



The Identification and Quantitation of Components in Essential Oils of *Citrus kinokuni* Tanaka Peel

Zheng Pan^{1,2}, Ting-Li Han^{3,4} and Jian Wang^{1,2*}

¹Department of Chinese Medicine and Chinese Material Medica, Chongqing Medical University, Chongqing China

²Chongqing Key Laboratory of Traditional Chinese Medicine for Prevention and Cure of Metabolic Diseases, Chongqing, China

³First Affiliated Hospital of Chongqing Medical University, Chongqing, China

⁴Liggins Institute, University of Auckland, Auckland, New Zealand

***Corresponding Author:** Jian Wang, Department of Chinese medicine and Chinese Materia Medica (CMM), Chongqing Medical University, Chongqing, China.

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Abstract

The extracted essential oils from the peels of *Citrus kinokuni* Tanaka collected from the same plant grown in Nancheng town, Fuzhou city, Jiangxi province of China in different time, respectively, were separated by column chromatography through different eluents. The chemical compositions in the essential oils and its separated parts were investigated by gas chromatography-mass spectrometry (GC-MS). The Automated Mass Spectral Deconvolution and Identification System (AMDIS) combined in the GC-MS chemstation was used to deconvolve the overlapped or even embedded peaks in the total ion chromatogram (TIC) obtained by GC-MS, and to extract the characteristic ion peaks of corresponding compound. A total of 183 compounds were quantified in which 109 components were identified and 74 components with unknown identities were detected. The essential oils from *C. kinokuni* Tanaka peel were high in β -pinene, p-cymene, d-limonene, γ -terpinene, α -terpineol, linalool, thymol, (E,E)- α -farnesene, spathulenol, and (-)-spathulenol. At the same time, the chemotype of the essential oils was analyzed, which showed (E,E)- α -farnesene played an important role in differentiating the essential oils from the peel of *C. kinokuni* Tanaka from that of other varieties of *C. reticulata* Blanco. The study demonstrated the separation of essential oils by column chromatography through suitable eluents can differentiate the components effectively. In sequence, they are the hydrocarbon terpenes, the other oxygenated terpenes but not the alcohol of terpenes, the alcohol of terpenes, and acids, respectively.

Keywords: Citrus kinokuni Tanaka; Citri Pericarpium Reticulatae; Citri Pericarpium Reticulatae Viride; Essential Oil; Automated Mass Spectral Deconvolution and Identification System (AMDIS); Chemotype

Introduction

The peel of *Citrus reticulata* Blanco and its varieties is abundance in essential oil, which play an important role in the world flavor market and has important value in medicine. *C. kinokuni* Tanaka is a native variety, which usually planted in Nanfeng city or nearby, Jiangxi province of China. Despite its abundant growth and recognized use, few studies have reported on the chemicals in the essential oils from the peel of *C. kinokuni* Tanaka. The peel collected in different time points from some varieties such as *C. reticulata* 'Chachi', *C. reticulata* 'Dahongpao', *C. Erythrosa* Tanaka, etc., can be used as two kinds of Chinese material medica (CMM) such as Citri Pericarpium Reticulatae (CPR) and Citri Pericarpium Reticulatae Viride (CPRV). The dried pericarp of ripe fruits (CPR) is

usually collected from September to December. The dry pericarp of immature fruits (CPRV) can be subdivided into Fructus Citri Immaturus (FCI) and CPRV. The little fruit (FCI) was collected in May or June, and the dry pericarp (CPRV) was collected in July or August usually [1-4]. As a result, the peel can be classified into three kinds: FCI, CPRV, and CPR. CPR and CPRV have different effects and indications. They both belong to the *qi*-regulating CMM, while CPR is good at regulating the flow of *qi* and energizing the spleen function, and CPRV is adept in relaxing the liver and disintegrating stagnated *qi* [3,4]. The dissimilar effects and indications between CPR and CPRV should be associated with their bioactive components such as essential oil which is the main active component of CPRV and CPR.

