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Volatile Constituents of Wild Citrus Mangshanyegan (*Citrus nobilis* Lauriro) Peel Oil

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ABSTRACT: Volatiles of a wild mandarin, Mangshanyegan (*Citrus nobilis* Lauriro), were characterized by GC-MS, and their aroma active compounds were identified by aroma extract dilution analysis (AEDA) and gas chromatography−olfactometry (GC-O). The volatile profile of Mangshanyegan was compared with those of other four citrus species, Kaopan pummelo (*Citrus grandis*), Eureka lemon (*Citrus limon*), Huangyanbendizao tangerine (*Citrus reticulata*), and Seike navel orange (*Citrus sinensis*). Monoterpene hydrocarbons predominated in Mangshanyegan, in particular *d*-limonene and *β*-myrcene, which accounted for 85.75 and 10.89% of total volatiles, respectively. Among the 12 compounds with flavor dilution factors (FD) = 27, 8 oxygenated compounds, including (*Z*)- and (*E*)-linalool oxides, were present only in Mangshanyegan. The combined results of GC-O, quantitative analysis, odor activity values (OAVs), and omission tests revealed that *β*-myrcene and (*Z*)- and (*E*)-linalool oxides were the characteristic aroma compounds of Mangshanyegan, contributing to the balsamic and floral notes of its aroma.

KEYWORDS: *Mangshanyegan (Citrus nobilis), volatiles, GC-MS, GC-O, AEDA, characteristic aroma compounds*

INTRODUCTION

The wild citrus Mangshanyegan (*Citrus nobilis* Lauriro) is native to the forests of Mangshan, Yizhang County, Hunan Province, China.[1] The yellow fruits of Mangshanyegan are somewhat rounded, and the petiole end of the fruit is radially sulcate. The surface of the fruit is uneven, and the inedible segments are almost completely filled with seeds. The phylogeny of Mangshanyegan has been investigated via molecular markers,[2−4] whereas analyses of pollen morphology[5] and botanical characters[6] have also been carried out. Previous studies revealed that this species is more primitive than Mangshanyeju (*Citrus reticulata* Blanco). Although the phylogeny of Mangshanyegan has been investigated, there have not been studies on the distinctive aroma of this fruit. This prompted us to investigate the volatile profile and aroma active compounds of Mangshanyegan to understand the factors that shape the aroma of its fruits.

In the global flavor market, citrus flavors are among the most important flavors, along with vanilla, representing about 25% of the market.[7] The fruits, leaves, and flowers of various citrus genotypes are all raw materials for many essential oils, such as bergamot oil, grapefruit oil, lemon oil, terpene-including or terpeneless lime oil, orange bitter oil, neroli oil (distilled from the flowers of the bitter orange tree), orange sweet oil, and others.

Volatiles of many commercial citrus cultivars have been analyzed by gas chromatography−mass spectrometry (GC-MS) alone or coupled with gas chromatography−olfactometry (GC-O) and aroma extract dilution analysis (AEDA) to identify odorous compounds.[8−10] Previous analyses have shown that the characteristic aromas of some citrus varieties are defined. Nguyen et al.[11] reported that (*R*)-(+)-citronellal was the characteristic aroma compound of Kabosu peel oil. However, olfactory flavors of other citrus fruits are the result of the presence of various aroma active compounds. Chisholm et al.[12] stated that no individual odorant could be singled out as the characteristic aroma compound of Clementine produced in Spain. Sawamura et al.[9] found that the most probable aroma model of an Italian bergamot (*Cittrus bergamia* Risso) essential oil included the contributions of limonene, linalool, *γ*-terpinene, (*Z*)-limonene oxide, decanal, linalyl acetate, and geraniol.

So far, numerous papers on citrus aroma have focused on commercial citrus cultivars,[13−15] whereas papers on wild citrus genotypes are scarce. For a long period of time, citrus breeding has focused on high yields or taste flavor while ignoring sensory flavor. However, elite citrus cultivars with excellent sensory traits are desirable for essential oil production as well as fresh consumption. Due to its special balsamic and floral odor, Mangshanyegan could be expected to be a promising and valuable raw material for essential oil production. Thus, GC-MS, AEDA, and GC-O analyses were performed to investigate the volatile profile and the characteristic aroma compounds of the Mangshanyegan peel oil. In addition, the volatile profile of Mangshanyegan was compared with those of other four citrus, including Kaopan pummelo (*Citrus paradisi*), Eureka lemon (*Citrus limon*), Huangyanbendizao tangerine (*Citrus reticulata*), and Seike navel orange (*Citrus sinensis*), which represent the commonly cultivated citrus species in the world.

MATERIALS AND METHODS

Plant Material. Ripe fruits of Mangshanyegan (*C. nobilis*), Kaopan pummelo (*C. paradisi*), Eureka lemon (*C. limon*), Huangyanbendizao tangerine (*C. reticulata*), and Seike navel orange (*C. sinensis*) were

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harvested from the National Citrus Breeding Centre of China in Wuhan. For the GC-MS analysis, all samples were harvested in 2009, while fully ripe Mangshanyegan fruits picked in both 2009 and 2010 were used for AEDA and GC-O analysis. After washing with tap water, the peels were separated longitudinally into three parts by cutting around the circumference of the fruit without touching the inner part of it (segment and juice sacs) and carefully removing only the middle section for analysis.[16] The peels (including flavedo and albedo) were ground immediately, transferred into glass tubes, sealed, and kept at −80 °C until extraction. Three biological replicates were prepared.

Standards and Reagents. Internal standards of chlorononane and methyl nonanoate were obtained from Sigma Co. Ltd. (St. Louis, MO, USA). A standard series of C_7−C_{30} saturated alkanes from Supelco (Bellefonte, PA, USA) was used for retention index (RI) determination. Methyl *tert*-butyl ether (MTBE) (HPLC grade) from Tedia (Fairfield, OH, USA) was used for volatiles extraction. Anhydrous sodium sulfate, dichloromethane, and propylene glycol were from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). The sources of volatile standards are listed in Table 1.

The linear regression range, equations of the standard curves, regression coefficients (R^2), total ion current (TIC), correction factor (CF) for each compound, and quantifying ions (QI) of standard solutions are listed in Table 1. The internal standard of chlorononane was used to calculate the concentrations of *β*-myrcene and *d*-limonene, whereas methyl nonanoate was used to calculate the concentrations of the other volatiles.

In addition, the following authentic compounds were also employed but not identified, and they were all obtained from Sigma: *allo*-ocimene, benzyl nitrile, butyl butyrate, butyraldehyde, carvone, (*Z*)- and (*E*)-carvyl acetate, caryophyllene oxide, decanoic acid, decenal, dimethyl anthranilate, 2-dodecenal, epiglobulol, farnesal, farnesol, geranyl isovalerate, 2,4-hexadienal, irone, limettin, linalyl acetate, 7-methoxy-coumarin, (*Z*)-nerolidol, (*E*,*E*)-2,4-nonadienal, (*Z*)-2-penten-1-ol, *α*-terpinyl acetate, thymol, thymol methyl ether.

Volatile Extraction and GC-MS Analysis. The volatile compounds were extracted from 3 g of citrus peel using 15 mL of MTBE containing 8697 μg of chlorononane and 400 μg of methyl nonanoate as internal standards.[12] The extraction was carried out in an ultrasonic cleaner model FS60 (Fisher Scientific, Pittsburgh, PA, USA) for 1 h, and the organic layer was collected, dried over Na_2SO_4, and concentrated to a final volume of 1.4 mL under a gentle stream of nitrogen.[17,18]

An aliquot of 1 μL of sample was analyzed using a TRACE GC Ultra GC coupled to a DSQ II mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). The GC was fitted with a TRACE TR-5 MS column (30 m × 0.25 mm × 0.25 μm, Thermo Scientific, Bellefonte, PA, USA). Helium was used as the carrier gas, with a split ratio of 50:1, at a flow rate of 1 mL/min. The temperatures of the injection port, ion source, and MS transfer line were 250, 260, and 280 °C, respectively. The oven temperature program was as follows: kept at 40 °C for the initial 3 min, followed by a ramp of 3 °C/min to reach 160 °C, kept at 160 °C for 1 min, then increased to 200 °C at a rate of 5 °C/min, kept at 200 °C for 1 min, raised to 240 °C at a rate of 8 °C/min, and finally kept at 240 °C for 3 min. The MS was operated in positive electron ionization mode at 70 eV, obtaining spectra with an m/z range of 45−400.

