.32	linalool	78-70-6	others	0.22	0.04	0.05	0.10	0.07	0.06	0.24	0.16	0.01	0.03	0.14	0.04
34	25.07	L-4-terpineol	20126-76-5	others	0.15	0.03	0.03	0.13	0.06	0.08	0.02	0.03	0.01	0.01	0.01	0.01
35	27.15	α-terpineol	98-55-5	others	0.12	0.02	0.02	0.05	0.02	0.02	0.03	0.03	0.00	0.01	0.01	0.01
37	29.53	decanal	112-31-2	aldehydes	0.13	0.01	0.01	0.06	0.02	0.03	0.01	0.01	0.01	0.00	0.00	0.00
60	44.48	valencen	4630-07-3	alkenes	0.28	0.10	0.14	0.03	0.11	0.03	0.08	0.02	0.00	0.00	0.00	0.01

					tangerines (*C. reticulata*)						hybrids (*C. reticulata*)			
no.	RT (min)	component	CAS no.	type	TB1	TS1	TM1	TP1	TP2	TH1	HS1	HB1	HO1	HC1
5	6.55	3-thujene	2867-05-2	alkenes	0.07	0.11	0.00	0.12	0.11	0.10	0.01	0.01	0.00	0.00
6	6.85	D-α-pinene	7785-70-8	alkenes	0.48	0.69	0.37	0.68	0.63	0.66	0.37	0.43	0.42	0.35
7	8.58	sabinene	3387-41-5	alkenes	0.03	0.25	0.13	0.08	0.15	0.08	0.65	0.48	0.06	0.10
8	8.82	L-β-pinene	18172-67-3	alkenes	0.20	0.27	0.02	0.28	0.28	0.28	0.06	0.06	0.01	0.01
9	9.50	β-pinene	127-91-3	alkenes	1.20	1.81	1.13	1.25	1.20	1.84	1.92	1.68	1.75	1.25
12	10.40	α-phellandrene	99-83-2	alkenes	0.04	0.05	0.03	0.04	0.04	0.18	0.05	0.05	0.11	0.02
13	10.51	3-carene	13466-78-9	alkenes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
14	11.05	α-terpinene	99-86-5	alkenes	0.06	0.05	0.02	0.08	0.08	0.07	0.09	0.04	0.01	0.01
16	11.56	o-cymene	527-84-4	alkanes	0.08	0.18	0.00	0.04	0.02	0.02	0.00	0.00	0.00	0.00
17	12.20	D-limonene	5989-27-5	alkenes	93.44	92.66	97.61	92.75	92.71	89.89	93.81	96.21	97.28	98.03
19	13.11	β-*cis*-ocimene	3338-55-4	alkenes	0.45	0.06	0.03	0.02	0.03	0.09	0.68	0.09	0.05	0.05
21	13.84	γ-terpinene	99-85-4	alkenes	3.39	3.07	0.05	4.27	4.36	4.80	0.21	0.09	0.02	0.04
24	15.92	terpinolene	586-62-9	alkenes	0.13	0.13	0.01	0.14	0.14	0.19	0.04	0.02	0.01	0.01
26	17.32	linalool	78-70-6	others	0.05	0.13	0.37	0.12	0.07	0.74	0.08	0.07	0.03	0.01
34	25.07	L-4-terpineol	20126-76-5	others	0.03	0.06	0.05	0.02	0.03	0.07	0.30	0.15	0.02	0.03
35	27.15	α-terpineol	98-55-5	others	0.04	0.03	0.06	0.02	0.01	0.13	0.04	0.03	0.03	0.02
37	29.53	decanal	112-31-2	aldehydes	0.05	0.23	0.01	0.02	0.03	0.17	0.40	0.07	0.09	0.01
60	44.48	valencen	4630-07-3	alkenes	0.00	0.00	0.00	0.00	0.00	0.00	0.18	0.12	0.00	0.00

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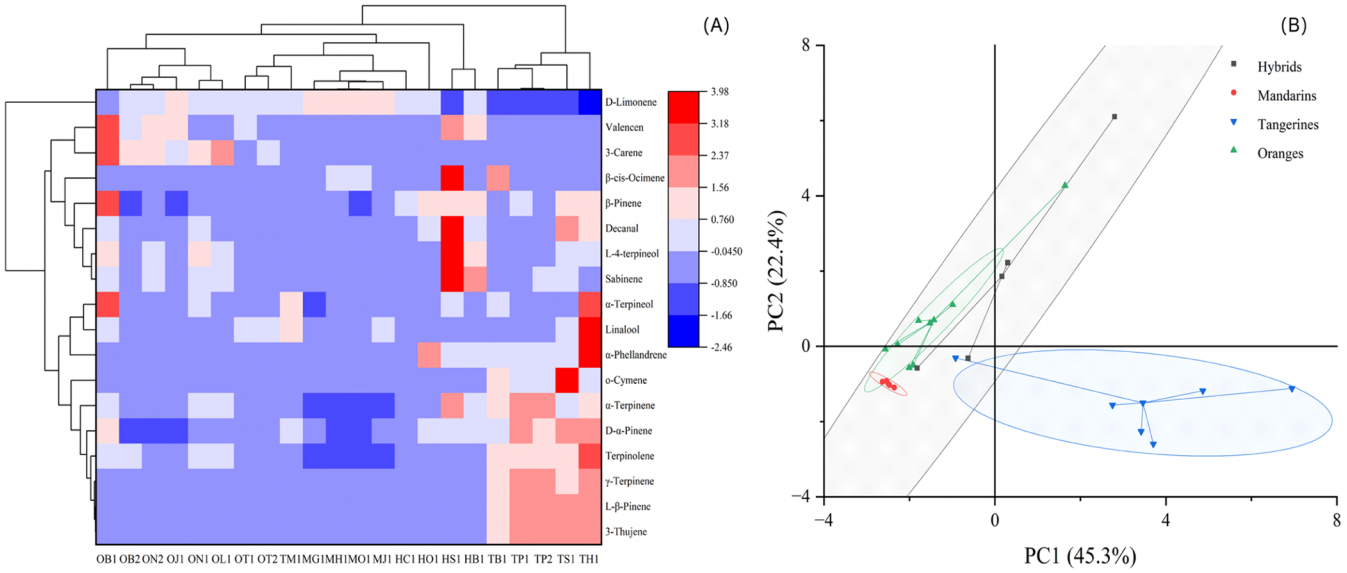

Figure 2. Heat map, dendrogram (A), and PCA scores plot (B) of 18 main volatile compounds in Citrus peels from 18 cultivars.

and hybrids, which are called gan, ju, and zagan in Chinese.[16] In a study published in 2024, Deng et al. used genetic sequencing information to analyze the differences in the genetic compositions of tangerines and mandarins.[17] However, there are still no reports of chemical composition differences of peels between mandarins, tangerines, and hybrids. In this study, 5 cultivars of sweet oranges, 4 cultivars of mandarins, 5 cultivars of tangerines, and 4 cultivars of hybrids were sampled, analyzed for the differences in volatile compounds using GC-MS, and analyzed for the differences in nonvolatile compounds using UHPLC-Q-Exactive Orbitrap-MS and HPLC-PDA, so as to provide information for its resource recycling.

2. RESULTS AND DISCUSSION

2.1. Volatile Compounds from Citrus Peels of 18 Cultivars Analyzed by GC-MS. The extraction rates of volatile substances vary among different citrus peels (Table 1). *C. reticulata* 'ponkan' (TP1) had the highest extraction rate (7.50%), and *C. reticulata* 'Shiranui' (HS1) had the lowest extraction rate (1.66%). Overall, the order of average volatile oil extraction rate from high to low is tangerine (5.71%), mandarin (4.71%), hybrid (4.40%), and sweet orange (3.87%). GC-MS analysis of citrus peels produced representative total ion chromatograms of volatile components in sweet orange, mandarin, tangerine, and the hybrid (Figure 1).

