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Multiple headspace solid-phase microextraction for quantifying volatiles in *Citrus reticulata* 'Chachi' peel of different storage time*

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Abstract: The dried pericarp of *Citrus reticulata* 'Chachi' (GCP, "Guangchenpi" in Chinese) is widely used in traditional Chinese medicine and cuisine. It has been generally recognized that its health benefits are dependent on storage time. As storage years increase, the most relevant components that are volatile oils. However, there are very few reports on the change in the content of volatiles in GCP with the increase of storage years. In this study, we examined the volatiles of GCP based on the headspace solid-phase microextraction method and the gas chromatography-mass spectrometry (HS-SPME-GC-MS). Factors of the extraction duration and temperature, sample size, and the type of fiber coatings were evaluated to quantify the D-Limonene, γ-Terpinene, L-α-Terpineol, and 2- (Methylamino) -benzoic acid-methyl ester in the GCP using the multiple headspace solid-phase microextraction approach (MHS-SPME). A total of 50 volatile compounds were identified, including terpenic, alcohol, aldehyde, and ester series. Results show that the terpenic series in the GCP decays much quicker than the ester and alcohol. The findings in the study shall help discriminate the behavior of the volatile substance during the GCP aging.

Key words: MHS-SPME; GC-MS; *Citrus reticulata* 'Chachi' peel; volatiles
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The dried pericarp of *Citrus reticulata* 'Chachi' (GCP, "Guangchenpi" in Chinese) is widely used in traditional Chinese medicine and cuisine. Valued for its nutritional and medical qualities, GCP is also used for flavoring, snacks, and tea in China and other Asian regions (He et al. , 2019). Generally, GCP is distinguished from the pericarps derived from other cultivars of *Citrus reticulata* Blanco (collectively called Chenpi in Chinese, CP) and GCP has always been well-regarded as the best national product with respect to geo-herbalism (Liu et al. , 2013; Zheng et al. , 2018; Su et al. , 2023). It is commonly believed that the longer it is stored, the higher quality it is (Zhang et al. ,2022). Pharmacological studies demonstrated that CP has been widely used to treat dyspepsia diseases, and compared with the results of the

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recent 5 and 10 years of the CP study, the 20-year CP is more effective in treating functional dyspepsia in clinical practice (Wang et al. , 2016; Fu et al. , 2017; Shi et al. ,2018; Bian et al. ,2022; Zhang et al. ,2022). In the market, the price of 20-year GCP is much higher than fresh GCP. Therefore, there exists considerable interest in developing accurate methods distinguishingthe storage years of GCP.

The main chemical components of GCP are flavonoids, volatile oils, and alkaloids. As storage years increase, the most relevant components that change are volatile oils (Zheng et al. ,2018; Yu et al. ,2022). Such volatile oils that consist of mainly monoterpenes, sesquiterpenes, and oxygenate compounds (Gorinstein et al. ,2001;Tranchida et al. ,2012), are acting antioxidant, antibacterial and antiviral functions by synergy or duplicate effect (Wang et al. ,2016). Limonene predominates among monoterpenes, followed by γ -terpinene(Yu et al. , 2009). Large changes are found in oxygenated compounds of the GCP from different origins, with principal ingredients like Linalool, Thymol, and α -Terpineol included (Minh Tu et al. ,2002). Sesquiterpenes, account for the less α-Farnesene and germacrene D. Special compound like nitrogenous compound 2-methyla minomethyl ester is also included. Therefore, variations in essential oils can serve as important indicators for determining the quality and age of CP.

With the expansion of the CP industry, the correlation between the quality of CP and its aging years has aroused increasing attention. The longer the aging period, the higher its economic value (Yu et al. , 2022). To meet market demands, the development of rapid and accurate identification techniques is of great significance for ensuring the quality of GCP and protecting consumer rights. Headspace solid-phase microextraction (HS-SPME) is a technique used for the extraction of volatile compounds. It is characterized by its simplicity, rapid analysis, high sensitivity, high throughput, low sample requirements, and a solvent-free operation, making it an efficient and effective method in various analytical applications (Zheng et al. ,2018). As an improvement over HS-SPME, multiple HS-SPME (MHS-SPME) has been proposed to successfully perform quantitation of volatile analytes from solid samples overcoming the matrix effect (Lei et al. ,2012; Costa et al. ,2013). It has been successfully applied in the quantitation of a number of volatiles in a variety of solid matrices including tomatoes (Serrano et al. , 2009) and cheeses (Rincón et al. , 2014).