Simultaneous Distillation Extraction (SDE) and GC-O and AEDA Analyses. A modified Likens−Nickerson apparatus was used to extract the volatiles. The sample flask contained 100 g of the Mangshanyegan peel with 250 mL of distilled water; dichloromethane (40 mL) was used as solvent. SDE was carried out for 2.2 h.[19,20] After cooling to room temperature, the dichloromethane extract was collected and dried over anhydrous Na_2SO_4. The extract was concentrated in a rotary evaporator over a 30 °C water bath to a volume of 1.5 mL and further concentrated to a final volume of 1 mL under a gentle stream of nitrogen.

For AEDA, a series of 3-fold dilutions of the Mangshanyegan extract were prepared using dichloromethane as a solvent. Samples were analyzed by an expert in fragrance chemicals and flavor technology. The odor characters of the volatiles were evaluated by sniffing, and flavor dilution (FD) factors were obtained.[21,22]

For the GC-O analysis, an Agilent 6890 GC coupled with an Agilent 5973 Network mass spectrometer (Agilent Technologies, Palo Alto, CA, USA) was equipped with an olfactory detection port Gerstel ODP-2 (Gerstel AG Enterprise, Mülheim an der Ruhr, Germany). The GC was fitted with an HP-Innowax column (60 m × 0.25 mm × 0.25 μm, Agilent, Palo Alto, CA, USA). The oven temperature was programmed as follows: an initial temperature of 50 °C was kept for 3 min, the temperature was then increased to 230 °C at a rate of 4 °C/min, and finally kept at 230 °C for 10 min. Helium was used as the carrier gas at a flow rate of 1 mL/min. The temperature of the injector port was 250 °C, and a 2 μL sample was injected into the GC-MS/O system in splitless mode. The ion source and MS transfer line were kept at 230 and 280 °C, respectively. The MS was operated in electron ionization mode at 70 eV, and the scan range was m/z 19−350. The SDE and AEDA and GC-O analyses were carried out at the Shanghai Institute of Technology (Shanghai, China).

Identification and Quantification of Volatiles. The raw data obtained from the GC-MS were processed using Xcalibur and AMDIS software, whereas those from the GC-MS/O were processed using ChemStation and AMDIS software. Volatiles were identified on the basis of the database of NIST/EPA/NIH Mass Spectral Library (NIST 2008) and the Wiley Registry of Mass Spectral Data (8th ed.). RIs were calculated using a homologous series of *n*-alkanes (C_7−C_{30}), analyzed on the TR-5 and HP-Innowax columns.[23,24]

The authentic standards of 62 compounds were available (Table 1). The concentrations of these compounds were calculated using standard curves obtained in the selective ion monitoring (SIM) mode. The equations of standard curves obtained from the TIC (Table 1) were used for the quantification of the rest of the volatiles (Table 2).

Odor activity values (OAVs) were calculated using the concentrations of the aroma active compounds and their respective odor threshold values in water obtained from the literature (Table 2).[25,26]

Omission Experiment. Model samples of Mangshanyegan aroma were prepared with authentic compounds, on the basis of the results of the chemical and sensory analyses. The stock solutions of *β*-myrcene, linalool, and (*Z*)- and (*E*)-linalool oxides were prepared on the basis of their concentrations determined by GC-MS analysis using the TR-5 column. Every standard was first diluted in propylene glycol and adjusted to a 10 mL volume with Milli-Q water. Both propylene glycol and water are odorless and do not react with essential oil components. The final model solutions contained 10 μL of the corresponding stock solutions. The compositions of the aroma model solutions are described in Table 3. Trained panelists evaluated the similarity between the scents of the model samples and the scent of the Mangshanyegan peel. The assigned scores ranged from 1 (extremely different) to 9 (extremely similar).[27]

Data Analysis. The equations of the standard curves obtained in SIM and TIC modes were processed using Xcalibur, and the rest of the data were processed with Microsoft Excel. Analysis of variance (ANOVA) was carried out at $p < 0.05$ using SAS (SAS Institute Inc., Cary, NC, USA). The analysis of the effect of limonene on the aroma model solutions was performed with the Student *t* test at $p = 0.05$, using SAS.

RESULTS

The presence of aroma active compounds is a prerequisite for flavor. In many cases, compounds with low odor thresholds, which even occur at low concentrations, are critical to flavor.[28] Compared with the headspace solid phase microextraction (HS-SPME) method, the use of solvent is a better option for the extraction of trace compounds, because the SPME fiber has a very limited extraction capacity.[29] Hence, solvent extraction with MTBE was selected for this study.

Volatile Content. A total of 102 volatiles were detected in the peel oil of the five citrus fruits. The numbers of volatiles

Table 1. Authentic Standard Compounds Used in the GC-MS Analysis and Equations of Standard Curves