Essential oil from the peel of *C. reticulata* is a complicated system and contains hundreds of chemical components. The overlapped or even embedded peaks extensively exist in the total ion chromatogram (TIC) obtained by gas chromatography-mass spectrometry (GC-MS), especially in the section of sesquiterpenoid, which would make the accurate quantitative and qualitative analyses more difficult [5,6]. To solve such problem, the essential oils extracted by hydrodistillation were separated into several parts by column chromatography through suitable eluents, and then the essential oils and its separated parts were subjected to GC-MS analysis. The Automated Mass Spectral Deconvolution and Identification System (AMDIS) combined with GC-MS ChemStation is an excellent tools analyzing the overlapped peaks in TIC [7]. In this study, the GC-MS ChemStation and AMDIS were used to deconvolve the overlapped or even embedded peaks in the TIC, and to extract the characteristic ion peaks of corresponding compound in the peaks.

Aim of the Study

The aim of this study was to investigate the chemicals in essential oils from the peel of *C. kinokuni* Tanaka thoroughly, and to analyze its chemotype.

Materials and Methods

Plant materials

The peels of *C. kinokuni* Tanaka were collected in July, September, and November in 2012 from the same plant grown in Nancheng town, Fuzhou city, Jiangxi province of China, respectively (Table 1).

Sample No.	Collected time	Color of peel	Classification by the collected status	Oil extraction rate % (Volume mL/Weight g * 100%)
Ck7	12-07-15	Black and green	FCI	3.00
Ck9	12-09-15	Yellow with green	CPRV	1.25
Ck11	12-11-15	Yellow	CPR	2.14

Table 1: Information about the peel collected in different time from *C. kinokuni* Tanaka and its oil extraction rate.

Chemical Standards and Reagents

n-Nonane (98%) used as an internal standard and Hexane (HPLC (High pressure liquid chromatography) grade) were pur-

chased from Adamas Reagent Company, Ltd. n-Alkane standard solution of C10-C25 purchased from Dr. Ehrenstorfer GmbH, Germany, was used to determine the linear retention index (LRI). Hexane (AR) and Ethyl Acetate (EtoAc) (AR) were purchased from Shanghai Titan Scientific Co., Ltd., China. Methanol (MeOH) (AR) was purchased from Chongqing chemical group, China. Silical gels which mesh number are 100-200 or 200-300 were purchased from Shanghai Titan Scientific Co., Ltd., China, and the department of Qingdao marine chemical industry, China, respectively.

Chemical standards were listed as the following. Linalool (98%+) and nerolidol (95%+) were purchased from Adamas Reagent Company, Ltd. d-Limonene (96%) was purchased from Acros organics, USA. Thymol was purchased from Shanghai Titan Chemical Company, Ltd., China. Carvacrol was purchased from Tokyo Chemical Industry, Japan. γ -Terpinene (97%) was purchased from wako pure chemical industries, Ltd., Japan. α -Caryophyllene ($\geq 96\%$) was purchased from Sigma-aldrich.

The extraction of essential oil

The peels of *C. kinokuni* Tanaka divided into about 0.4 cm \times 0.4 cm sections weighed between 30.0 - 40.0g were swollen with 10 times volume (volume/weight) 300 - 400 mL of pure water in a 500 or 1000 mL round-bottomed flask, then soaked for 0.5 h at 40°C. The essential oil was extracted by Clevenger-type apparatus for 3 - 4h. Subsequently, the essential oil was prepared according to the procedure described in Chinese pharmacopoeia [1]. The essential oil was stored in separate screw-capped vials in a refrigerator at 4°C until needed. The essential oil was diluted as Volume_{oil}: Volume_{hexane} equal to 1: 50 for detection by GC-MS.

Separation of the essential oil

The essential oil was separated by column chromatography, the eluents were decided by the retardation factor (Rf) value recorded in the thin layer chromatography (TLC), which was n-Hexane: EtoAc with different volume ratio in sequence, that was 97: 3, 9: 1, 8: 2, and 0: 1. MeOH was used as the eluent in the end. Every eluent was eluted by two column volumes, and every column volume was received by a flask.