There were 66 volatile compounds tentatively identified in 18 citrus cultivars peels (Supporting Information, Table S1), including 32 alkenes, 4 aldehydes, 2 alkanes, 2 alcohols, 1 ester, 1 ether, 1 ketone, and 23 other compounds. Seven compounds were detected (relative content greater than or equal to 1%) in each citrus cultivar, namely, D-Limonene, γ-Terpinene, D-α-Pinene, Sabinene, β-Pinene, Linalool, and L-4-terpineol. D-Limonene exhibited the highest relative content (89.89−98.56%) among the 18 citrus varieties, and it had many pharmacological activities such as antioxidation, anticancer, antidiabetic, and liver protection.[1]

Eighteen volatile compounds with higher contents (relative contents greater than 4% in 2 or more cultivars) were considered as major volatile compounds (Table 2), and heat map clustering and principal component analysis were used for them. The heat map cluster analysis showed that five out of six tangerines samples were classified into one group, namely, *C. reticulata* 'Bendizao' (TB1), *C. reticulata* 'Ponkan' (TP1 and TP2), *C. reticulata* 'Shatangju' (TS1), and *C. reticulata* 'Hongju' (TH1); the other cultivars were classified into one group (Figure 2A). In other cultivar groups, *C. sinensis* 'Bingtangcheng' (OB1 and OB2), *C. sinensis* 'Newhall Navel Orange' (ON1 and ON2), *C. sinensis* 'Jincheng' (OJ1), and *C. sinensis* 'Lane Late Navel Orange' (OL1) were classified into one subgroup; *C. reticulata* 'Shiranui' (HS1) and *C. reticulata* 'Beni Madonna' (HB1) were classified into one subgroup; the other cultivars were classified into one subgroup. The principal component analysis also obtained similar results, with five out of six tangerines samples located in the fourth quadrant and the remaining samples located in the third, second, and first quadrants (Figure 2B).

Based on the above analysis, except *C. reticulata* 'Mashuiju', the rest of the tangerines were classified into one group, which may be related to the higher relative contents of o-Cymene (0.02−0.18%), α-Terpinene (0.05−0.08%), D-α-Pinene (0.48−0.69%), Terpinolene (0.13−0.19%), γ-Terpinene (3.07−4.80%), L-β-Pinene (0.20−0.28%), and 3-Thujene (0.07−0.12%) in tangerines peels (Table 2). Except for *C. sinensis* 'Tarrocco Blood Orange', other sweet oranges are classified into one group, which might be due to their higher content of Valencen (0.03−0.28%) and 3-Carene (0.08−0.49%) in their peels. Mandarins were grouped into one class in the last level clustering heat map and were also closely distributed in the principal component analysis, which might be due to their higher content of D-Limonene (98.37−98.56%) and the lower content of α-Terpinene (<0.01%), D-α-Pinene (0.20−0.28%), and Terpinolene (<0.01%) in their peels. The volatile compounds contents of hybrids were very different and could not be clustered. The principal component analysis also observed that its distribution range was large. This may be due to its complex parental origin. In addition, *C. sinensis* 'Tarrocco Blood Orange' (OT1 and OT2) and *C. reticulata* "mashuiju" (TM1) were not classified into their categories, suggesting that there were individual differences in individual varieties.

In general, there were differences in the volatile components between tangerines and other citrus cultivars (mandarins, sweet oranges, and hybrids). Tangerines with higher volatile oil

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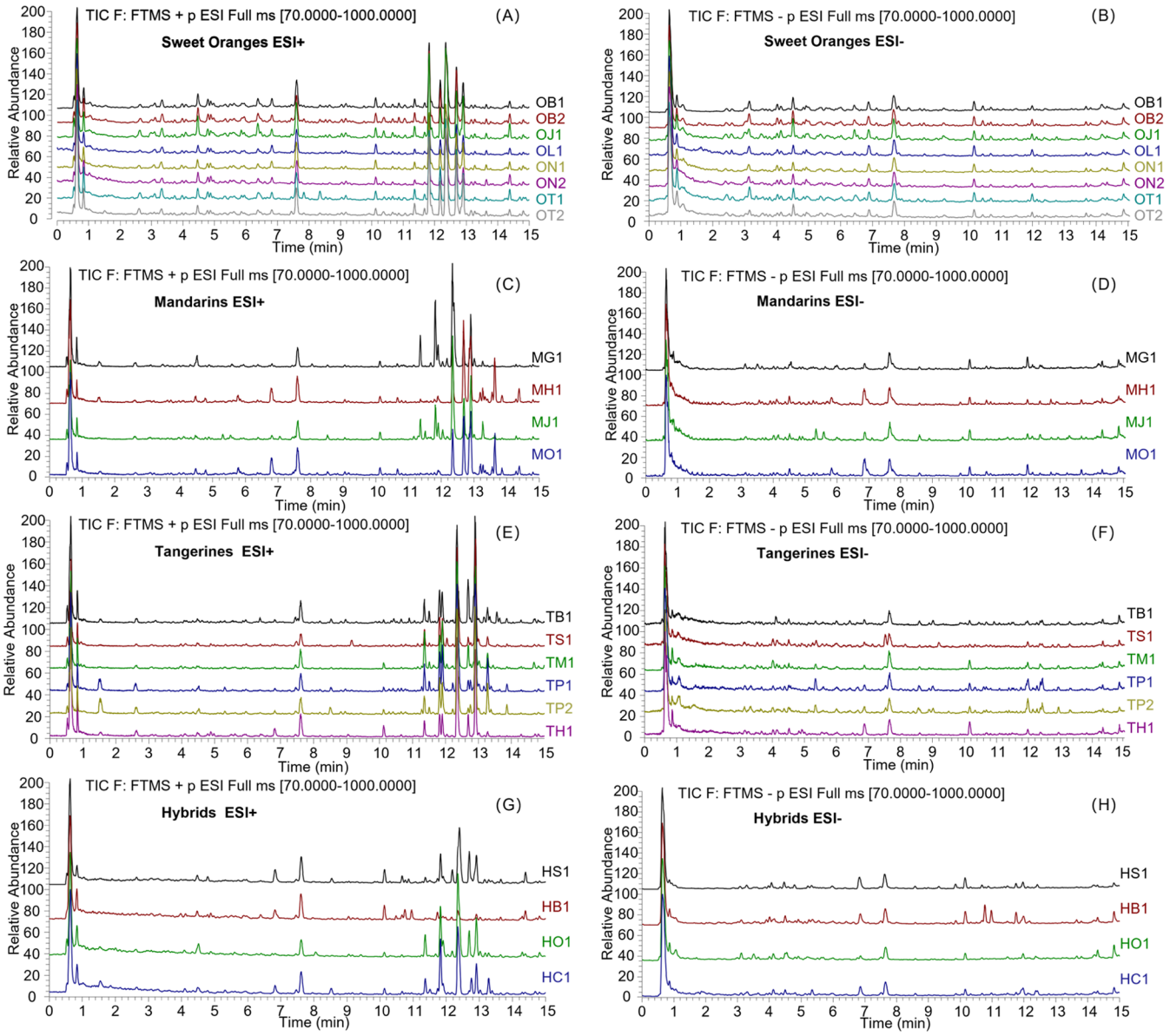

Figure 3. Total ion chromatograms of the nonvolatile compounds of 18 Citrus peels in positive and negative ion modes.

extraction rates and relative contents of o-Cymene, α-Terpinene, D-α-Pinene, Terpinolene, γ-Terpinene, L-β-Pinene, and 3-Thujene. Especially, although both belong to *C. reticulata* Blanco, there is a significant difference between the volatile components of mandarins and tangerines. Tangerines, sweet oranges, and mandarins could be mostly internal clustering of volatile substances, and there were some differences between clusters.