chemical	source[a]	range (μg/mL)	curves[b]	QI[c]	CF[d]	R^2
borneol	Sigma	0.48−7.73	$y = -0.000327 + 0.00223x$	95	0.798	1.000
camphene	Sigma	0.81−14.83	$y = 0.000276 + 0.0007624x$	93	0.399	1.000
d-camphor	Sigma	0.96−15.42	$y = -0.000250 + 0.000799x$	95	0.283	1.000
(E)-carveol	*Sigma*	*0.87−221.81*	$y = 0.00143 + 0.00908x^{T1}$		*3.247*	*0.999*
caryophyllene	Sigma	0.22−13.86	$y = -0.00138 + 0.000727x$	93	0.278	0.999
			$y = -0.0248 + 0.0101x^{T2}$		*3.563*	*0.999*
α-caryophyllene	Sigma	0.22−14.06	$y = -0.00662 + 0.00231x$	93	0.855	0.999
			$y = -0.0112 + 0.00851x^{T3}$		*3.059*	*0.999*
citronellal	Sigma	0.83−13.24	$y = 0.000187 + 0.000684x$	69	0.358	1.000
β-citronellol	Sigma	0.81−13.03	$y = -0.000518 + 0.000734x$	69	0.205	0.997
citronellyl acetate	Alfa	0.42−6.74	$y = -0.000118 + 0.000588x$	69	0.196	0.999
coniferyl alcohol	Sigma	17.12−247.67	$y = -0.00906 + 0.000386x$	137	0.091	1.000
p-cymene	Sigma	0.83−13.34	$y = -0.00471 + 0.00968x$	119	3.224	0.999
decanal	Sigma	0.80−12.84	$y = -0.0009731 + 0.00149x$	70	0.467	0.997
decanol	Sigma	0.40−6.42	$y = -0.000297 + 0.000358x$	70	0.111	1.000
dodecanal	Sigma	0.81−12.89	$y = 0.0000476 + 0.000265x$	57	0.081	1.000
farnesene	Sigma	*e*				
β-farnesene	Chroma-Dex	0.84−12.86	$y = -0.00457 + 0.00570x$	69	1.662	0.997
		0.80−51.43	$y = -0.0336 + 0.0277x^{T4}$		*8.855*	1.000
geranial	Sigma	2.25−144.08	$y = -0.00199 + 0.000994x$	69	0.322	1.000
geraniol	Alfa	0.22−14.14	$y = -0.00120 + 0.00192x$	69	0.634	0.999
			$y = -0.0389 + 0.00743x^{T5}$		*2.515*	*0.999*
geranyl acetate	Sigma	0.88−56.63	$y = -0.000375 + 0.00111x$	69	0.374	0.999
geranyl-linalool	Sigma	0.22−14.12	$y = -0.0145 + 0.00246x$	69	0.823	0.999
			$y = -0.0636 + 0.0129x^{T6}$		*4.042*	*0.999*
hexanal	Sigma	0.80−50.89	$y = -0.000490 + 0.00123x$	56	0.457	1.000
hexanoic acid	Sigma	115.30−461.18	$y = -0.0595 + 0.000553x$	60	0.131	1.000
hexanol	Alfa	0.24−15.36	$y = -0.0171 + 0.00184x$	56	0.446	0.999
(*E*)-2-hexenal	Sigma	3.26−13.06	$y = -0.0000512 + 0.000210x$	69	0.083	1.000
(*Z*)-3-hexenol	Alfa	0.23−14.74	$y = -0.00687 + 0.000896x$	67	0.264	0.999
			$y = -0.0215 + 0.00351x^{T7}$		*0.914*	*0.914*
(*E*)-2-hexenol	Alfa	0.23−14.83	$y = -0.0237 + 0.00101x$	57	0.171	0.964
(*Z*)-3-hexenyl hexanoate	Sigma	0.43−6.80	$y = -0.00295 + 0.00664x$	67	1.972	0.995
hexyl acetate	Sigma	0.85−13.54	$y = 0.000190 + 0.000597x$	56	0.306	0.999
hexyl butanoate	Sigma	0.41−6.62	$y = 0.000177 + 0.00121x$	71	0.477	1.000
indole	Alfa	0.20−12.50	$y = -0.00949 + 0.00361x$	117	1.306	0.999
			$y = -0.0192 + 0.00912x^{T8}$		*3.109*	*0.999*
d-limonene	Alfa	22.98−1470.59	$y = 0.0207 + 0.0000484x$	68	0.510	0.997
(*Z*)-limonene oxide	Sigma	0.29−4.63	$y = -0.000105 + 0.00123x$	79	0.431	1.000
(*E*)-limonene oxide	Sigma	0.60−9.64	$y = -0.000651 + 0.00118x$	79	0.356	0.997
linalool	Alfa	0.23−14.45	$y = -0.00626 + 0.00131x$	93	0.454	0.999
			$y = -0.0414 + 0.00870x^{T9}$		*2.872*	*0.999*
(*Z*)-linalool oxide	Sigma	0.11−7.22	$y = -0.00353 + 0.00118x$	59	0.396	0.998
(*E*)-linalool oxide	Sigma	0.09−6.01	$y = -0.00370 + 0.00122x$	59	0.404	0.998
methyl palmitate	Sigma	0.98−15.63	$y = -0.00751 + 0.00764x$	74	2.299	0.996
β-myrcene	Sigma	4.88−312.5	$y = -0.00257 + 0.000129x$	93	1.045	0.999
neral	Sigma	0.30−76.37	$y = -0.000485 + 0.000746x$	69	0.232	1.000
nerol	Sigma	0.85−217.03	$y = -0.00847 + 0.00107x$	69	0.221	0.995
(*E*)-nerolidol	Sigma	0.47−7.58	$y = -0.00204 + 0.00366x$	69	1.045	0.994
neryl acetate	Alfa	0.88−905.45	$y = -0.00197 + 0.00129x$	69	0.444	1.000
nonanal	Sigma	0.80−51.48	$y = -0.000264 + 0.000309x$	57	0.106	1.000
nonanol	Sigma	0.80−12.86	$y = -0.000639 + 0.000661x$	70	0.149	0.998
nootkatone	Alfa	0.20−12.50	$y = -0.00163 + 0.000348x$	147	0.126	0.998
(*Z*)-β-ocimene	Sigma	0.26−16.41	$y = -0.00214 + 0.00183x$	93	0.394	0.988
(*E*)-β-ocimene	Sigma	3.26−834.81	$y = -0.00358 + 0.00105x$	93	0.359	0.999
octanal	Sigma	3.19−51.10	$y = -0.000125 + 0.000271x$	69	0.089	0.999
octanoic acid	Sigma	14.20−56.82	$y = -0.0132 + 0.000671x$	101	0.106	1.000
octanol	Sigma	3.23−51.67	$y = -0.00287 + 0.000514x$	69	0.118	0.997
1-penten-3-ol	Sigma	0.82−13.04	$y = -0.000239 + 0.000621x$	57	0.205	1.000
α-phellandrene	Sigma	0.23−14.71	$y = -0.0108 + 0.00234x$	93	0.838	0.999
			$y = -0.0270 + 0.00644x^{T10}$		*2.404*	*0.999*

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Table 1. continued

chemical	source[a]	range (μg/mL)	curves[b]	QI[c]	CF[d]	R^2
α-pinene	Sigma	0.23−14.57	$y = -0.0178 + 0.00363x$	93	1.296	0.999
			$y = -0.0506 + 0.0108x^{T11}$		*4.047*	*0.998*
β-pinene	Alfa	0.23−14.53	$y = -0.0113 + 0.00244x$	93	0.875	0.999
sabinene hydrate	Sigma	0.96−61.50	$y = -0.000114 + 0.000558x$	71	0.210	1.000
		0.96−984.00	*$y = -0.000424 + 0.00547x^{T12}$*		*2.140*	*1.000*
α-terpinene	Sigma	0.75−192.30	*$y = -0.00135 + 0.000895x$*	121	0.329	1.000
γ-terpinene	Sigma	0.82−841.50	$y = -0.000904 + 0.00132x$	93	0.519	1.000
α-terpineol	Alfa	0.21−13.32	$y = -0.00486 + 0.00133x$	59	0.470	0.999
			$y = -0.0273 + 0.00939x^{T13}$		*3.415*	*0.999*
4-terpineol	Sigma	0.90−14.44	$y = -0.00226 + 0.00317x$	111	1.017	0.997
terpinolene	Sigma	0.77−48.97	$y = -0.000721 + 0.00130x$	136	0.493	0.999
undecane	Sigma	0.72−11.49	$y = 0.00152 + 0.00109x$	57	0.815	0.999
undecanal	Sigma	0.78−12.53	$y = -0.00124 + 0.00159x$	57	0.473	0.996
valencene	Sigma	2.93−46.81	$y = -0.00507 + 0.000984x$	161	0.235	0.990

[a]Sigma, Sigma-Aldrich Co. Ltd. (St. Louis, MO, USA); Alfa, Alfa Aesar Co. Ltd. (Heysham, Lancashire, U.K.); ChromaDex, ChromaDex, Inc. (Irvine, CA, USA). [b]The equations of curves without a superscript character were calculated in SIM mode; the equations of curves with T1−T13 were obtained in TIC mode (italic); y is the ratio of the area of the peak of an authentic standard to that of the internal standard; x is the weight of an authentic standard in grams. [c]Quantifying ion for SIM mode. [d]Correction factor were calculated as means using this formula: $[(C^1/A^1)/(C^2/A^2)]$, where C^1 is the concentration of the authentic standard, A^1 is the peak area of the authentic standard (TIC) or the peak area of the corresponding quantifying ion (SIM mode), C^2 is the concentration of the internal standard, A^2 is the peak area of the quantifying ion of the internal standard (QI of chlorononane, 91; QI of methyl nonanoate, 74). [e]The standard was used for identification, but the corresponding standard curve was not built.

detected in Mangshanyegan, Kaopan pummelo, Eureka lemon, Seike navel orange, and Huangyanbendizao tangerine were 70, 46, 69, 63 and 64, respectively (Table 2). Among these volatiles, 62 compounds were quantified in SIM mode using standard curves. The regression coefficients were generally beyond 0.990, except those of (*E*)-2-hexenol, (*Z*)-3-hexenol, and (*Z*)-β-ocimene, which were 0.964, 0.914, and 0.988, respectively (Table 1). The rest of the compounds were quantified in TIC mode (Table 1).

The total volatile contents of the peels of the five citrus fruits ranged from 5638.12 μg/g (Kaopan pummelo) to 15560.02 μg/g (Eureka lemon). Mangshanyegan, Seike navel orange, and Huangyanbendizao tangerine had similar levels of total volatiles, with values being 14696.20, 13369.17, and 12041.73 μg/g, respectively (Table 2). The relatively low content of volatiles in Kaopan pummelo might result from its thick albedo. The volatile profiles of the analyzed citrus are listed in Table 2.