GC-MS analysis

An Agilent 7890B gas chromatograph (USA) matched with an Agilent 5977A mass spectrometer was used for GC-MS analysis. The gas chromatograph was coupled with an Agilent fused silica capillary column DB-5 (3m \times 0.25 mm i.d., 0.25 μ m film thickness). The oven temperature was programmed from 60°C (3-min hold)

to 270°C at 3°C min⁻¹, and then held for 8 minutes. The carrier gas was helium at a constant flow of 1 ml min⁻¹. Injector and ion-source were remained at 280°C and 200°C, respectively. Transfer line was set to 280°C. Splitting ratio was 20:1. Solvent delay was 3 minutes. Electron impact mass spectra were taken at 70 eV. Scan at 3.9 scans s⁻¹ from m/z 30 to 400 amu. The injection volume was 1 µl of a 1: 50 (Volume_{oil}: Volume_{hexane}) solution to obtain the appropriate peak intensity [8].

Component Identification

The peaks in the TICs obtained by GC-MS were identified by probability based matching (PBM) searching first. The AMDIS was used to deconvolve the overlapped or even embedded peaks in TICs. After deconvolution, the purified mass spectrum of these peaks was compared with the National Institute of Standard and Technology (NIST) 08 database or the mass spectra of standards. The LRIs of peaks were calculated and also compared with the LRIs of the corresponding matched chemicals provided by NIST 08 database or the standards.

Quantitation of Components

The quantitative analysis was directly calculated by the overall volume integration method.

Results and Discussion

Identification and Quantitation Outcome

The identification and quantitation result was displayed in table 2. Since most components in the essential oils were eluted when the Ethyl Acetate (EtoAc) was eluted by the first column volume, the components in elution as the EtoAc by the second column volume, and the elution of Methanol (MeOH) was not displayed in table 2. As a result, seven separated parts of each essential oil sample were recorded in table 2. A total of 183 compounds were quantitated in which 109 components were identified and 74 components were unknown. With regards to their relative area ratio, β-pinene, p-cymene, d-limonene, γ-terpinene, linalool, α-terpineol, thymol, (E, E)-α-farnesene, spathulenol, and (-)-spathulenol were found in high levels.

The (E, E)-α-farnesene belonging to hydrocarbon sesquiterpene (HS) was relatively high compared with the essential oils from peels of other varieties such as *C. reticulata* 'Chachi', *C. reticulata* 'Da-hongpao', *C. erythrosa* Tanaka, etc., which would be an important characterization to distinguish the essential oil from the peel of *C. kinokuni* Tanaka from that of other varieties [6]. Linalool, thymol,

α-terpineol, spathulenol, and (-)-spathulenol were the prominent oxygenated compounds. The content of d-limonene was relatively low while the content of p-cymene was relatively high compared with that of other varieties [5,6,8-12].

Many compounds can't be identified through the probability based matching (PBM) searching or National Institute of Standard and Technology (NIST) 08 database searching directly, but their mass spectra can give much valuable information to their structure. Many of them can be elucidated to which structure type such as hydrocarbon monoterpene (HM), HS, alcohol of monoterpene (AM), alcohol of sesquiterpene (AS), other oxygenated monoterpene (OOM) but not the AM, other oxygenated sesquiterpene (OOS) but not the AS, etc [13]. For example, the ion peak such as 139 (81) in the mass spectrum of compound 176 as Unknown-70 should be an important hint to elucidate its structure. Its abundance was close to the base peak, which demonstrated that the compound most probably belongs to carotenoid, the elucidated plane structure is listed in the following [14]. The exact structure will be confirmed in the following research.

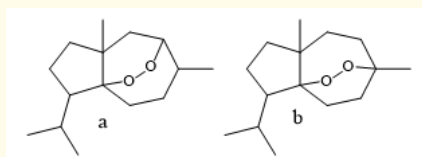


Figure 1: The probably plane structure of compound 176 (Unknown-70).