2.2. Nonvolatile Compounds from Citrus Peels of 18 Cultivars Analyzed Qualitatively. Figure 3 presents the total ion chromatograms from UHPLC-Q-Exactive Orbitrap-MS of nonvolatile compounds in the peels of sweet oranges, mandarins, tangerines, and hybrids. Utilizing data derived from fragment ion analysis, retention times, established reference standards, relevant literature, and the Orbitrap Traditional Chinese Medicine Library (OTCML), a total of 115 distinct components have been successfully tentatively identified within the methanol extracts obtained from the peels of 18 citrus varieties, including 15 polymethoxyflavones (PMFs), 13 flavonoid glycosides, 14 other flavonoids, 17 coumarins, 13 terpenoids, 16 organic acids, 3 alkaloids, 8 aldehydes, and 16 other compounds (Table 3). The detailed chemical structures of these tentatively identified components are illustrated in Figure S1 (Supporting Information). Table S2 displays the fragment information and retention time of the compounds that were detected. Nine compounds, including pterostilbene, sibiricose A5, ferulaldehyde, parthenolide, fraxinellone, skimming, 2″-*O*-β-L-galactopyranosylorientin, casticin, and iristectorigenin B, were tentatively identified for the first time in citrus peels.

PMFs, the main cleavage process is the process of losing methoxy or neutral water molecules in the parent nucleus. For example, the molecular formula $C_{21}H_{22}O_8$ was inferred from the m/z 403.13782$[M + H]^+$ of Peak 98. The Major MS2 fragment ions were mainly 388.11417, 373.09076, 355.08002, 327.08521, etc. The fragmentation pathways were that the m/z 403.13782$[M + H]^+$ lost one methoxy neutrally to form a m/z 373.09076, and then lost one molecule of water to form a m/z 355.08002, which continued to lose one carbon oxygen fragments to obtain a m/z 327.08521; the m/z 403.13782[M

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Table 3. Nonvolatile Compound Differences among Citrus Peels from 18 Cultivars

Peak	RT (min)	Component name	Sweet Oranges (*Citrus sinensis*)								Mandarins (*Citrus reticulata*)				Tangerines (*Citrus reticulata*)					Hybrids (*Citrus reticulata*)				Ref
			OB1	OB2	OJ1	ON1	ON2	OL1	OT1	OT2	MG1	MH1	MJ1	MO1	TB1	TS1	TM1	TP1	TP2	TH1	HS1	HB1	HO1	HC1	
PMFs																									
83	10.92	Irigenin													✓	✓	✓	✓	✓			✓		✓	[24,25],*b*
84	10.95	Iristectorigenin B														✓			✓			✓			[26],*b*
85	10.98	Jaceosidin	✓		✓			✓	✓									✓	✓		✓	✓		✓	[27],*b*
89	11.66	5,7,3'-Trihydroxy-6,4',5'-trimethoxyflavone										✓	✓	✓	✓			✓							*b*
91	11.83	Sinensetin	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	[28],*b*
92	11.91	6-Demethoxytangeretin	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	*a*
95	12.01	Pectolinarigenin			✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	[29],*b*
96	12.15	Lysionotin			✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓			✓	✓	✓	[30],*b*
97	12.28	Casticin																				✓			[31],*b*
98	12.35	Nobiletin	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	*a*
99	12.44	Artemetin				✓					✓														[32],*b*
102	12.70	3,5,6,7,8,3',4'-Heptamethoxyflavone	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	*a*
105	12.91	Tangeretin	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	*a*
106	13.02	Gardenin B															✓					✓	✓		[33,34],*b*
108	13.29	5-hydroxy-6,7,8,3',4'-pentamethoxyflavone	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	*a*
Flavonoid glycoside																									
27	3.90	2''-O-β-L-galactopyranosylorientin			✓	✓	✓	✓	✓	✓			✓					✓	✓		✓			✓	[35],*b*
32	4.32	Vicenin-2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	[36],*b*
37	4.74	Isoquercitrin										✓		✓										✓	[37],*b*
38	4.92	Orientin			✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓		✓	✓	✓	✓	[35],*b*
47	5.64	Rutin	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	*a*
48	5.71	Vitexin	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	[35],*b*
49	5.78	Eriocitrin	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	[38],*b*
59	6.82	Narirutin	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	[39],*b*
63	7.11	Iridin	✓	✓		✓			✓													✓			[24],*b*
68	7.45	Diosmin	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	*a*
71	7.60	Hesperidin	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	*a*
73	8.00	Neohesperidin	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓			✓	✓	✓	✓	✓	✓		✓	[40],*a*
76	9.05	Methyl hesperidin	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	*b*
Other flavonoids																									
22	3.40	Taxifolin										✓			✓										[41],*b*
35	4.69	Apigenin																				✓	✓	✓	[42],*b*
36	4.74	Morin	✓		✓	✓	✓	✓	✓	✓	✓	✓		✓	✓					✓	✓	✓	✓	✓	[37],*b*
45	5.50	Isorhamnetin							✓											✓					[43],*b*
50	5.78	Eriodictyol			✓	✓		✓	✓				✓	✓		✓		✓	✓	✓	✓	✓		✓	[44],*b*
51	5.79	Kaempferol										✓													[45],*b*
70	7.60	Hesperetin	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	[46],*b*

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Table 3. continued

Peak	RT (min)	Component name	Sweet Oranges (*Citrus sinensis*)								Mandarins (*Citrus reticulata*)				Tangerines (*Citrus reticulata*)					Hybrids (*Citrus reticulata*)					Ref
			OB1	OB2	OJ1	ON1	ON2	OL1	OT1	OT2	MG1	MH1	MJ1	MO1	TB1	TS1	TM1	TP1	TP2	TH1	HS1	HB1	HO1	HC1	
77	9.97	Pinocembrin	✓	✓	✓	✓		✓	✓	✓		✓	✓	✓			✓		✓	✓	✓	✓		✓	[47],*b*
79	10.12	Isosakuranetin	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	*b*
81	10.53	Naringenin	✓	✓		✓	✓	✓	✓		✓	✓	✓	✓	✓					✓	✓			✓	*a*
82	10.56	Diosmetin										✓		✓	✓			✓	✓						[37,48],*b*
86	11.21	5-Methyl-7-methoxyisoflavone	✓				✓																		[49],*b*
101	12.45	Acacetin																✓	✓			✓			[50],*b*
112	14.23	Kanzonol C									✓														[51],*b*
Coumarins																									
18	2.98	Skimmin									✓							✓	✓	✓			✓	✓	[52],*b*
23	3.44	Isoscopoletin									✓		✓			✓						✓	✓		[18],*b*
25	3.50	Scopolin									✓		✓			✓						✓	✓		*b*
26	3.54	Scopoletin											✓		✓		✓								[18],*b*
29	4.08	Citropten	✓	✓	✓	✓	✓	✓	✓		✓	✓		✓						✓			✓	✓	*b*
40	5.17	7-Hydroxycoumarin																		✓			✓		[53],*b*
41	5.19	7-Methoxy-4-methylcoumarin	✓	✓	✓				✓	✓	✓		✓			✓	✓			✓					*b*
42	5.37	Nodakenin			✓	✓			✓																[54],*b*
56	6.45	Marmesin																					✓		[55],*b*
64	7.29	Fraxinol											✓		✓	✓									*b*
66	7.43	Scoparone											✓			✓	✓	✓	✓		✓	✓			[18],*b*
75	8.50	Isomeranzin									✓										✓				[56],*b*
80	10.18	Isopsoralen																					✓		[57],*b*
87	11.39	Isopimpinellin																					✓		[58],*b*
103	12.75	7-Demethylsuberosin											✓										✓		[55],*b*
111	13.82	Osthole	✓	✓	✓				✓	✓	✓	✓									✓			✓	[19],*b*
113	14.26	Suberosin											✓			✓							✓		[59],*b*
Terpenoids																									
31	4.18	Camphor									✓									✓					[60],*b*
43	5.45	Agnuside									✓		✓				✓			✓			✓		*b*
46	5.53	Curdione								✓															[61],*b*
53	6.08	Atractylenolide II										✓													[62],*b*
58	6.76	Curcumenol	✓																			✓			*b*
61	6.92	Fraxinellone		✓																					[63],*b*
65	7.30	Parthenolide														✓									[64],*b*
78	10.00	Atractyloside A			✓	✓	✓	✓		✓															*b*
88	11.61	Rutaevin																	✓						*b*
90	11.81	Glycyrrhizic acid									✓														[65],*b*
94	11.95	Limonin	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	*a*
100	12.45	Nomilin									✓	✓	✓	✓			✓	✓	✓	✓	✓	✓	✓	✓	*a*
114	14.39	α-Cyperone		✓		✓						✓	✓	✓							✓	✓	✓		[66],*b*
Organic acids																									
5	0.65	Quinic acid		✓	✓	✓		✓																	[67,68],*b*
6	0.84	Nicotinic acid														✓					✓	✓		✓	[8,69],*b*
8	0.85	L-Tyrosine	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	[70],*b*
12	0.86	p-Coumaric acid																			✓				[71],*b*
13	1.02	L-Leucine					✓		✓	✓						✓						✓	✓		[72],*b*