Volatile Composition. Ninety-five of the 102 detected compounds were identified and grouped into 14 classes: monoterpenes, monoterpene alcohols, monoterpene aldehydes, monoterpene ketones, monoterpene oxides, monoterpene esters, sesquiterpenes, sesquiterpene alcohols, alcohols, aldehydes, acids, esters, unknowns, and others. It is worth noting that the levels of sesquiterpene alcohols, monoterpene oxides, acids, esters, geranyl linalool (a diterpene, listed as "others"), and undecane (an alkane, listed as "others") in Mangshanyegan peel oil were significantly higher than those in the other four citrus. These volatile compounds are described in the following sections.

Sesquiterpene Alcohols. In this study, four sesquiterpene alcohols were detected. The levels of elemol, (*E*)-nerolidol, germacrene D-4-ol, and total sesquiterpene alcohols were significantly higher in Mangshanyegan than in the other citrus species (Table 2). However, α-bisabolol was found only in the Eureka lemon (3.17 μg/g).

Monoterpene Oxides and the Unique Diterpene. In Mangshanyegan, monoterpene oxides (5.43 μg/g) were found at concentrations 3−13 times higher than in the other citrus. Four monoterpene oxides, (*E*)- and (*Z*)-limonene oxides and (*E*)- and (*Z*)-linalool oxides, were identified. There were no significant differences between the concentrations of limonene oxides in Mangshanyegan and the other four citrus. However, both (*Z*)- and (*E*)-linalool oxides were found only in Mangshanyegan (2.53 and 2.03 μg/g, respectively) (Table 2). Geranyl linalool, the only positively identified diterpene, was detected only in Mangshanyegan (4.31 μg/g) (Table 2).

Acids, Esters, and the Only Alkane. Two organic acids, hexanoic acid and octanoic acid, were detected in Mangshanyegan with a total concentration of 51.29 μg/g. The concentration of hexanoic acid in Mangshanyegan was significantly higher than that in the other samples. However, the levels of octanoic acid in Mangshanyegan, Seike navel orange, and Huangyanbendizao tangerine were not significantly different.

Four esters were identified in this study: hexyl acetate, hexyl butanoate, (*Z*)-3-hexenyl hexanoate, and methyl hexadecanoate. The first three esters were found only in Mangshanyegan. On the other hand, the concentration of methyl hexadecanoate in Mangshanyegan was significantly lower than that in Huangyanbendizao tangerine (Table 2).

Undecane was found only in Mangshanyegan (0.80 μg/g). Choi et al.[30] reported this compound in *Citrus flaviculpus* Hort. ex Tanaka. Undecane was described as having a green and perfume-like aroma and an FD factor of 27 in AEDA.[30]

Major Volatile Compounds of Mangshanyegan. The most abundant volatile in Mangshanyegan was *d*-limonene, which had a significantly higher concentration in this fruit than in Kaopan pummelo. β-Myrcene was the second most abundant compound and also had a significantly higher concentration in Mangshanyegan (1600.29 μg/g) than in the other citrus (57.29−171.77 μg/g). With a concentration of 82.64 μg/g, β-pinene was the third most abundant volatile. The level of β-pinene in Mangshanyegan was significantly lower than in Eureka lemon (524.17 μg/g) but significantly higher than in Seike navel orange (5.47 μg/g).

Additionally, another 14 volatile compounds, including δ-cadinene, α-caryophyllene, α-copaene, β-cubebene, elemol, elixene, germacrene D, germacrene D-4-ol, α-guaiene, hexanol,

Table 2. Volatile Compounds in the Peel of the Citrus Fruits Analyzed (Micrograms per Gram)

compound[a]	RI[b]	RI*[c]	Mangshanyegan	Kaopan pummelo	Eureka lemon	Seike navel orange	Huangyanbendizao tangerine	ID[d]
monoterpenes								
α-thujene[T10]	931	931	2.01 ± 0.22	19.30 ± 2.97	34.43 ± 4.80	2.10 ± 0.11	11.98 ± 3.28	T, P1
α-pinene	938	939	25.41 ± 8.56	30.11 ± 5.29	69.54 ± 9.80	28.49 ± 3.38	31.03 ± 10.26	R, P2
camphene	956	953	1.28 ± 0.70	0.50 ± 0.16	6.07 ± 0.98	0.38 ± 0.06	0.42 ± 0.14	R, P2
sabinene[T11]	979	976	21.18 ± 8.88	5.44 ± 0.78	84.88 ± 13.2	63.82 ± 12.76	7.70 ± 2.31	T, P2
β-pinene	982	980	82.64 ± 40.45	30.82 ± 4.60	524.17 ± 75.32	5.47 ± 0.60	16.10 ± 4.57	R, P2
β-myrcene*	994	991	1600.29 ± 703.48	57.29 ± 14.12	171.77 ± 34.26	169.74 ± 23.37	148.61 ± 45.48	R, P2
α-phellandrene	1011	1005	4.79 ± 1.19	3.22 ± 0.40	5.04 ± 0.70	12.00 ± 3.46	4.13 ± 0.90	R, P2
α-terpinene	1023	1018	U[e]	11.55 ± 3.20	23.71 ± 4.36	0.52 ± 0.46	7.49 ± 2.15	R, P2
d-limonene	1038	1031	12601.77 ± 3677.26	4582.76 ± 1298.53	12606.21 ± 2182.10	12613.02 ± 506.29	11206.15 ± 2962.64	R, P2
β-phellandrene[T11]*	1040	1031	2.65 ± 0.20	2.21 ± 0.12	3.15 ± 0.09	2.27 ± 0.27	2.28 ± 0.24	T, P2
(*Z*)-β-ocimene	1044	1040	0.77 ± 0.15	0.58 ± 0.04	3.24 ± 0.70	0.67 ± 0.08	1.61 ± 0.32	R, P2
(*E*)-β-ocimene	1055	1050	14.97 ± 5.55	6.68 ± 1.42	11.58 ± 2.34	7.24 ± 0.84	70.36 ± 19.53	R, P1
γ-terpinene	1066	1062	0.85 ± 0.23	742.60 ± 121.03	946.60 ± 174.02	0.99 ± 0.18	289.47 ± 77.41	R, P2
terpinolene	1090	1070	1.06 ± 0.35	13.94 ± 2.90	22.96 ± 4.01	1.62 ± 0.49	7.55 ± 1.96	R, P2
cosmene[T1]	1139	1132[n]	U	0.10 ± 0.04	U	U	U	T, P3
sum			**14359.67**	**5507.10**	**14513.35**	**12908.33**	**11804.88**	
monoterpene alcohols								
sabinene hydrate	1079	1068	2.10 ± 0.61	3.24 ± 0.31	13.65 ± 1.45	5.58 ± 0.89	1.10 ± 0.19	R, P2
β-linalool	1107	1098	10.38 ± 2.20	5.03 ± 0.40	12.60 ± 1.85	34.74 ± 4.29	25.39 ± 4.28	R, P2
(*Z*)-*p*-menth-2-en-1-ol[T12]	1109	1121	0.84 ± 0.12	4.87 ± 1.44	11.98 ± 1.68	U	U	T, P2
borneol	1184	1165	U	U	1.66 ± 0.25	U	U	R, P2
4-terpineol	1190	1177	0.32 ± 0.02	0.74 ± 0.01	0.84 ± 0.04	0.33 ± 0.02	0.32 ± 0.01	R, P2
α-terpineol	1206	1189	6.36 ± 0.50	12.89 ± 1.03	32.74 ± 2.94	11.22 ± 1.04	6.46 ± 0.85	R, P2
(*E*)-piperitol[T1]	1210	1205	U	U	U	U	0.49 ± 0.17	T, P4
nerol	1234	1228	U	U	48.17 ± 3.93	U	U	R, P2
β-citronellol	1236	1228	U	0.76 ± 0.06	5.12 ± 0.67	1.17 ± 0.13	2.55 ± 0.86	R, P4
geraniol	1261	1255	3.94 ± 0.80	16.02 ± 3.47	47.14 ± 5.39	2.08 ± 0.16	0.34 ± 0.02	R, P2
p-mentha-1(7),8(10)-dien-9-ol[T1]	1303		U	U	U	0.81 ± 0.21	U	T, P5
8-hydroxylinalool[T7]*	1376	1336[n]	1.85 ± 1.60	U	3.43 ± 0.64	U	U	T, P6
sum			**25.79**	**43.55**	**177.33**	**55.93**	**36.65**	
monoterpene aldehydes								
citronellal	1164	1153	0.94 ± 0.48	U	13.86 ± 2.03	5.03 ± 1.85	0.85 ± 0.64	R, P2
neral	1251	1240	4.52 ± 1.40	1.35 ± 0.68	185.57 ± 32.64	19.60 ± 4.48	0.60 ± 0.22	R, P2
geranial	1281	1270	21.32 ± 7.19	2.76 ± 1.24	399.81 ± 76.98	36.85 ± 8.59	1.66 ± 0.32	R, P1
perillal[T13]*	1291	1271	1.24 ± 0.04	U	3.11 ± 0.32	2.33 ± 0.16	2.73 ± 0.45	T, P2
sum			**28.02**	**4.11**	**602.35**	**63.81**	**5.84**	
monoterpene ketones								
d-camphor	1160	1146	U	U	2.18 ± 0.14	U	U	R, P4
piperitone[T9]*	1269	1252	2.30 ± 0.21	U	2.00 ± 0.02	U	U	T, P2
p-mentha-1,8-dien-3-one[T5]	1284	1272[n]	41.33 ± 2.81	U	U	115.85 ± 27.63	1.74 ± 0.03	T, P7
sum			**43.63**	**U**	**4.18**	**115.85**	**1.74**	