The aim of this work is to develop an approach to reach the changes in the content of volatiles in GCP with the increase of storage years. Accurate and quick quantification of the main volatile compounds in the GCP, such as D-Limonene, γ-Terpinene, L-α-Terpineol, and 2-(Methylamino)-benzoic acid-methyl ester was based on the MHS-SPME method.

1 Experimental

1. 1 Materials

GCP samples in different storage years (1−8 years) were supplied by Guangzhou Baiyunshan Chenliji Pharmaceutical Factory Co. , Ltd. (Guangdong, China). They were all officially authenticated.

D-Limonene and 2-(Methylamino)-benzoic acid-methyl ester were purchased from the National Institute for Control of Biological and Pharmaceutical Products of China (Beijing, China). γ-Terpinene and L- α -Terpineol and Caryophyllene were purchased from Yuanye Bio-Technology (Shanghai, China). Decanal and Tridecane were purchased from Sigma-Aldrich (St. Louis. , MO, USA), and methyl alcohol was obtained from Macklin (Shanghai, China). The suppliers stated purity of all standards was above 95%.

Three types of SPME fibers were used: 65 μm divinylbenzene/polydimethylsiloxane (DVB/PDMS), 80 μm divinylbenzene /carbon /polydimethylsiloxane (DVB/C-WR/PDMS), 95 μm carbon/polydimethylsiloxane (C-WR/PDMS) fibers, all obtained from Agilent (Palo Alto, USA).

1. 2 HS-SPME extraction

Prior to the HS-SPME analysis, the samples were powdered by a mill and passed through a 60-mesh sieve. Then 20 mg of sample powders were placed in a 20 mL headspace, and adding 5 μL Tridecane (0. 166 4 mg/mL dissolved in methyl alcohol) as an internal standard.

According to the previous studies (Zheng et al. , 2018), GC-MS analysis was carried out using a Trace GC Ultra gas chromatograph coupled with a triple

quadrupole mass spectrometer (Agilent Technologies Inc, Palo Alto, USA). Injections were performed in split mode (1 ∶ 50), and volatile compounds were chromatographed on an HP-5MS column (30 m × 0. 25 mm × 0. 25 μm) provided by Agilent Technologies Inc (Palo Alto, USA), with helium as the carrier gas at a constant flow of 1. 0 mL/min. The oven temperature was maintained at 40 ℃ for 3 min, and then increased to 200 ℃ at a rate of 5 ℃/min before being subsequently increased to 250 ℃ at a rate of 10 ℃/min, and then finally held at 250 ℃ for 3 min. The inlet and ion source temperatures were both set at 230 ℃, and MS was scanned at 70 eV in the electronic ionization mode. The mass spectra were acquired using the full-scan-monitoring mode, with a mass-scan range of *m*/*z* 29−448, equipped using the Agilent MassHunter Workstation (NIST17. L, Agilent Technologies Inc). The extraction parameters were set as: Fiber type 80 μm DVB/C-WR/PDMS-gray, sampling temperature 50 ℃, sampling time 30 min. Then, the volatile compounds were desorbed in the vaporizing chamber at 220 ℃ for 10 min.

1. 3 MHS-SPME extraction

A certain amount of samples were placed into a 20 mL headspace vial, repeating the procedures in 1. 2, and four consecutive extractions were performed in the headspace of the vials to ensure the peak area of analyte mass will decrease along with the extraction frequency.

1. 4 Single-factor experiments

The effects of four single factors (fiber type, sampling temperature, sampling time and sample mass) on volatile extraction efficiency were investigated using MHS-SPME-GC-MS extraction from the pericarps of powder GCP. When one factor changed, others were fixed. For example, when fiber type changed (65 μm DVB/PDMS-purple/80 μm DVB/C-WR/PDMS-gray/95 μm C-WR/PDMS-blue) , other factors (sampling temperature, sampling time and Sample size) were fixed at 50 ℃, 30 min and 20 mg, respectively; when sampling temperature varied (30−100, in 10 ℃ step size), other factors (fiber type, sampling time and sample size) were set at 80 μm DVB/C-WR/PDMS-gray, 30 min and 20 mg, respectively. The experimental conditions were optimized based on single-factor experiments.

1. 5 Methodology

Three-year-aged GCP was taken for methodological verification, according to The Pharmacopoeia of China in 2020.

A calibration curve was constructed with seven concentration levels of the standard samples covering the range. Each concentration level was prepared in triplicate. Meanwhile, the peak area was derived from the characteristic ions of each analyte. Calibration curves were constructed to plot the peak area against the mass of the compound.