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Table 3. continued

Peak	RT (min)	Component name	Sweet Oranges (*Citrus sinensis*)								Mandarins (*Citrus reticulata*)				Tangerines (*Citrus reticulata*)					Hybrids (*Citrus reticulata*)					Ref
			OB1	OB2	OJ1	ON1	ON2	OL1	OT1	OT2	MG1	MH1	MJ1	MO1	TB1	TS1	TM1	TP1	TP2	TH1	HS1	HB1	HO1	HC1	
15	2.53	Vanillic acid																				✓		✓	*b*
16	2.64	L-Tryptophan	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	[70,73],*b*
17	2.71	Caffeic acid																	✓						[74],*b*
21	3.31	Chlorogenic acid										✓		✓	✓		✓	✓	✓		✓			✓	[74,75],*b*
28	3.97	Isoferulic acid	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	[76],*b*
30	4.09	Sinapic acid	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	[77],*b*
44	5.48	Ferulic acid	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	*a*
55	6.42	Abscisic acid			✓	✓		✓	✓	✓	✓		✓	✓	✓	✓		✓	✓						[78],*b*
67	7.44	Azelaic acid		✓																					*b*
69	7.54	Isochlorogenic acid C															✓	✓	✓		✓			✓	[79],*b*
115	14.82	α-Linolenic acid		✓		✓	✓		✓	✓												✓	✓	✓	*b*
Alkaloids																									
2	0.63	Stachydrine	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	*a*
4	0.65	Synephrine	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	*a*
52	6.02	6-Hydroxyindole								✓															*b*
Aldehydes																									
1	0.61	5-Hydroxymethylfurfural						✓				✓			✓						✓				[80],*b*
24	3.48	Asarylaldehyde			✓						✓	✓		✓	✓	✓	✓		✓	✓					*b*
34	4.68	Vanillin	✓	✓			✓	✓	✓		✓	✓			✓			✓	✓	✓	✓				[20],*b*
39	5.15	Syringaldehyde									✓														*b*
93	11.91	Cinnamaldehyde																		✓					[81],*b*
104	12.85	Isovanillin														✓						✓			*b*
109	13.30	Ferulaldehyde	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓		✓				✓	✓	✓	✓		[82],*b*
110	13.42	p-Hydroxybenzaldehyde								✓					✓	✓	✓	✓	✓						*b*
Others																									
3	0.63	Sucrose			✓	✓	✓	✓				✓	✓	✓											[83–85],*b*
7	0.84	Adenosine										✓					✓	✓	✓		✓			✓	[86],*b*
9	0.85	Nicotinamide										✓		✓	✓										[87,88],*b*
10	0.86	Guanine	✓	✓			✓	✓	✓	✓											✓	✓		✓	[89],*b*
11	0.86	Guanosine	✓	✓			✓											✓		✓	✓	✓		✓	[89],*b*
14	1.08	Acetophenone	✓	✓			✓		✓						✓					✓					[90],*b*
19	3.09	Coniferin			✓	✓	✓	✓			✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓		[91],*b*
20	3.31	Perillene										✓													[92],*b*
33	4.45	Sibiricose A5									✓														[93],*b*
54	6.38	Butylphthalide		✓					✓			✓		✓				✓							[94],*b*
57	6.59	Adamantanone																		✓					*b*
60	6.93	Emodin									✓											✓			*b*
62	6.99	Pinoresinol 4-O-glucoside			✓	✓	✓	✓	✓		✓	✓	✓	✓		✓	✓			✓	✓	✓	✓		*b*
72	7.92	α-Asarone									✓												✓		*b*
74	8.04	Aurantio-obtusin β-D-glucoside																				✓			*b*
107	13.11	Pterostilbene													✓	✓	✓	✓	✓	✓					[21],*b*

[a]Confirmation in comparison with standard substances. [b]Refers to the database. The retention time and fragment information on the detected compounds are shown in Supporting Information Table S2.

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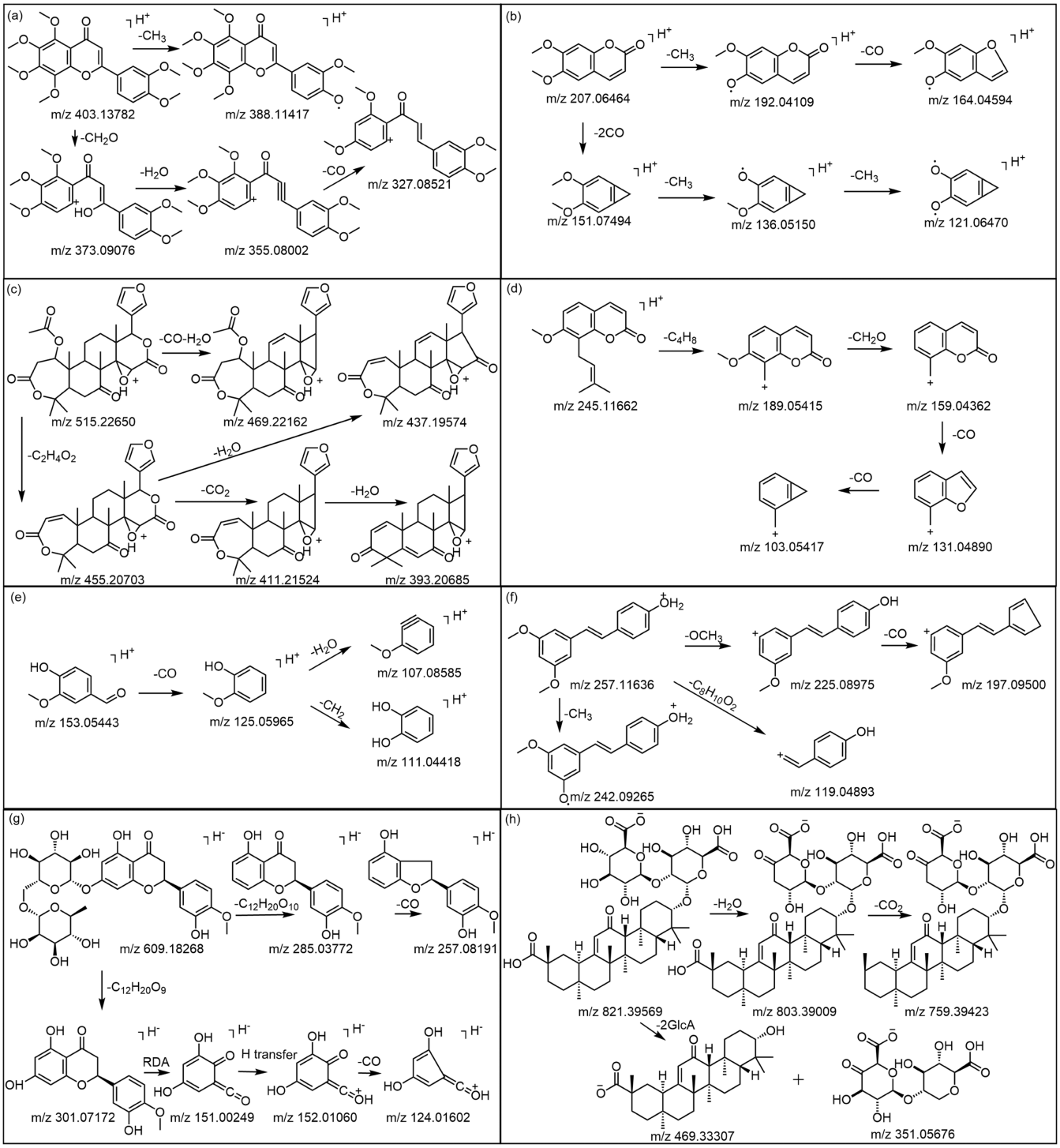