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Table 2. continued

compound[a]	RI[b]	RI*[c]	Mangshanyegan	Kaopan pummelo	Eureka lemon	Seike navel orange	Huangyanbendizao tangerine	ID[d]
monoterpene oxides								
(*Z*)-linalool oxide*	1078	1074	2.53 ± 0.54	U	U	U	U	R, P2
(*E*)-linalool oxide*	1093	1088	2.03 ± 0.37	U	U	U	U	R, P2
(*Z*)-limonene oxide	1143	1134	0.25 ± 0.12	U	0.13 ± 0.04	0.64 ± 0.42	0.31 ± 0.31	R, P2
(*E*)-limonene oxide	1148	1139	0.62 ± 0.08	0.43 ± 0.02	0.33 ± 0.04	1.17 ± 0.17	1.22 ± 0.38	R, P2
sum			**5.43**	**0.43**	**0.46**	**1.81**	**1.53**	
monoterpene esters								
methyl geranate[T1]	1330	1323	3.44 ± 0.60	U	3.04 ± 0.26	U	U	R,P1
citronellyl acetate	1357	1354	U	U	3.86 ± 0.92	U	0.89 ± 0.38	R, P1
neryl acetate	1366	1365	U	0.94 ± 0.19	48.75 ± 8.07	1.31 ± 0.27	3.17 ± 0.93	R, P1
geranyl acetate	1385	1383	U	4.69 ± 2.56	22.62 ± 5.30	0.91 ± 0.50	2.76 ± 1.02	R, P1
geranyl isobutyrate[T6]	1517	1514	U	U	2.24 ± 0.09	U	U	T, P8
sum			**3.93**	**5.63**	**80.51**	**2.22**	**6.82**	
sesquiterpenes								
δ-elemene[T13]	1338	1339	1.54 ± 0.24	2.42 ± 0.47	U	U	5.91 ± 1.38	T, P2
α-copaene[T13]*	1380	1376	13.69 ± 4.51	U	U	3.19 ± 0.63	2.18 ± 0.39	T, P2
β-cubebene[T3,]*	1391	1390	17.03 ± 5.67	U	U	2.23 ± 0.56	U	T, P2
β-elemene[T13]	1393	1391	4.33 ± 0.92	1.23 ± 0.02	1.33 ± 0.10	2.38 ± 0.47	8.07 ± 2.32	T, P2
(*Z*)-α-bergamotene[T13]	1416	1415	U	U	2.60 ± 0.38	U	U	T, P1
caryophyllene	1424	1418	3.57 ± 1.16	2.76 ± 0.97	11.32 ± 2.43	2.97 ± 0.39	1.19 ± 0.24	R, P2
(*E*)-α-bergamotene[T13]	1436	1436	U	U	26.36 ± 4.67	U	U	T, P2
α-guaiene[T2,]*	1439	1439	1.21 ± 0.10	U	U	U	U	T, P9
(*Z*)-β-farnesene [T4]	1441	1443	U	U	0.70 ± 0.06	U	U	T, P1
β-farnesene	1457	1458	U	U	1.09 ± 0.19	0.55 ± 0.02	U	R, P4
α-caryophyllene*	1461	1454	3.20 ± 0.82	1.26 ± 0.11	1.87 ± 0.17	1.43 ± 0.16	2.00 ± 0.30	R, P10
germacrene D[T2]*	1487	1480	10.50 ± 3.88	2.12 ± 0.4	U	2.13 ± 0.34	6.69 ± 1.65	T, P2
valencene	1497	1491	U	U	4.02 ± 0.38	10.13 ± 1.36	U	R, P4
elixene[T13]*	1500	1445[n]	6.51 ± 1.75	U	3.54 ± 0.30	1.88 ± 0.03	2.07 ± 0.60	T, P11
α-muurolene[T3,]*	1505	1499	1.39 ± 0.39	U	U	0.81 ± 0.02	U	T, P2
(*Z*)-α-bisabolene[T5]	1504	1504	U	U	14.58 ± 0.85	U	U	T, P1
α-farnesene[T4]	1508	1508	0.66 ± 0.10	U	U	0.74 ± 0.05	U	R, P1
β-bisabolene[T6]	1512	1509	2.44 ± 0.30	U	29.42 ± 4.15	U	U	T, P1
δ-cadinene[T13]*	1524	1524	15.35 ± 5.33	U	U	3.36 ± 0.75	2.46 ± 0.55	T, P12
germacrene B[T13]	1566	1556	U	1.33 ± 0.08	U	U	U	T, P2
cadalene[T8]*	1685	1674	1.94 ± 0.10	1.15 ± 0.07	U	3.07 ± 0.14	3.35 ± 0.30	T, N
sum			**83.36**	**12.27**	**96.83**	**34.87**	**33.92**	
sesquiterpene alcohols								
elemol[T2]*	1558	1549	4.96 ± 1.76	U	U	1.52 ± 0.24	1.16 ± 0.12	T, P2
(*E*)-nerolidol*	1567	1564	0.49 ± 0.11	U	U	U	0.29 ± 0.02	R, P2
germacrene D-4-ol[T3]*	1585	1574	2.36 ± 0.69	U	1.02 ± 0.05	0.76 ± 0.07	0.74 ± 0.10	T, P13
α-bisabolol[T13]	1698	1683	U	U	3.17 ± 0.73	U	U	T, P2
sum			**7.81**	**U**	**4.19**	**2.28**	**2.19**	