Repeatability was evaluated by calculating RSD with six parallel injections. Intermediate precision and accuracy were appraised by calculating RAD with parallel injections (n=6) by two different operators. Recovery of analyses was assessed at medium concentration levels with 6 replicates.

Then, after all the samples' analysis using freshly prepared calibration curves, imprecision, and accuracy were expressed based on the RSD.

1. 6 Statistical analysis

Qualitative analysis: Compounds with matching degree over 80 were tentatively identified in NIST17. L standard reference database. The compounds were further quantified by greater relative peak area and comparison of control samples.

Quantitative analysis: Multiple HS-solid-phase microextraction (MHS-SPME) is a technique where analyte mass will decrease exponentially along with the extraction frequency and the peak area, after 3 to 4-time consecutive extractions from the same vial, will be calculated based on Eq. (1)

$$A_{\mathrm{T}} = \sum_{N=1}^{i} A_N = A_1 \sum_{N=0}^{i-1} \beta^N = \frac{A_1}{1-\beta}, \qquad (1)$$

where A_{T} is the total area of the chromatographic peak for the target compound, A_N represents the area of chromatographic peak for the compound in the Nth extraction, A_1 is the area of extraction chromatographic peak at first time, N represents the number of extraction times; and constant β can be determined as given by

$$\ln A_i = \ln A_1 + (i-1)\ln\beta, \qquad (2)$$

where A_i corresponding to peak area extraction at i time(s), $\ln A_i$ the Logarithmic value of the peak area at i time(s), (i−1) the extraction time i minus 1. β can be

obtained based on the slope ($\ln\beta$) in linear regression equation [$\ln A_i - (i-1)$].

With Eq. (1) and (2) combined, final contents of the target analytes were determined by the calculation A_T and substitution of external standard curve.

2 Results and discussion

2.1 GC MS analysis

Fig. 1 shows the ionic chromatograms of the GCP. A total of 50 volatile compounds were identified composed of 95.1% of the peak area, as shown in Supply: Appendix table 1. The volatile profile consists of 50 compounds, predominantly olefinic in nature, with terpenoids being the dominant class. Specifically, the composition includes 26 monoterpenoid compounds, 17 sesquiterpenoid compounds, 2 alcohol compounds, 1 aldehyde compound, 1 ester compound, and 1 ether compound. The classification employed in this study is to categorize both terpenes and their derivatives as terpenoid compounds. In summary, the volatile compounds include monoterpenoids, sesquiterpenoids olefin, alcohols, aldehyde, and esters, which is consistent with those detected using vapor distillation (Yi et al., 2015).

Of the 50 volatile compounds identified, D-Limonene, γ-Terpinene, L-α-Terpineol, 2-(Methylamino)-benzoic acid-methyl ester, and Caryophyllene revealed greater contents, which can be provided as an index for further quantitative analysis, compared to the control samples.

2.2 MHS-SPME

The volatile extraction profiles obtained using SPME vary with fiber type, temperature, time, and mass during experiment. To evaluate the feasibility of the MHS-SPME conditions, volatile profiles of a GCP sample (three years old) were obtained through the MHS-SPME, to examine the effects of all four considered factors (fiber type, sampling temperature, time, and mass taken).

2.2.1 Selection of SPME fiber D-Limonene, γ-Terpinene, L-α-Terpineol, 2-(Methylamino)-benzoic acid-methyl ester, and Caryophyllene, as described in the previous study (Zheng et al., 2018), were set as target compounds for quantitative analysis to study the volatile compositions of GCP. Samples from the same batch (GCP aged 3 years) was employed to investigate the extraction effect of coatings in three extraction fibers (80 μm DVB/C-WR/PDMS, 95 μm C-WR/PDMS, 65 μm DVB/PDMS) on the compounds to quantity. The extraction fiber 80 μm DVB/C-WR/PDMS was observed to provide a better response (Table 1) and more abundant types of absorption (Fig. 2), and hence, applied for quantitative analysis to obtain more compositions of GCP.