Figure 4. Detail fragmentation pathways of 8 compounds (a) nobiletin, (b) scoparone, (c) nomilin, (d) osthole, (e) vanillin, (f) pterostilbene, (g) hesperidin, and (h) glycyrrhizic acid.

+ H]$^+$ lost one methyl to form a m/z 388.11417. After verification by the reference substance, peak 98 was identified as nobiletin. Its possible fragmentation mechanism is shown in Figure 4a.

Flavonoid glycosides respond better in negative ion mode, and obvious [M − H]$^-$ can be observed. The glycosyl is often removed to obtain flavonoid aglycones, and fragment ions that lose neutral CO can also be observed. In addition, Retro Diels−Alder (RDA) cleavage is also the main mode of cleavage of this type of compound. The m/z of [M − H]$^-$ of Peak 71 was 609.18268 and the molecular formula was inferred to be $C_{28}H_{34}O_{15}$. Major fragments m/z 301.07172 were judged to be the deglycosylation product. M/z 151.00249 was A typical flavonoid RDA cleavage and contains two hydroxyl substitutions in the A ring. M/z 152.01060, m/z 124.01602 were the products of the hydrogen transfer process and further loss of CO, which are the characteristic cracking reactions of the flavonoid A ring. M/z 285.03772 was another deglycosylation process of flavonoid glycosides, and m/z 257.08191 was the result of preion CO loss. Based on the above fragment

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Table 4. Determination of Nine Main Nonvolatile Components in 18 Cultivars by HPLC-PDA[a]

	HPLC-PDA (mg·g^{-1})								
ID	synephrine	hesperidin	ferulic acid	limonin	sinensetin	nobiletin	HMF	tangeretin	5-HPMF
OB1	1.302 ± 0.005^{n}	32.041 ± 0.113^{k}	0.348 ± 0.001^{c}	0.246 ± 0.013^{klm}	0.818 ± 0.004^{e}	1.309 ± 0.006^{m}	0.632 ± 0.004^{h}	0.216 ± 0.001^{opq}	0.071 ± 0.001^{n}
OB2	1.583 ± 0.005^{l}	39.620 ± 0.453^{e}	0.324 ± 0.004^{e}	0.433 ± 0.010^{h}	0.980 ± 0.007^{c}	1.540 ± 0.010^{k}	0.897 ± 0.002^{e}	0.229 ± 0.001^{nop}	0.093 ± 0.001^{j}
OJ1	0.947 ± 0.011^{r}	35.395 ± 0.250^{g}	0.130 ± 0.001^{n}	0.341 ± 0.020^{ij}	0.752 ± 0.004^{g}	1.374 ± 0.008^{l}	0.501 ± 0.003^{j}	0.247 ± 0.002^{no}	0.074 ± 0.001^{mn}
ON1	1.004 ± 0.006^{q}	27.335 ± 0.174^{n}	0.252 ± 0.002^{h}	0.301 ± 0.008^{ijk}	0.581 ± 0.006^{j}	1.131 ± 0.017^{o}	0.494 ± 0.010^{j}	0.204 ± 0.003^{pq}	0.084 ± 0.001^{k}
ON2	0.840 ± 0.002^{s}	23.144 ± 0.025^{q}	0.381 ± 0.001^{a}	0.208 ± 0.022^{m}	0.353 ± 0.001^{o}	0.677 ± 0.001^{r}	0.409 ± 0.001^{l}	0.135 ± 0.001^{r}	0.071 ± 0.001^{n}
OL1	1.256 ± 0.006^{o}	36.010 ± 0.236^{f}	0.268 ± 0.001^{g}	0.524 ± 0.004^{g}	0.494 ± 0.003^{m}	0.995 ± 0.003^{p}	0.505 ± 0.001^{j}	0.188 ± 0.001^{q}	0.082 ± 0.001^{kl}
OT1	2.262 ± 0.011^{j}	34.092 ± 0.056^{i}	0.116 ± 0.001^{o}	0.449 ± 0.014^{h}	0.788 ± 0.002^{f}	1.217 ± 0.003^{n}	0.653 ± 0.005^{g}	0.208 ± 0.002^{pq}	0.073 ± 0.002^{mn}
OT2	1.504 ± 0.002^{m}	31.754 ± 0.062^{k}	0.148 ± 0.001^{m}	0.218 ± 0.008^{lm}	0.997 ± 0.002^{b}	1.970 ± 0.005^{j}	0.980 ± 0.003^{d}	0.347 ± 0.002^{l}	0.098 ± 0.002^{j}
MG1	4.383 ± 0.003^{b}	30.775 ± 0.029^{l}	0.215 ± 0.001^{j}	0.843 ± 0.010^{d}	1.392 ± 0.001^{a}	4.214 ± 0.005^{f}	0.608 ± 0.007^{i}	0.896 ± 0.006^{j}	0.139 ± 0.001^{i}
MH1	1.070 ± 0.004^{p}	39.154 ± 0.093^{e}	0.117 ± 0.001^{o}	0.128 ± 0.024^{n}	0.072 ± 0.001^{r}	0.754 ± 0.002^{q}	1.861 ± 0.003^{a}	1.452 ± 0.003^{g}	0.354 ± 0.002^{e}
MJ1	1.719 ± 0.006^{k}	25.469 ± 0.020^{o}	0.177 ± 0.001^{l}	0.724 ± 0.023^{ef}	0.520 ± 0.001^{l}	5.799 ± 0.008^{e}	1.304 ± 0.002^{c}	1.401 ± 0.002^{h}	0.429 ± 0.002^{c}
MO1	0.787 ± 0.005^{t}	34.772 ± 0.014^{h}	0.077 ± 0.001^{q}	0.756 ± 0.007^{e}	0.042 ± 0.001^{s}	0.506 ± 0.002^{s}	1.339 ± 0.001^{b}	0.990 ± 0.002^{i}	0.283 ± 0.001^{g}
TB1	2.977 ± 0.023^{e}	43.578 ± 0.271^{b}	0.097 ± 0.003^{p}	0.354 ± 0.006^{i}	0.438 ± 0.011^{n}	2.450 ± 0.016^{i}	0.707 ± 0.010^{f}	2.202 ± 0.017^{e}	0.289 ± 0.002^{g}
TS1	2.649 ± 0.022^{f}	33.247 ± 0.234^{j}	0.248 ± 0.002^{h}	0.660 ± 0.019^{f}	0.551 ± 0.004^{k}	6.365 ± 0.048^{d}	0.140 ± 0.001^{p}	5.660 ± 0.043^{b}	0.266 ± 0.003^{h}
TM1	4.145 ± 0.010^{c}	41.375 ± 0.104^{d}	0.240 ± 0.001^{i}	0.309 ± 0.021^{ijk}	0.512 ± 0.004^{l}	6.629 ± 0.017^{c}	0.366 ± 0.006^{m}	3.508 ± 0.010^{d}	0.413 ± 0.001^{d}
TP1	4.004 ± 0.017^{d}	42.602 ± 0.170^{c}	0.133 ± 0.002^{n}	0.865 ± 0.022^{d}	0.664 ± 0.004^{h}	7.990 ± 0.027^{a}	0.350 ± 0.003^{n}	5.877 ± 0.024^{a}	1.643 ± 0.005^{b}
TP2	4.580 ± 0.017^{a}	64.923 ± 0.092^{a}	0.145 ± 0.002^{m}	1.790 ± 0.019^{b}	0.600 ± 0.006^{i}	7.770 ± 0.006^{b}	0.360 ± 0.002^{mn}	5.484 ± 0.003^{c}	1.784 ± 0.004^{a}
TH1	4.012 ± 0.012^{d}	39.180 ± 0.096^{e}	0.335 ± 0.001^{d}	0.282 ± 0.013^{jkl}	0.212 ± 0.003^{p}	2.713 ± 0.005^{g}	0.230 ± 0.001^{o}	1.568 ± 0.003^{f}	0.079 ± 0.001^{klm}
HS1	0.721 ± 0.001^{u}	24.326 ± 0.057^{p}	0.201 ± 0.001^{k}	0.279 ± 0.006^{jkl}	0.350 ± 0.001^{o}	1.019 ± 0.005^{p}	0.459 ± 0.002^{k}	0.266 ± 0.002^{mn}	0.095 ± 0.001^{j}
HB1	2.358 ± 0.007^{i}	31.045 ± 0.183^{l}	0.371 ± 0.001^{b}	6.648 ± 0.060^{a}	0.122 ± 0.002^{q}	0.125 ± 0.001^{t}	0.022 ± 0.001^{q}	0.090 ± 0.001^{s}	0.035 ± 0.001^{o}
HO1	2.567 ± 0.016^{g}	14.112 ± 0.046^{r}	0.287 ± 0.003^{f}	0.256 ± 0.009^{klm}	0.833 ± 0.005^{d}	2.533 ± 0.008^{h}	0.367 ± 0.002^{m}	0.511 ± 0.002^{k}	0.076 ± 0.001^{lmn}
HC1	2.483 ± 0.015^{h}	28.221 ± 0.042^{m}	0.251 ± 0.001^{h}	0.935 ± 0.037^{c}	0.812 ± 0.003^{e}	1.512 ± 0.004^{k}	0.373 ± 0.003^{m}	0.293 ± 0.001^{m}	0.346 ± 0.001^{f}