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Table 2. continued

compound[a]	RI[b]	RI*[c]	Mangshanyegan	Kaopan pummelo	Eureka lemon	Seike navel orange	Huangyanbendizao tangerine	ID[d]
alcohols								
1-penten-3-ol	696	693[n]	2.03 ± 0.66	U	U	1.44 ± 0.12	1.17 ± 0.33	R, P14
(*Z*)-3-hexenol	868	851	11.3 ± 1.83	19.5 ± 0.67	4.24 ± 0.25	9.01 ± 0.87	10.79 ± 0.77	R, P15
(*E*)-2-hexenol*	878	880[n]	13.98 ± 1.92	10.13 ± 0.30	U	8.81 ± 0.34	8.37 ± 0.05	R, P16
hexanol*	881	867	10.11 ± 0.82	7.96 ± 1.47	U	4.92 ± 0.32	5.76 ± 0.99	R, P15
octanol	1081	1070	2.48 ± 0.20	U	6.31 ± 0.79	15.17 ± 3.06	12.61 ± 1.56	R, P16
nonanol	1181	1171	U	U	1.87 ± 0.12	U	Tr[f]	R, P17
decanol	1281	1272	U	U	U	U	0.55 ± 0.10	R, P4
coniferyl alcohol	1761	1729	14.88 ± 3.61	8.28 ± 0.18	8.61 ± 0.17	8.85 ± 0.08	9.66 ± 1.71	R, N
sum			**54.78**	**45.87**	**21.03**	**48.20**	**48.91**	
aldehydes								
3-hexenal[T7]	811	801[n]	3.02 ± 0.14	2.46 ± 0.01	2.84 ± 0.25	3.10 ± 0.17	2.76 ± 0.19	T, P18
hexanal	812	800	3. 03 ± 0.73	1.30 ± 0.41	1.13 ± 0.39	4.99 ± 1.88	7.04 ± 1.43	R, P10
(*E*)-2-hexenal	870	854	8.07 ± 0.48	U	3.41 ± 1.10	8.85 ± 1.16	3.14 ± 0.75	R, P10
octanal	1014	1001	U	U	6.79 ± 1.01	38.23 ± 7.96	13.26 ± 1.08	R, P13
nonanal	1116	1102	4.38 ± 0.27	U	25.52 ± 4.17	16.47 ± 1.66	6.26 ± 4.65	R, P5
decanal	1216	1204	0.62 ± 0.11	U	1.46 ± 0.13	8.30 ± 0.19	5.98 ± 1.88	R, P13
undecanal	1318	1306	U	U	1.19 ± 0.18	0.59 ± 0.02	0.54 ± 0.09	R, P15
dodecanal	1418	1416[n]	1.43 ± 0.21	0.80 ± 0.34	1.54 ± 0.41	8.89 ± 0.21	8.53 ± 3.30	R, P13
sum			**17.52**	**4.56**	**43.88**	**89.42**	**47.51**	
acids								
hexanoic acid	990	990[n]	44.45 ± 3.49	U	U	36.34 ± 0.17	37.24 ± 0.58	R, P19
octanoic acid	1181	1179[n]	6.84 ± 0.21	U	U	6.88 ± 0.09	6.93 ± 0.07	R, P6
sum			**51.29**	**U**	**U**	**43.22**	**44.17**	
esters								
hexyl acetate*	1022	1008	1.14 ± 0.21	U	U	U	U	R, P1
hexyl butanoate*	1197	1191	1.02 ± 0.60	U	U	U	U	R,P17
(*Z*)-3-hexenyl hexanoate*	1386	1383[n]	0.49 ± 0.09	U	U	U	U	R, P3
methyl hexadecanoate	1932	1927	0.98 ± 0.26	1.11 ± 0.68	1.44 ± 0.38	0.73 ± 0.18	1.73 ± 0.08	R, P9
sum			**3.63**	**1.11**	**1.44**	**0.73**	**1.73**	
unknowns								
unknown[T11]	1295		U	U	3.25 ± 0.45	U	U	
unknown[T13]	1333		U	U	U	U	2.25 ± 0.36	
unknown[T11]	1488		U	U	3.17 ± 0.27	U	U	
unknown[T4]	1546		U	U	2.32 ± 0.18	U	U	
unknown[T11]	1548		4.67 ± 0.71	U	U	U	U	
unknown[T1]	1245		U	0.30 ± 0.04	U	U	U	
unknown[T6]	1599		U	U	2.24 ± 0.09	U	U	
sum			**4.67**	**0.30**	**10.98**	**U**	**2.25**	
others								
p-cymene	1033	1022	U	2.67 ± 0.71	1.42 ± 0.13	U	3.59 ± 0.58	R, P2
undecane*	1100	1100	0.80 ± 0.28	U	U	U	U	R, P10
indole	1312	1288	2.05 ± 0.66	1.58 ± 0.43	U	U	U	R, P1

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Table 2. continued

compound[a]	RI[b]	RI*[c]	Mangshanyegan	Kaopan pummelo	Eureka lemon	Seike navel orange	Huangyanbendizao tangerine	ID[d]
nootkatone	1824	1800	U	8.94 ± 2.25	2.07 ± 0.17	2.50 ± 0.29	U	R, P1
geranyl linalool*	1987	2008[n]	4.31 ± 1.19	U	U	U	U	R, N
sum			**7.16**	**13.19**	**3.49**	**2.50**	**3.59**	
total volatiles			**14696.20**	**5638.12**	**15560.02**	**13369.17**	**12041.73**	

[a]Tn (n = 1–13) corresponds to the equations of standard curves obtained in TIC mode listed in italics in Table 1. The asterisk (*) indicates significant differences between the volatile concentrations in Mangshanyegan and the other citrus ($p < 0.05$). [b]Retention index on TR-5 MS column. [c]Retention index on DB-5 column. Values were obtained from ref 47 except for RIs with a superscript n, which were obtained from the NIST Chemistry WebBook (http://webbook.nist.gov/chemistry). [d]ID method: T, tentatively identified compounds; R, compounds positively identified using authentic standards; P, compounds previously reported in citrus peel oil (references as follows: P1,[36] P2,[48] P3,[49] P4,[46] P5,[50] P6,[51] P7,[52] P8,[37] P9,[35] P10,[53] P11,[54] P12,[55] P13,[12] P14,[56] P15,[57] P16,[11] P17,[58] P18,[59] P19.[60] [e]Not detected. [f]Trace.

(E)-2-hexenol, α-muurolene, (E)-nerolidol, and piperitone, also had significantly higher levels in Mangshanyegan than in the other citrus (Table 2). Compounds found only in Mangshanyegan included (Z)-3-hexenyl caproate, geranyl linalool, hexyl acetate, hexyl butanoate, undecane, α-guaiene, and (Z)- and (E)-linalool oxides.

Major Volatile Compounds in the Other Citrus Analyzed. Nootkatone, a sesquiterpene ketone identified as one of the characteristic aroma compounds of pummelo or grapefruit,[31,32] was found at a significantly higher concentration in Kaopan pummelo than in the other citrus species.

Kaopan pummelo had the lowest levels of monoterpenes, at 5507.10 μg/g, whereas other citrus had levels ranging from 11804.88 to 14513.35 μg/g.

Monoterpene alcohols, monoterpene aldehydes, and monoterpene esters had extremely high concentrations in Eureka lemon. Nerol (48.17 μg/g), geraniol (47.14 μg/g), and α-terpineol (32.74 μg/g) together represented 72% of the total monoterpene alcohols of this fruit. Geranial (399.81 μg/g) and neral (185.57 μg/g) accounted for 97% of its total monoterpene aldehyde content. In addition, geranyl acetate (22.62 μg/g) and neryl acetate (48.75 μg/g) represented >88% of its monoterpene ester content. Nerol and geranyl isobutyrate were unique to Eureka lemon.

In Seike navel orange, the total amount of aldehydes was 89.42 μg/g, being significantly higher than in the other citrus. Among the eight aldehydes identified, octanal (38.23 μg/g) and nonanal (16.47 μg/g) predominated, together accounting for 61% of the total aldehyde content. Additionally, decanal had a significantly higher concentration in Seike navel orange (8.30 μg/g) than in the other citrus.

In Huangyanbendizao tangerine, p-cymene, β-elemene, δ-elemene, and (E)-β-ocimene had significantly higher levels than in the other citrus. In particular, the concentration of (E)-β-ocimene (70.36 μg/g) in this fruit was 4-fold higher than in the other citrus. Interestingly, (E)-piperitol and decanol were detected only in the Huangyanbendizao tangerine.

In this study, three volatile compounds, cadalene, coniferyl alcohol, and geranyl linalool, were identified for the first time in citrus peel. Cadalene was tentatively identified, whereas coniferyl alcohol and geranyl linalool were positively identified using authentic standards. Cadalene was reported in *Cistus ladaniferus* L. essential oil by Simon-Fuentes et al.[33] Coniferin (coniferyl alcohol-4-β-O-glucoside) was reported in lemon, orange, and grapefruit,[34] but this is the first report of free coniferyl alcohol in citrus.