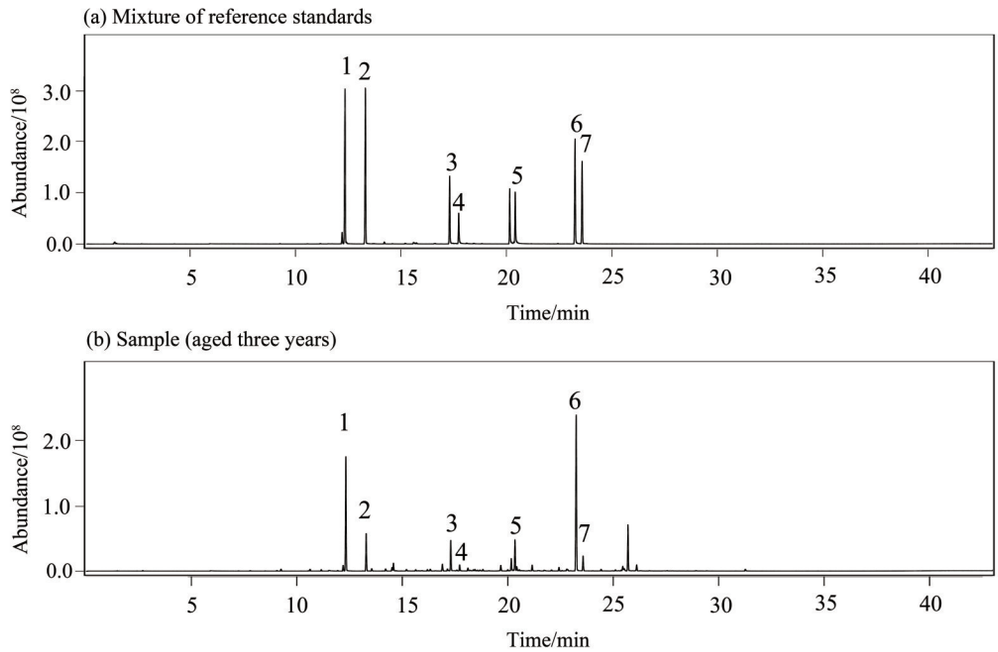
2.2.2 Optimization of extraction temperature Table 2 presents the type and abundance of the thermally extracted proposed quantitative compound, showing the relatively suitable abundance of the species obtained at 50 ℃. Because the abundance response of the individual component is proportional to its boiling point. Extraction is easier at the temperature near the boiling point. The components with higher boiling points received a relatively high response at 50 ℃. Therefore, 50 ℃ was chosen as the operation temperature for the subsequent procedures.

2.2.3 Optimization of extraction time Table 3 shows the extraction time-resolved rate of the considered volatile substance at the optimal temperature of 50 ℃. The optimal duration is set at 30 min.

2.2.4 Optimization of sampling amount In the quantitative analysis of the MHS-SPME, β value ($0.40 < \beta < 0.95$) is the crux for evaluation of approach feasibility(Rincón et al.,2014). If there is a linear relationship ($R^2 > 0.9$) between peak area log

Table 1 Relative peak areas of the proposed components using different fiber type

Fiber type	Relative area /%				
	D-Limonene	γ-Terpinene	L-α-Terpineol	2-(Methylamino)-benzoic acid-methyl ester	Caryophyllene
80 μm(Dark grey)	21.24	6.70	5.56	32.46	2.74
95 μm(Dark blue)	31.83	7.69	4.21	24.09	2.70
65 μm(Violet)	0.99	0.52	6.89	46.97	1.45



Peak 1: D- Limonene; Peak 2: γ-Terpinene; Peak 3: L-α-Terpineol; Peak 4: Tridecane (Internal standard); Peak 5: Carvacrol; Peak 6: 2-(Methylamino)-benzoic acid-methyl ester; Peak 7: Caryophyllene.