[a]Different letters indicate significant differences ($P < 0.05$). Data are presented as mean ± standard deviation ($n = 3$). HMF, 3,5,6,7,8,3′,4′-heptamethoxyflavone; 5-HPMF, 5-hydroxy-6,7,8,3′,4′-pentamethoxyflavone.

information and reference substance, the substance was identified as hesperidin, and its possible cleavage pathway is shown in Figure 4g.

Limonoids in plants are a class of highly oxidized tetracyclic triterpenoid metabolites; the main lost neutral fragments are H_2O, CO, CO_2, and CH_3COOH. For example, the m/z of $[M + H]^+$ of Peak 100 was 515.22650 and the molecular formula was inferred to be $C_{28}H_{34}O_9$. Its $[M + H]^+$ lost CO and H_2O to form m/z = 469.22162. It could also form m/z 455.20703 by losing CH_3COOH via the McLafferty rearrangement. Then, it lost H_2O, CO_2, and so on in its lactone ring. Based on the above fragments and standards, the substance was identified as nomilin, and the possible cleavage pathways are shown in Figure 4c.

Coumarin is a characteristic component of Umbelliferae plants, and the continuous loss of neutral CO is its main mass spectrometric feature. For example, the molecular formula $C_{11}H_{10}O_4$ was inferred from the m/z 207.06464$[M + H]^+$ of Peak 66. M/z 192.04109 and m/z 164.04594 were believed to be the products of methyl loss and further loss of CO. The m/z 151.07494, 136.05150, and 121.06470 were believed to be the product of m/z 207.06464$[M + H]^+$ losing 2CO on the lactone ring and then losing the methyl groups at the 6 and 7 positions. Combined with the above fragments and references,[18] the substance was tentatively identified as scoparone, and the possible cleavage pathway is shown in Figure 4b. The molecular formula $C_{15}H_{16}O_3$ was inferred from the m/z = 245.11662$[M + H]^+$ of Peak 111. M/z 189.05415 and m/z 159.04362 were inferred to be the product of the removal of the substituent at position 8 and the subsequent removal of the methoxy group at position 7. M/z 131.04890 and m/z 103.05417 were inferred to be the products of the continuous loss of CO from the precursor ion. The fragmentation information was consistent with the relevant literature,[19] Peak 111 was tentatively identified as osthole, and the possible fragmentation pathway is shown in Figure 4d.

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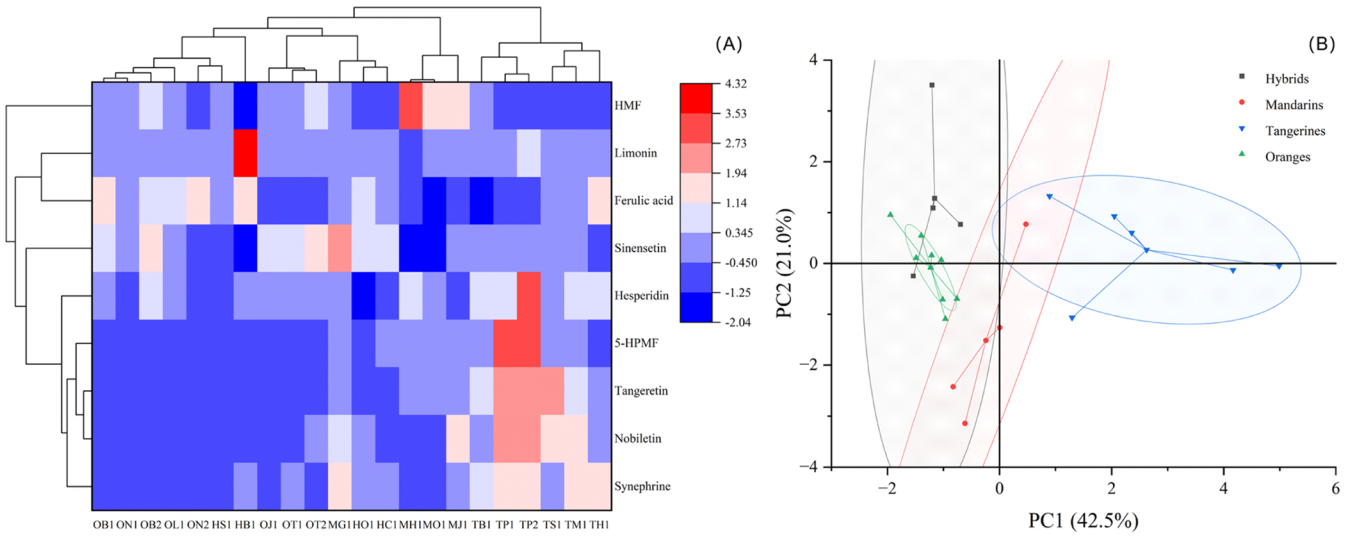

Figure 5. Heat map, dendrogram (A) and PCA scores plot (B) of 9 main nonvolatile compounds in Citrus peels from 18 cultivars. HMF: 3,5,6,7,8,3′,4′-Heptemthoxyflavone, 5-HPMF: 5-hydroxy-6,7,8,3′,4′-pentamethoxyflavone.