AEDA of Mangshanyegan Peel Oil. GC-O coupled with AEDA is a method commonly used for the identification of characteristic aroma compounds.[35−37] In this study, the SDE extract was serially diluted in a 1:3 ratio. FD factors and odor descriptors are listed in Table 4. In the original SDE extract, 48 odor-active compounds were detected by GC-O, and 47 of them were identified by their aromas, RIs, and mass spectra. Twelve compounds were detected by sniffing in the 1:27 dilution (FD = 27). These compounds were β-pinene, β-myrcene, d-limonene, γ-terpinene, hexanol, (Z)- and (E)-linalool oxides, decanal, β-linalool, geraniol, dodecanol, and 2,4-di-*tert*-butylphenol, and their corresponding odor descriptors are given in Table 4. β-Myrcene, d-limonene, β-pinene, and γ-terpinene are monoterpenes. β-Myrcene, d-limonene, and β-pinene were the three most abundant compounds in Mangshanyegan, although the other monoterpene, γ-terpinene, was found at a low concentration (0.85 μg/g). Furthermore, 2,4-di-*tert*-butylphenol and

Table 3. Composition of the Aroma Model of Mangshanyegan

compound	volume in 10 mL of stock solution[b] (chemical/propylene glycol)	content in 1 mL of aroma model (μg)	no. of the aroma model solution[a] 1	2	3	4	5	6
limonene	10.00 mL/0 mL	8413.00	+	+	+	−	−	−
myrcene	1.35 mL/3.00 mL	1068.36	+	+	+	+	+	+
linalool	7.97 μL/1.00 mL	6.93	+	−	+	+	−	+
(*Z*)- and (*E*)-linalool oxides	3.15 μL/1.00 mL	2.98	+	+	−	+	+	−

[a]"+" indicates that an odorant was added to the model; "−" indicates that it was not added. [b]The stock solution was adjusted to 10mL with Milli-Q water.

Table 4. Odorants Detected in the Dilutions of the Mangshanyegan Peel Extract

compound[a]	RI[b]	RI*[c]	odor quality	FD
methyl acctate	839	816[n]	fruity, bitter	3
α-pinene	1028	1026[a]	pine-like	1
2-methyl-3-buten-2-ol	1038	1038[n]	fruity, green, sweet	9
unknown	1071		green, sweet	3
butyl acctate	1078	1085[b]	fruity, sweet	3
hexanal	1088	1092[b]	green, sweet, leafy	9
β-pinene	**1113**	**1113[a]**	**resinous, pungent, green, pine-like**	**27**
undecane	1145	1152[n]	green, dry	3
β-myrcene	**1185**	**1209[a]/1169[b]**	**balsamic, woody, floral, sweet, herbal, fruity, gardenia-like**	**27**
d-limonene	**1225**	**1215[a]**	**lemon-like, green**	**27**
β-phellandrene	1228	1222[a]	pungent	3
2-hexenal	1230	1212[n]	leafy, green	3
γ-terpinene	**1254**	**1254[a]**	**pine-like, lemon-like**	**27**
p-cymene	1285	1282[c]	lemon, green	1
terpinolene	1289	1288[a]	pine, sweet	9
octanal	1299	1296[a]	spicy, fruity-peel, green	9
hexanol	**1325**	**1355[b]**	**fruity, green, sweet, fresh**	**27**
(*Z*)-3-hexenol	1385	1379[b]	leafy, green	1
nonanal	1401	1392[a]	sweet, green	3
(*Z*)-2-hexen-1-ol	1403	1397[n]	spicy, fruity-ester	1
Z-linalool oxide	**1452**	**1445[a]**	**flower, woody, green, linalool-like**	**27**
(*E*)-linalool oxide	**1479**	**1473[a]**	**flower, woody, sweet, green, linalool-like**	**27**
citronellal	1486	1488[c]	lemon, sweet	3
ethyl hexanol	1489	1488[n]	green sweet	3
α-copaene	1504	1501[d]	green, pine-like, waxy, pungrnt	9
decanal	**1505**	**1504[d]**	**sweet, fruity, waxy, peel-like**	**27**
β-linalool	**1549**	**1544[a]**	**woody, muguet, green, fruit-like, flower**	**27**
(*Z*)-2-menthenol	1569		Lemon-like	3
β-elemene	1599	1597[d]	fresh, green	3
4-terpineol	1610	1612[b]	pine-like, sweet, green	9
hexyl caproate	1615	1601[n]	fruity, ester, sweet	9
(*Z*)-2-decenal	1655	1651[d]	fruity	9
(*Z*)-3-hexenyl hexanoate	1661	1646[n]	fruity	9
α-caryophyllene	1685	1681[c]	fruity, fresh, sweet	3
(*Z*)-citral	1692	1695[c]	lemon, fresh, sweet	3
α-terpineol	1702	1696[a]	pine-like	3
α-muurolene	1736	1734[d]	bay-leaf like	3
geranial	1743	1732[b]	lemon, fresh, sweet, citronella	9
elixene	1750	1646[d]	fruity green	1
δ-cadinene	1769	1766[d]	green, fruity, sweet	9
nerol	1800	1794[d]	flower, orange-sweet	3
geraniol	**1847**	**1861[d]**	**bay-leaf like, fruity, sweet**	**27**
dodecanol	**1967**	**1950[n]**	**fruity, sweet, pungent**	**27**
(*E*)-nerolidol	2041	2048[d]	flower, orange-sweet	3
elemol	2090	2091[d]	fruity, sweet	3
cembrene	2213	2175[n]	pine-like,sweet,pungent	9
geranyl linalool	2263	/	sweet	3
2,4-di-*tert*-butylphenol	**2306**	**2317[n]**	**phenol-like, pungent**	**27**

[a]Compounds were identified by their RI, mass spectra, and odor, except for (*Z*)-2-menthenol and geranyl linalool, which were identified only by mass spectra and odor. Compounds in bold had FD = 27. [b]Retention index on HP-Innowax column. [c]Retention index from ref [a]42, [b]61, [c]35, [d]39; [n]average values from NIST Chemistry WebBook (http://webbook.nist.gov/chemistry).

dodecanol could not be detected by GC-MS (using the TR-5 MS column). The rest of the compounds had concentrations ranging from 0.62 to 10.38 μg/g.

OAVs and Sensory Attributes. OAVs can be used to complement the results of the GC-O analysis. The OAVs of the aroma compounds detected by GC-O in the 1:27 dilution, together with their odor threshold values, are listed in Table 5.[26]

In Mangshanyegan, the OAVs for *β*-myrcene, *d*-limonene, and *β*-linalool were all beyond 1000. The OAV of *β*-myrcene was the highest, at 16165, although in the other citrus the OAV of this compound ranged from 579 to 1735. The OAVs of *d*-limonene and *β*-linalool in Mangshanyegan were only significantly higher than those in the Kaopan pummelo. Notably, the OAVs of *d*-limonene in Mangshanyegan, Eureka lemon, Seike navel orange, and Huangyanbendizao tangerine were very similar. However, the OAV of *β*-linalool in Mangshanyegan was significantly lower than the OAVs of this compound in Eureka lemon, Seike navel orange, and Huangyanbendizao tangerine. The OAVs of (*Z*)- and (*E*)-linalool oxides in Mangshanyegan were 25 and 11, respectively. Therefore, of all compounds with FD = 27 in Mangshanyegan, *β*-myrcene was the only one with a significantly higher OAV than in the other citrus. Additionally, (*Z*)- and (*E*)-linalool oxides were detected only in Mangshanyegan. The OAVs indicate that *β*-myrcene, (*Z*)-linalool oxide, and (*E*)-linalool oxide may be the compounds that differentiate the aroma of Mangshanyegan from those of the other citrus. Furthermore, the balsamic note of Mangshanyegan was found to be produced by *β*-myrcene, because this was the only aroma compound with balsamic smell found in the GC-O analysis, whereas

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Table 5. Odor Activity Values (OAVs) of Odorants with FD = 27 in Mangshanyegan and Comparison with Other Citrus

compound	Mangshanyegan	Kaopan pummelo	Eureka lemon	Seike navel orange	Huangyanbendizao tangerine	threshold in water from the literature (mg/kg)
β-myrcene	16165	579	1735	1715	1501	0.099
d-limonene	10501	3819	10505	10511	9338	1.200
β-linalool	1297	629	1575	4342	3173	0.008
decanal	310	0	730	4150	2990	0.002
geraniol	99	400	1178	52	8	0.040
(Z)-linalool oxide	25	0	0	0	0	0.100
β-pinene	20	7	126	1	4	4.160
(E)-linalool oxide	11	0	0	0	0	0.190
hexanol	6	5	0	3	4	1.620
γ-terpinene	3	2856	3640	4	1113	0.260

(Z)- and (E)-linalool oxide seemed to be responsible for the floral notes perceived in Mangshanyegan. To confirm the identities of the characteristic aroma compounds of Mangshanyegan, an omission test was carried out.