In the separated parts of essential oils, it can be seen that most hydrocarbon terpene (HT) including HM and HS were eluted in the eluent as n-Hexane: EtoAc (volume: volume equal to 97: 3), and more HS were detected in the second column volume eluted by n-Hexane: EtoAc (97: 3). There is none or few oxygenated compounds in the eluent as n-Hexane: EtoAc (97: 3), except the second column volume eluted by n-Hexane: EtoAc (97: 3) from the essential oil of the peel of *C. kinokuni* Tanaka collected in July, which area percentage of total oxygenated compounds is as high as 91.55%, and 70.71% of it was thymol and its 3 isomers. Thymol and its 3 isomers were mainly quantified in the eluent as n-Hexane: EtoAc (volume: volume equal to 9: 1), usually they are quantified in the second column volume of n-Hexane: EtoAc (9: 1). Their MS spectra were highly similar with each other (seen in Table 2). The

41	Cyclohexanol, 5-methyl-2-(1-methylethyl)-, (1 α ,2 β ,5 α)-(.+/-)-	1164-E	1168			+			0.02					8.87			12.14			1.96			2.29				
42	Octanoic Acid	1156	1169	+	0.06	0.04														+			2.98	7.98			
43	Epoxylinolol	1136	1169	+		+														+	+						
44	<i>cis</i> -Citral	1208	1172	+		+																					
45	Terpinen-4-ol	1162	1173	0.79	0.81	0.57								7.99	5.41												
46	<i>p</i> -Cymen-8-ol	1172	1179	0.09	0.12	0.08											0.34	2.07	0.56	1.49			1.74	0.56			
47	Cyclohexanol, 2-methylene-5-(1-methylethenyl)-	1186	1183	0.08	0.23	0.14											1.14	1.29	3.24	0.92			0.57				
48	α -Terpineol	1172	1186	2.12	1.12	0.81											4.97	51.75	6.03	19.99	24.61	15.47	4.62		+	1.75	
49	Dihydrocarveol	1182	1190	+																+							
50	<i>p</i> -Menth-1-en-3-ol, <i>cis</i> -	1190	1191		0.15												0.08										
51	Unknown-7(AM)		1191	0.07	+	0.10																					
52	Myrtenol	1191	1192																								
53	Unknown-8(AM)		1194	+	+	+																					
54	Unknown-9(AM)		1195	0.05	0.18	0.14																					
55	Unknown-10(OOM)		1199	+	0.01	0.01																					
56	Decanal	1184	1202	0.04	0.06	0.07	0.09																				
57	<i>trans-p</i> -Menth-1-en-3-ol	1196	1203			+																					
58	Eucarvone	1248	1212	+													0.65	+									
59	<i>trans</i> -Carveol	1206	1214	0.15	0.28	0.21																					
60	Unknown-11(OOM)		1214																								
61	Unknown-12(AM)		1222	0.06	0.19	0.13																					
62	Nerol	1215	1224	+	+	+																					
63	β -Citronellol	1208	1224	+	+	+																					
64	<i>cis</i> -Carveol	1208	1225	0.10	0.18	0.13																					
65	Thymol methyl ether	1215	1231	0.05	0.05	0.07	0.58																				
66	Unknown-13(OOM)		1232		0.11																						
67	Propanal, 2-methyl-3-phenyl-	1244	1233		+																						
68	Carvone	1220	1237	0.17	0.12	0.09																					
69	Unknown-14(OOM)		1247		0.05	0.03																					
70	Piperitone	1243	1248																								
71	<i>trans</i> -Geraniol	1238	1250	0.04	0.03	0.02																					
72	Perillaldehyde	1246	1267	0.02	0.21	0.13																					
73	1,7-Octadiene-3,6-diol, 2,6-dimethyl-	1265	1267		+																						7.53
74	Unknown-15(OOM)		1268																								
75	Unknown-16(OOM)		1268	+		+																					
76	Nonanoic acid	1268	1268																							4.25	2.21
77	Unknown-17(IT)		1282	0.03	0.12	0.09			1.36		1.90															+	
78	<i>p</i> -Mentha-1(7),8(10)-dien-9-ol	1287	1284	0.02	0.05	0.04																					
79	Unknown-18		1288	+																							1.97
80	Thymol	1266	1289	0.94	2.28	1.90	0.18		66.51		66.70			2.97	53.84	31.61	0.46	1.58	6.87			0.26	1.26		+	2.82	
81	<i>p</i> -Menth-1-en-9-ol	1285	1290																								
82	Unknown-19(IT)		1291	0.02	0.10	0.06																				+	+
83	Carvacrol	1278	1297	0.07	0.42	0.23			2.84		5.58																0.64

two components such as thymol and carvacrol were identified by comparison with the corresponding standards, respectively. Such exception is due to the essential oil from the peel of *C. kinokuni* Tanaka collected in July was eluted after the elution of the essential oil from the peel of *C. kinokuni* Tanaka collected in September by EtoAc for two column volumes in the same column with the same silica gel. The existed little EtoAc in the column may increase the polarity of the eluent as n-Hexane: EtoAc (97: 3) for the essential oil from the peel of *C. kinokuni* Tanaka collected in July, and more oxygenated compounds would be eluted especially for thymol and its 3 isomers.