The parent ions of aromatic aldehyde compounds show a high-intensity response value. If the *ortho*-substituent is a hydroxyl group or a methoxy group, the dehydrated fragment ions can be further generated. For example, Peak 34 exhibited the peak of $[M + H]^+$ at *m/z* 153.05443, its characteristic fragments involved *m/z* 125.05965 $[M + H-CO]^+$, 111.04418 $[M + H-CO-CH_2]^+$, 107.08585$[M + H-CO-H_2O]^+$ and 93.03381$[M + H-CO-CH_3-OH]^+$. Based on existing study,[20] it was tentatively identified as vanillin, and its possible detailed fragmentation mode is shown in Figure 4e.

The identification of other compounds was based on their precise *m/z* values, fragments of MS/MS, relevant literature, and the data from the OCTML databases. For example, Peak 107 had a $[M + H]^+$ of 257.11636, while its major MS2 fragment ions were mainly *m/z* 242.09265, 225.08975, 197.09500, and 119.04893, which was in agreement with the literature[21] and was tentatively identified as pterostilbene. Its possible cleavage pathway is shown in Figure 4f.

In general, 20 compounds were detected in each citrus cultivars, namely, sinensetin, 6-demethoxytangeretin, nobiletin, Tangeretin, 3,5,6,7,8,3′,4′-heptemthoxyflavone, 5-hydroxy-6,7,8,3′,4′-pentamethoxyflavone, vicenin-2, rutin, vitexin, eriocitrin, narirutin, diosmin, hesperidin, hesperetin, isosakuranetin, limonin, L-tryptophan, sinapic acid, ferulic acid, and synephrine (Table 3). Among these, 15 compounds were flavonoids, which exhibit a range of biological activities, including antioxidant, antibacterial, anti-inflammatory, and anticancer effects, among others.[10]

There were differences in the compounds of sweet oranges, mandarins, tangerines, and hybrids. Regarding the overall count of recognized compounds, tangerines (79) were the most abundant, followed by hybrids (78), mandarins (75), and sweet oranges (65). When it comes to the average number of compounds identified per sample, hybrids (average 51, range 49−54) contained the most compounds, followed by mandarins (average 50, range 47−53), tangerines (average 47, range 43−50), and sweet oranges (average 44, range 40−49). Overall, *C. sinensis* (sweet oranges) contained fewer compounds than did *C. reticulata* (tangerines, mandarins, or hybrids), similar results were obtained in a previous study.[13] This may be attributed to the fact that there are a number of flavonoids and coumarins in *C. sinensis* was less than that in *C. reticulata*. Since they all belong to *C. reticulata*, the types and quantities of compounds among tangerines, mandarins, and hybrids were very similar. However, pterostilbene was detected in all tangerines but not in mandarins and hybrids, suggesting its potential as a marker compound for differentiating tangerines from other *C. reticulata*. It surpasses resveratrol in alleviating inflammation and has multiple biological activities, including anticancer, antioxidant, lipid-lowering, antidiabetic, and neuroprotective effects.[22]

2.3. Quantification of 9 Major Nonvolatile Components in 18 Citrus Cultivars Peels. In this study, the HPLC-PDA external standard method was used to determine 9 main nonvolatile components in peels of sweet oranges, mandarins, tangerines, and hybrids. The chromatogram is shown in Figure S2 (Supporting Information). Table S3 (Supporting Information) presents the calibration curves along with the correlation coefficients (R^2) for synephrine, hesperidin, ferulic acid, limonin, sinensetin, nobiletin, tangeretin, 3,5,6,7,8,3′,4′-heptemthoxyflavone (HMF), and 5-hydroxy-6,7,8,3′,4′-pentamethoxyflavone (5-HPMF). The linearity was excellent across the tested concentration range, with all R^2 values exceeding 0.999. A signal-to-noise ratio (S/N) of 3 was established as the limit of detection (LOD), while a ratio of 10 was set as the limit of quantification (LOQ). The relative standard deviations of precision (0.50−0.71%), repeatability (0.26−2.49%), stability (0.20−2.53%), and recovery (0.25−2.87%) of all standards were lower than 3.00%. The average extraction recovery rate was 90.91−100.35%. Therefore, this method showed good feasibility and reliability.

Table 4 presents the results of the quantitative analysis of nine nonvolatile compounds found in citrus peels. Hesperidin had the highest content among all samples, ranging from 14.112 to 64.923 mg·g^{-1}. The content order of the remaining 8 compounds varied from cultivars to cultivars. Generally, they were as follows from high to low: nobiletin (0.125−7.990 mg·g^{-1}), synephrine (0.721−4.580 mg·g^{-1}), tangeretin (0.090−5.877 mg·g^{-1}), HMF (0.022−1.861 mg·g^{-1}), sinensetin (0.42−1.392 mg·g^{-1}), limonin (0.128−6.648 mg·g^{-1}), 5-HPMF (0.035−1.784 mg·g^{-1}), and ferulic acid (0.077−0.381 mg·g^{-1}). Research indicates that flavones derived from citrus

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fruits possess various biological activities, including anti-inflammatory and antioxidant effects.[10] Reports suggest that synephrine demonstrates anticancer properties and could serve as a framework for developing a new class of nonsteroidal selective glucocorticoid receptor agonists.[9] Additionally, limonin shows promise for neuroprotection and anti-inflammatory benefits in the treatment of Parkinson's and Alzheimer's diseases.[11]

The content of the above 9 nonvolatile components differed among peels of sweet oranges, mandarins, tangerines, and hybrids. The analysis of the heat map indicated that the samples were categorized into two primary clusters, one cluster was tangerines (TB1, TP1, TP2, TS1, TM1, and TH1), and the other cluster was other cultivars (Figure 5A). Similar results were observed in the principal component analysis diagram, with tangerines in the first and fourth quadrants and the rest in the second and third quadrants (Figure 5B). It may be caused by the higher content of synephrine, nobiletin, tangeretin, and 5-HPMF in tangerines. In the other cultivars, three-quarters of the mandarins (MH1, MJ1, and MO1) were clustered in one cluster, and the rest were clustered in another cluster (Figure 5A). From the principal component analysis diagram, the confidence ellipses of mandarins and the other samples (sweet oranges and hybrids) did not coincide completely (Figure 5B). This may be attributed to mandarins' higher HMF content and lower ferulic acid content. In short, the contents of the 9 compounds in tangerines differed from other cultivars (sweet oranges, mandarins, and hybrids). Except for *C. reticulata* 'Gonggan' (MG1), there were certain differences in the contents of the 9 compounds between mandarins and the remaining varieties (sweet oranges and hybrids).

3. CONCLUSIONS

The study involving 22 batches of citrus peel samples collected from 19 prominent origins in China, nonvolatile and volatile components, was performed across 18 distinct cultivars, which comprised 5 cultivars of sweet oranges, 4 cultivars of mandarins, 5 cultivars of tangerines, and 4 cultivars of hybrids. 66 volatile compounds were tentatively identified using GC-MS. The volatile components exhibited variations between tangerines and other citrus varieties (mandarins, sweet oranges, and hybrids), tangerines with higher volatile oil extraction rates, and relative contents of o-Cymene, α-Terpinene, D-α-Pinene, Terpinolene, γ-Terpinene, L-β-Pinene, and 3-Thujene.