It is important to note that the OAVs of decanal, geraniol, β-pinene, and hexanol had a high variability among the citrus species analyzed, whereas γ-terpinene had the lowest OAV. 2,4-Di-*tert*-butylphenol and dodecanol could not be detected in the GC-MS analysis using the TR-5 MS column.

Omission Experiment and Characteristic Aroma Compounds of Mangshanyegan. The aroma of Mangshanyegan has distinctive balsamic and floral notes. In this study, the compound responsible for the balsamic note was identified as β-myrcene. Attention was then focused on identifying the compound(s) responsible for the characteristic floral notes. The omission experiment was performed by excluding linalool or (Z)- and (E)-linalool oxides. In addition, the influence of limonene, the most abundant aroma compound, was also evaluated to confirm whether this compound increased the intensity of the background lemon aroma.

ANOVA was used to evaluate the scores of six aroma model solutions (Table 3). The aroma solution with limonene but without linalool (2) had the highest score (7.3), and the aroma solution with all four compounds (1) had the second highest score (6.7). There was no significant difference between model solutions 1 and 2. Both scores were significantly higher than those obtained in the other four model solutions. The above results indicated that the aroma model 2 had a very similar smell to that of the Mangshanyegan peel. This in turn indicated that (Z)- and (E)-linalool oxides were the compounds responsible for the floral notes, because the aroma solution 3 (containing limonene and linalool but no linalool oxides) had a significantly lower score. Although linalool also has a floral note, the results show that it is not a characteristic aroma compound responsible for the floral notes of Mangshanyegan. However, the lack of significant differences between model solutions 1 and 2 suggests that linalool may strengthen the floral notes of Mangshanyegan.

The Student *t* test was carried out to determine whether limonene affected the aroma model solutions by providing a sensory background. The results revealed that the group containing limonene had a significantly higher score than the group without it ($p < 0.05$), which suggests that limonene has an important role as the background aroma of Mangshanyegan.

DISCUSSION

Monoterpenes accounted for >97% of total volatiles in Mangshanyegan. The three most abundant monoterpenes were also the top three volatiles in Mangshanyegan, including d-limonene (12601.77 μg/g), β-myrcene (1600.29 μg/g), and β-pinene (82.64 μg/g). d-Limonene represented 85.75% of total volatiles of Mangshanyegan, whereas β-myrcene represented 10.89%; the latter had significantly higher levels in Mangshanyegan than in the other citrus. d-Limonene, β-myrcene, and β-pinene together with γ-terpinene were identified as the monoterpenes having high FD factors (FD = 27) These four compounds have odor thresholds of 1.200, 0.099, 4.160, and 0.260 μg/g, respectively (Table 5).

Interestingly, oxygenated compounds had higher concentrations in the extract of Mangshanyegan peel than in the extracts of the other citrus. These compounds include (Z)- and (E)-linalool oxides, (Z)-hexenyl caproate, piperitone, elemol, (E)-nerolidol, germacrene D-4-ol, geranyl linalool, hexanoic acid, hexyl acetate, and hexyl butanoate.

The high OAV of d-limonene (10501) may be explained by its high concentration (12601.77 μg/g), whereas the high OAV of β-myrcene (16165) may be the result of its high content (1600.29 μg/g) and low odor threshold. However, the OAVs of β-pinene and γ-terpinene were low, whereas their FD factors were high. The reason for this might be that the odor thresholds determined in water do not reflect the true values in citrus peel. The odor thresholds not only change for different media (water, citrus peel, juice, or organic solution)[26,38] but are also affected by both environmental and anthropogenic factors. The odor threshold of (Z)-linalool oxide in water was 0.006 μg/g according to Bonvehi,[39] whereas Boonbumrung et al.[40] reported a value of 0.10 μg/g. Furthermore, Plotto et al.[38] reported that the odor thresholds of limonene and myrcene in juice were 13.7 and 0.773 μg/g, respectively, whereas in another paper[40] these values were 1.200 and 0.099 μg/g, respectively.

Previous studies have reported that monoterpenes are the major volatile compounds of citrus peel, representing >90% of the total volatiles, with d-limonene usually being the dominant compound, accounting in most cases for >60% of total volatiles. Notwithstanding their high concentrations, monoterpenes have been considered to be minor players in citrus aroma.[11,12,35,37,41] In contrast, many oxygenated compounds with low concentrations, but high OAVs, have been recognized as important components of the aroma.[11,12,35,37,41] Nguyen et al.[11] found that (R)-(+)-citronellal, which accounted only for 0.005−0.05% of the total volatiles, was responsible for the characteristic aroma of Kabosu (*Citrus sphaerocarpa*) instead of the much more abundant limonene and myrcene, which represented 70.5 and 20.2% of total volatiles, respectively. On the other hand, some studies have revealed a close relationship between the high concentration of a specific volatile and the characteristic

aroma of the fruit. Nguyen et al.[42] also reported that in cold-pressed peel oil of *Citrus kiyookadaidai*, myrcene was not only abundant (62.4% of total volatiles) but was also one of the characteristic aroma compounds. Thus, OAV and FD factor are important parameters in aroma analysis, but alone they cannot answer the question of whether a volatile is a characteristic aroma compound.[11,43,44] It is suggested, then, that to identify characteristic aroma compounds, a study coupling these parameters with odor descriptions should be conducted.

In this study, GC-O analysis and omission test resulted in the identification of β-myrcene as one of the characteristic aroma compounds of Mangshanyegan, being responsible for the balsamic note in the aroma of this fruit. On the other hand, the pine-like smell of β-pinene and γ-terpinene was found to be too different from the odorous notes perceived in Mangshanyegan. The floral notes of (*Z*)- and (*E*)-linalool were identified as important contributors to the aroma of Mangshanyegan peel. Furthermore, although linalool also has a floral smell, it did not determine the floral character of Mangshanyegan; but it is possible that this compound acts synergistically, strengthening the floral aroma. Limonene was also important to the background aroma of Mangshanyegan.

Apart from the presence of specific volatiles, it is also possible that the unique aroma of Mangshanyegan results from the low concentration or absence of particular compounds. Among all of the citrus species investigated, Mangshanyegan had particularly low amounts of nerol, β-citronellol, neryl acetate, geranyl acetate, and octanal (Table 2). Nguyen et al.[45] found that β-citronellol, neryl acetate, and geranyl acetate contributed to the characteristic aroma of the essential oil of lime (*Citrus aurantifolia* Persa) from Vietnam. Octanal was a characteristic aroma compound of daidai (*Citrus aurantium* L. var. *cyathifera* Y. Tanaka) peel oil.[43] Minh Tu et al.[46] found that myrcene, (*E*)-ocimene, (*Z*)-linalool oxide, (*E*)-linalool oxide, β-copaene, perillaldehyde, and perillyl alcohol were the characteristic aroma compounds of Mochiyu (*Citrus inflata* Hort. ex Tanaka) and suggested that (*E*)-ocimene could be a key aroma compound of this fruit. However, (*E*)-ocimene and perillaldehyde were found at low concentrations in Mangshanyegan, whereas β-copaene and perillyl alcohol could not be detected. Thus, it was supposed that the low concentrations or absence of the compounds mentioned above might be important for the unique aroma of Mangshanyegan peel.

In summary, the combined results of GC-MS, AEDA, and GC-O analyses, together with OAVs and omission test, allow us to conclude that β-myrcene, (*Z*)-linalool oxide, and (*E*)-linalool oxide are the characteristic aroma compounds of Mangshanyegan.

It is important to note that orange peel oil, the predominant essential oil in the world, is commercially extracted as a byproduct of the juice industry. With its inedible fruit, Mangshanyegan is a promising raw material for essential oil extraction like bergamot.

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