From the eluents as n-Hexane: EtoAc (9: 1) to (8: 2), and to EtoAc, the total area percentage of oxygenated compounds was more than 90% or even equal to 100%, except the first column volume eluted by n-Hexane: EtoAc (9: 1) of essential oil from the peel of *C. kinokuni* Tanaka collected in July, and the first column volume eluted by EtoAc of essential oil from the peel of *C. kinokuni* Tanaka collected in September, which is 74.17% and 65.95%, respectively. In the first column volume eluted by n-Hexane: EtoAc (9: 1) of essential oil from the peel of *C. kinokuni* Tanaka collected in July, the area percentage of (E,E)- α -Farnesene is 23.91%, which may be caused by the fact listed above. While for the first column volume eluted by EtoAc of essential oil from the peel of *C. kinokuni* Tanaka collected in September, the area ratio of compound 133 (Unknown-38) is 32.08%, which structure class can't be deduced by its mass spectrum, and it is not be classified as the oxygenated compound although it should be. The main oxygenated compound is oxygenated terpene in the elution as n-Hexane: EtoAc (9: 1) and the first column volume of n-Hexane: EtoAc (8: 2), while the content of oxygenated terpene in the second column volume of n-Hexane: EtoAc (8: 2) and the first column volume of EtoAc was decreased compared with that in the elution as n-Hexane: EtoAc (9: 1) and the first column volume of n-Hexane: EtoAc (8: 2), except the second column volume of n-Hexane: EtoAc (8: 2) of essential oil from the peel of *C. kinokuni* Tanaka collected in September. The area percentage of acid and ester in the second column volume of n-Hexane: EtoAc (8: 2) and the first column volume of EtoAc is relatively high compared with that in the elution as n-Hexane: EtoAc (9: 1) and the first column volume of n-Hexane: EtoAc (8: 2), except the value in the elution as the second column volume of n-Hexane: EtoAc (8: 2) of the essential oil from the peel of *C. kinokuni* Tanaka collected in September which is 0%. It can be seen that the acids such as n-hexadecanoic acid were mainly eluted by the elution as

the second column volume of n-Hexane: EtoAc (8: 2) and the first column volume of EtoAc. As for the oxygenated terpene in the elution as n-Hexane: EtoAc (9: 1) and the first column volume of n-Hexane: EtoAc (8: 2), the main compound is the other oxygenated terpene but not the alcohol of terpene in the elution as the first column volume of n-Hexane: EtoAc (9: 1), the area ratio of alcohol of terpene is increasing while the content of the other oxygenated terpene is decreased in the elution as the second column volume of n-Hexane: EtoAc (9: 1), especially for the elution as the second column volume of n-Hexane: EtoAc (9: 1) of the essential oil from the peel of *C. kinokuni* Tanaka collected in July. The main compound is the alcohol of terpene but not the other oxygenated terpene in the elution as the first column volume of n-Hexane: EtoAc (8: 2), two. In conclusion, the components in the essential oil can be effectively separated by the successive elutions in this study, and they can be divided into HM and HS, other oxygenated monoterpene (OOM) and other oxygenated sesquiterpene (OOS), alcohol of monoterpene (AM) and alcohol of sesquiterpene (AT), and acids, in sequence in a whole.

Chemical Variability of essential oil in CPRV and CPR of *C. kinokuni* Tanaka

Three samples have almost the equivalent qualitative constituents of HM, which represent the main compound family in all essential oils. Three specimens all differed qualitatively and quantitatively in HS to some extent which amounts were relatively poor. Three samples varied qualitatively and quantitatively in oxygenated compounds greatly which contents (7.88%, 15.81%, 11.01%) were relatively medium compared with the other essential oil from peel of *C. reticulata* [5,6,8-12]. Alcohol terpene including 58 compounds, and thymol and its 3 isomers were the main groups.