115 nonvolatile compounds were detected through UHPLC-Q-Exactive Orbitrap-MS. *C. sinensis* (sweet oranges) contained fewer compounds than *C. reticulata* (tangerines, mandarins, or hybrids). The types and quantities of compounds among tangerines, mandarins, and hybrids were very similar. Pterostilbene was detected in all tangerines, but not in mandarins and hybrids, suggesting its potential as a marker compound for differentiating tangerines from other *C. reticulata*. In addition, HPLC-PDA was used to quantify 9 major nonvolatile components. Heat map and principal component analysis showed the contents of tangerines differed from other cultivars (sweet oranges, mandarins, and hybrids). It may be caused by the higher content of synephrine, nobiletin, tangeretin, and 5-hydroxy-6,7,8,3′,4′-pentamethoxyflavone in tangerines. It is worth noting that according to the findings from GC-MS and HPLC-PDA, tangerines can be distinguished from other *C. reticulata* (mandarins or hybrids). The study may obtain certain reference information for the application of *C. sinensis* (sweet oranges) peels and different types of *C. reticulata* (tangerines, mandarins, or hybrids) peels, thereby promoting their recycling.

4. MATERIALS AND METHODS

4.1. Reagents and Materials. Samples of citrus peel were gathered in 22 batches from 18 different cultivars sourced from 19 primary regions across China (Table 1), including 5 cultivars of sweet oranges, 4 cultivars of mandarins, 5 cultivars of tangerines, and 4 cultivars of hybrids. All of these samples were identified by Prof. Guodong Zheng and preserved at the Pharmacognostic Laboratory, which is located at the Guangzhou Medical University in Guangdong Province, China.

The standards listed below (with a purity of at least 98%) were obtained from Sichuan Weikeqi Biotechnology: stachydrine, synephrine, rutin, diosmin, hesperidin, neohesperidin, naringenin, Sinensetin, 6-demethoxytangeretin, limonin, nobiletin, nomilin, tangeretin, and 3,5,6,7,8,3′,4′-heptemthoxyflavone. The remaining standards (with a purity of at least 98%) indicated that 5-hydroxy-6,7,8,3′,4′-pentamethoxyflavone was acquired from Nanjing Chunqiu Biotechnology, while ferulic acid was sourced from Chengdu Must Biotechnology. Water was purified with a Milli-Q system provided by Merck Millipore. Merck supplied chromatographically grade methanol, formic acid, and hexane. Phosphoric acid and acetonitrile were sourced from Tianjin Damao Chemicals Reagent Factory and Thermo Fisher Scientific, respectively.

4.2. Preparation of Samples and Standards. A suitable quantity of 15 reference materials was measured and subsequently dissolved in methanol to create a mixed reference solution containing 0.2 mg·ml^{-1} of each reference substance for UPLC-Q-Exactive orbitrap-MS analysis. Additionally, a mixed reference solution comprising 9 standard compounds (namely, synephrine, hesperidin, ferulic acid, limonin, sinensetin, nobiletin, tangeretin, 3,5,6,7,8,3′,4′-heptamethoxyflavone, and 5-hydroxy-6,7,8,3′,4′-pentamethoxyflavone) was prepared and subsequently diluted to suitable concentrations in methanol for HPLC-PDA analysis.

Citrus samples were washed with distilled water and peeled. After natural sun drying for about 2 weeks to a constant weight, the material is ground and then sieved through a 40-mesh screen to produce a fine powder for further analysis.

100 g of fine powder was used to extract volatile components from each sample. It was transferred into a 2-L round-bottom distillation flask, where it was immersed in 1.5 L of distilled water for 12 h. The mixture was then heated to reflux for about 1.5 h, and the extract was collected. The content of volatile compounds (%) is determined by dividing the weight of the extracted volatile oil by the weight of the sample powder. An aliquot of 100 μL of the extract was mixed with 900 μL of hexane, subsequently filtered using 0.22 μm PTFE membrane, and thereafter employed for GC-MS analysis.

A 0.2 g sample of powder was loaded into a 50 mL conical flask, followed by the addition of 7 mL of methanol. The mixture underwent ultrasonication at 320 W (40 kHz) for a duration of 30 min using a KQ-800 KDE instrument from Kunshan Ultrasonic Instruments Co. Ltd. Subsequently, the solution was filtered, and the resulting filtrate was transferred to an additional 50 mL conical flask. The residue was subjected to a second extraction with 7 mL of methanol, and this extraction process was carried out one more time. The

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gathered filtrates were united in a conical flask and then filtered using 0.22 μm PTFE membrane in anticipation of UPLC-Q-Exactive orbitrap-MS analysis.

Approximately 0.3 g of sample powder was precisely measured and transferred to a stoppered conical flask. Subsequently, 20 mL of methanol was added to the flask, which was then securely sealed, and the combined weight of the flask and its contents was recorded. Ultrasonic extraction was conducted at a power of 320 W (40 kHz) for a duration of 60 min. Following the extraction, methanol was used to compensate for the lost mass. The filtrate was subsequently filtered using a 0.22 μm PTFE membrane for HPLC-PDA analysis.

4.3. Conditions of GC−MS Analysis. GC-MS was composed of the following equipment, including a DB-5MS ultrainert capillary chromatographic column (30 m × 0.25 mm × 0.25 μm), the Agilent 7890A gas chromatograph system, and a 5975C Triple-Axis mass selective detector from Agilent. The operating conditions were as follows: high-purity helium serving as the carrier gas; the injection port temperature was maintained at a steady 270 °C; the injection volume remained at a precise 1 μL; the column flow rate was carefully controlled at 1 mL·min^{-1}; split ratio of 10:1. The mass spectrometry conditions and temperature programming mirrored those previously established by research team.[23]

4.4. Conditions of UHPLC-Q-Exactive Orbitrap-MS System. An ACQUITY UPLC BEH C18 column (2.1 mm × 100 mm, 1.7 μm) was used for component separation, with 0.1% formic acid aqueous solution (phase A) and acetonitrile (phase B) as the mobile phases, a flow rate of 0.4 mL·min^{-1}, and a constant temperature of 40 °C. The gradient elution protocol was as follows: 5−15%(0−3 min) B, 15−25%(3−8 min) B, 25−35%(8−9 min) B, 35−85%(9−15 min) B. The sample and standard solution were both injected at 2.0 μL. For data acquisition, the mass spectrometer operated in both positive and negative ionization modes using full-scan mode. The analysis conditions included heated electrospray ionization source (HESI-II), spray voltage of 3.5 kV, capillary temperature of 320 °C, nitrogen gas was utilized as purge gas, auxiliary gas, and sheath gas with flow rates set at 3, 15, and 45 L·min^{-1}, respectively. The heating temperature is set to 350 °C. The mass scanning range is m/z 70−1000 Da.

4.5. HPLC−PDA Analysis Conditions. A Dikma Diamonsil C18 column (250 mm × 4.6 mm, 5 μm) was used for component separation with a flow rate of 0.7 mL min^{-1}, an injection volume of 10 μL, and a temperature of 40 °C. PH 3.70 phosphoric acid water (phase A) and methanol/acetonitrile (phase B, v/v = 1:1) were the mobile phases, with gradient variations as follows: 5%(0−5 min) B, 5−55%(5−10 min) B, 55−65%(10−20 min) B, 65−85%(20−30 min) B, 85%(30−35 min) B. The detection wavelengths: 330 nm (ferulic acid, sinensetin, nobiletin, tangeretin, 5-hydroxy-6,7,8,3′,4′-pentamethoxyflavone, and 3,5,6,7,8,3′,4′-heptemthoxyflavone), 283 nm (hesperidin), 224 nm (synephrine), and 209 nm (limonin).

4.6. Statistical Analysis. Origin 2024b (Northampton, Massachusetts) was used to analyze the data. Statistical significance was determined by using a one-way analysis of variance, $P < 0.05$ indicated statistical significance. Data obtained from the experiment were normalized using Z-score descriptive statistics. After data normalization, the principal component analysis and cluster heat maps were used to group samples according to their relative contents.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.4c09701.

Summary of all detected compounds, structures of all detected nonvolatile compounds, HPLC chromatograms, and methodological evaluation information (PDF)

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Notes

The authors declare no competing financial interest.

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