The chemotype of essential oil from peel of *C. kinokuni* Tanaka

Lota., *et al.* [16] differentiate the chemotype of essential oils from the peel of different cultivars of *C. reticulata* by the contents of d-limonene and γ -terpinene. In addition to these two important components, other prominent components such as linalool, methyl N-methylantranilate, and (E, E)- α -farnesene identified in this study should be considered also. The standard to classify the chemotype was established by the authors previously [17]. The current standard is listed in table 2, and there are three criteria for each component.

Component	Characteristical ion peaks (M/W, %)
Unknown-1 (OOM)	79 (100) 94 (57) 137 (35) 43 (17) 152 (17) 77 (17)
Unknown-2 (HM)	79 (100) 59 (35) 105 (22) 93 (21) 67 (19) 136 (5.7)
Unknown-3 (AM)	79 (100) 110 (65) 119 (3.9) 134 (2.5) 137 (1.8) 152 (1.8)
Unknown-4 (AM)	109 (100) 67 (63) 119 (52) 137 (45) 152 (23) 134 (16)
Unknown-5 (AM)	79 (100) 136 (60) 80 (50) 93 (34) 108 (16) 121 (11)
Unknown-6 (AM)	71 (100) 43 (96) 81 (51) 109 (45) 152 (9.5) 134 (6.6)
Unknown-7 (AM)	79 (100) 94 (82) 121 (60) 137 (22) 134 (11) 152 (9.6)
Unknown-8 (AM)	121 (100) 94 (82) 105 (35) 137 (16) 134 (9.0) 152 (2.2)
Unknown-9 (AM)	84 (100) 91 (20) 41 (14) 134 (12) 137 (2.9) 152 (1.8)
Unknown-10 (OOM)	79 (100) 122 (93) 93 (87) 150 (37) 132 (7.8) 135 (6.4)
Unknown-11 (OOM)	135 (100) 108 (31) 136 (8.8) 137 (6.4) 152 (3.1) 134 (1.8)
Unknown-12 (AM)	109 (100) 67 (55) 119 (43) 134 (24) 137 (14) 152 (3.3)
Unknown-13 (OOM)	121 (100) 136 (66) 93 (63) 152 (1.9) 150 (1.8) 168 (0.6)
Unknown-14 (OOM)	43 (100) 109 (79) 71 (71) 81 (49) 153 (7.4) 170 (1.1)
Unknown-15 (OOM)	43 (100) 91 (93) 79 (75) 77 (53) 135 (20) 150 (3.4)
Unknown-16 (OOM)	109 (100) 95 (32) 81 (30) 50 (12) 124 (11) 137 (8.6)
Unknown-17 (IT)	135 (100) 150 (35) 115 (15) 107 (7.8) 77 (6.7) 121 (2.8)
Unknown-18	43 (100) 107 (61) 58 (23) 82 (23) 95 (21) 122 (5.5)
Unknown-19 (IT)	135 (100) 150 (30) 107 (7.3) 115 (7.3) 77 (7.0) 121 (2.8)
Unknown-20 (OOM)	59 (100) 71 (88) 43 (73) 108 (30) 137 (18) 152 (2.2)
Unknown-21 (OOM)	43 (100) 97 (77) 69 (44) 125 (29) 139 (12) 168 (2.1)
Unknown-22 (OOM)	79 (100) 109 (62) 135 (41) 137 (35) 150 (2.2) 152 (2.8)
Unknown-23 (OOM)	100 (100) 69 (35) 41 (30) 82 (20) 152 (5.4) 168 (2.6)
Unknown-24 (OOM)	84 (100) 71 (80) 43 (75) 109 (68) 152 (21) 134 (16)
Unknown-25 (OOM)	109 (100) 81 (54) 67 (53) 43 (43) 137 (36) 152 (30)
Unknown-26 (HS)	107 (100) 121 (68) 204 (50) 189 (47) 148 (15) 175 (7.2)

Unknown-27	59 (100) 94 (54) 43 (41) 71 (28) 173 (9.7) 188 (3.7)
Unknown-28 (OOS)	145 (100) 157 (45) 160 (37) 168 (23) 200 (11), 185 (9.3)
Unknown-29	93 (100) 119 (83) 69 (43) 107 (42) 79 (35) 55 (25)
Unknown-30	83 (100) 111 (48) 55 (25) 112 (24) 182 (8.9) 153 (6.1)
Unknown-31 (AS)	91 (100) 105 (94) 119 (62) 202 (30) 205 (18) 220 (1.4)
Unknown-32 (AS)	43 (100) 161 (96) 107 (94) 189 (44) 204 (38) 222 (3.0)
Unknown-33 (AS)	161 (100) 105 (85) 109 (82) 93 (78) 204 (37) 222 (3.3)
Unknown-34 (AS)	149 (100) 105 (43) 161 (38) 177 (24) 204 (15) 222 (2.8)
Unknown-35 (OOS)	119 (100) 43 (80) 91 (72) 147 (64) 220 (13) 205 (9.2)
Unknown-36 (OOS)	79 (100) 43 (93) 69 (75) 41 (70) 119 (38) 205 (8.8)
Unknown-37 (OOS)	109 (100) 67 (82) 93 (77) 138 (73) 220 (7.0) 202 (2.1)
Unknown-38	247 (100), 248 (17), 262 (15), 231 (5.6), 159 (4.6), 157 (4.5)
Unknown-39 (AS)	119 (100) 59 (62) 147 (51) 187 (22) 202 (14) 220 (0.8)
Unknown-40 (OOS)	131 (100) 91 (91) 159 (86) 205 (14) 202 (9.8) 220 (4.8)
Unknown-41 (OOS)	79 (100) 69 (88) 93 (46) 134 (24) 202 (1.8) 220 (1.3)
Unknown-42 (OOS)	69 (100) 41 (35) 83 (29) 55 (22) 93 (19) 159 (8.1)
Unknown-43 (OOS)	119 (100) 93 (90) 134 (42) 187 (4.9) 220 (1.5) 202 (1.4)
Unknown-44 (OOS)	93 (100) 79 (52) 105 (49) 121 (38) 202 (2.2) 173 (1.7)
Unknown-45 (HS)	185 (100) 200 (54) 143 (30) 157 (29) 169 (11) 142 (17)
Unknown-46	119 (100) 161 (85) 204 (40) 159 (31) 179 (17) 189 (6.8)
Unknown-47	217 (100) 232 (45) 218 (22) 128 (9.9) 175 (8.4) 201 (6.1)
Unknown-48 (AS)	161 (100) 119 (57) 179 (49) 105 (44) 204 (30) 93 (22)
Unknown-49 (AS)	187 (100) 161 (30) 79 (28) 159 (20) 174 (16) 202 (13)
Unknown-50 (AS)	159 (100) 187 (78) 131 (70) 220 (65) 91 (56) 202 (36)
Unknown-51 (OOS)	123 (100) 82 (62) 177 (59) 205 (20) 220 (8.5) 202 (8.2)
Unknown-52 (OOS)	187 (100) 202 (96) 121 (36) 91 (33) 159 (20) 173 (19)
Unknown-53 (OOS)	159 (100) 91 (60) 105 (51) 177 (45) 220 (31) 202 (12)

Unknown-54 (AS)	149 (100) 59 (88) 161 (88) 189 (83) 204 (71) 222 (5.7)
Unknown-55 (AS)	91 (100) 105 (86) 93 (85) 43 (78) 159 (72) 202 (23)
Unknown-56 (AS)	159 (100) 41 (70) 69 (69) 93 (67) 162 (28) 202 (25)
Unknown-57 (OOS)	105 (100) 92 (49) 131 (43) 117 (43) 160 (29) 132 (18)
Unknown-58 (OOS)	145 (100) 105 (71) 91 (55) 119 (32) 160 (18) 218 (10)
Unknown-59	183 (100) 198 (45) 168 (28) 153 (20) 165 (19) 212 (3.9)
Unknown-60 (OOS)	159 (100) 91 (72) 220 (29) 177 (23) 202 (14) 205 (5.5)
Unknown-61 (OOS)	109 (100) 91 (87) 159 (77) 220 (5.9) 236 (5.8) 204 (3.8)
Unknown-62	129 (100) 185 (42) 101 (14) 56 (13) 231 (0.9) 202 (0.6)
Unknown-63 (OOS)	133 (100) 93 (77) 145 (68) 159 (45) 220 (25) 202 (20)
Unknown-64 (OOS)	159 (100) 187 (77) 131 (73) 220 (70) 145 (56) 202 (32)
Unknown-65	91 (100) 105 (92) 145 (87) 119 (78) 77 (57) 160 (30)
Unknown-66 (OOS)	93 (100) 81 (92) 107 (83) 159 (50) 202 (14) 220 (38)
Unknown-67 (OOS)	41 (100) 69 (89) 82 (53) 159 (9.4) 202 (5.1) 189 (2.1)
Unknown-68 (OOS)	69 (100) 41 (90) 82 (43) 121 (19) 159 (16) 202 (5.2)
Unknown-69 (OOS)	159 (100) 220 (97) 91 (67) 135 (47) 205 (12) 202 (8.2)
Unknown-70 (OOS)	55 (100) 95 (86) 139 (81) 167 (53) 238 (42) 195 (8.3)
Unknown-71 (OOS)	55 (100) 97 (73) 167 (67) 266 (38) 195 (34) 235 (3.1)
Unknown-72 (OOS)	55 (100) 83 (94) 139 (86) 181 (34) 266 (34) 223 (7.8)
Unknown-73	55 (100) 83 (91) 167 (61) 195 (33) 294 (30) 263 (3.9)
Unknown-74 (OOD)	107 (100) 91 (58) 243 (27) 286 (14) 271 (5) 268 (4.0)

Table 3: The characteristically ion peaks of unknown compounds in essential oil of peel collected in different time from *C. kinokuni* Tanaka.

By such standard, the essential oils from the peel of *C. kinokuni* Tanaka collected in July, September, and November, respectively, can be classified as the chemotype as limonene III/ γ -terpinene III/(E, E)- α -farnesene I, d-limonene III/linalool III/(E, E)- α -farnesene II, and d-limonene II/linalool III/(E, E)- α -farnesene I, respectively.

Component	Standard (by the relative area percentage % detected by GC-MS)	Chemotype classification
<i>d</i> -Limonene	≥ 85.0	limonene I
	$85.0 > \geq 70.0$	limonene II
	$70.0 > \geq 55.0$	limonene III
γ -Terpinene	≥ 12.0	γ -terpinene I
	$12.0 > \geq 8.0$	γ -terpinene II
	$8.0 > \geq 4.0$	γ -terpinene III
Linalool	≥ 6.0	linalool I
	$6.0 > \geq 4.0$	linalool II
	$4.0 > \geq 2.0$	linalool III
Methyl N-methylan-thranilate	≥ 2.5	methyl N-methylan-thranilate I
	$2.5 > \geq 1.5$	methyl N-methylan-thranilate II
	$1.5 > \geq 0.5$	methyl N-methylan-thranilate III
<i>(E,E)</i> - α -Farnesene	≥ 0.9	<i>(E,E)</i> - α -farnesene I
	$0.9 > \geq 0.6$	<i>(E,E)</i> - α -farnesene II
	$0.6 > \geq 0.3$	<i>(E,E)</i> - α -farnesene III

Table 4: The standard for the chemotype classification of essential oil EO from the peel of *C. reticulata*.

Conclusion

The various constituents in essential oils and its separated parts by column chromatography of CPRV and CPR of *C. kinokuni* Tanaka from the same origin were identified and quantified by GC-MS and AMDIS, and then compared based on their qualitative and relatively quantitative results. The AMDIS can effectively deconvolve the overlapped peaks in TIC, and can extract the characteristic ion peaks of corresponding compound. The essential oils' chemotypes of CPRV and CPR from *C. kinokuni* Tanaka were first analyzed. The amount of (E,E)- α -farnesene present in the peel essential oil of *C. kinokuni* Tanaka demonstrated that it is an important component for classifying the essential oil chemotype of peel of *C. kinokuni* Tanaka from that of other varieties. The separation by column chromatography of the essential oil can effectively separate the different type components and reduce the overlapped peaks in TIC.

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