Fig. 1 GC-MS ion chromatogram (TICs) of volatile compounds in the GCP

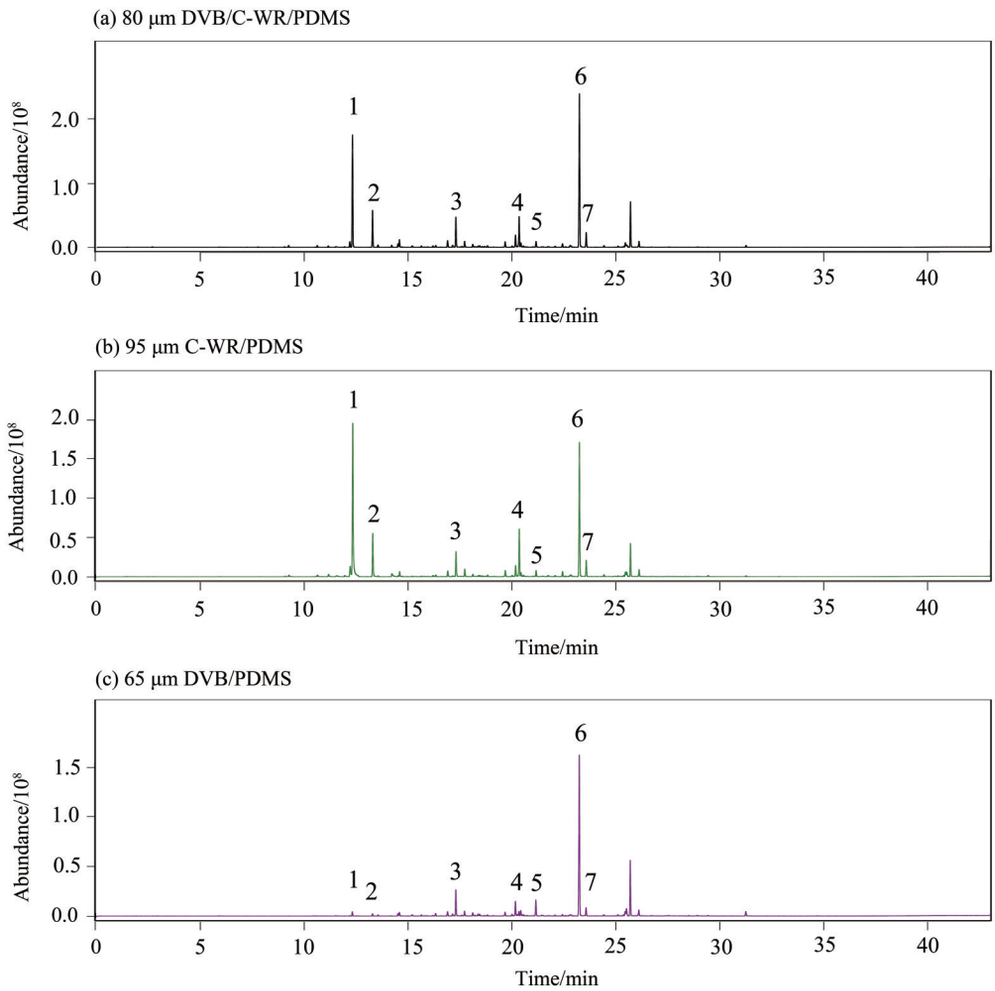
($\ln A_i$) extracted and extraction time ($i-1$), then the analytes can be accurately quantified. In addition, proper amount of analyte samples is the key to presenting a linear relationship between the above. The amount of anlytes at first extraction has been exhaustively extracted when β value was smaller than 0.4. The amount of analyte from multiple extractions can be proved to have no significant change when the β value is over 0.95; otherwise, it can be interpreted that the sample amount and the contents of the target analyte were overloaded (Rincón et al. ,2014).

To determine the β and R_2 values of the $[\ln A_i-(i-1)]$ profile, the results are shown in Table 4 To determine the β and R^2 values of the $[\ln A_i-(i-1)]$ profile, the results are shown in, injector β values of 2-(Methylamino)-benzoic acid-methyl ester and L-α-Terpineol were over 0.9 and R^2 is above 0.9 when the amount of analyte samples is beyond 30 mg, indicating that the amount of 2-(Methylamino)-benzoic acid-methyl ester and L-α-Terpineol were overwhelming as injection volume was equal to 30 mg or more. As the injection volume was in the range of 10−20 mg, which fits with the quantitative requirement, β value was in a reasonable range. Given that, sample mass was set at 20 mg. Chromatographic graph of four consecutive extractions of target compounds is shown in Fig. 3, in which the gradual decrease of peak-areas for the D-Limonene, γ-Terpinene, L-α-Terpineol, 2-(Methylamino)-benzoic acid-methyl ester, Caryophyllene in a specific GCP sample between successive extractions is clear (Rincón et al. , 2014). Fig. 4 shows typical plots related to Eq. 2(2), from which β is obtained.

From what has been discussed above, the optimal process were determined as: 20 mg (60-mesh screening) of the target compounds are placed in a 20 mL headspace vial, subsequently sealed with. Prior to GC-MS analysis, the vial is extracted for 30 min at 50 ℃ with a 80 μm DVB/C-WR/PDMS fiber and desorbed in the vaporizing chamber at 220 ℃ for 10 min.

2.3 Methodology

2.3.1 Linearity Before the GC-MS analysis, individual samples were precisely weighted and dissolved to be a solution as control samples. One microliter of the control samples were injected into the vials for GC-MS analysis, respectively. The procedure then conducted a regression analysis for injection concentration x using the peak area integration y. Table 5 presents the linear relationship and the interval of analytes. The peak area shows a linear dependence on the mass of the compounds within the individual range of concentration.



Peak 1: D-Limonene; Peak 2: γ-Terpinene; Peak 3: L-α-Terpineol; Peak 4: Tridecane (IS);
Peak 5: Carvacrol; Peak 6: 2-(Methylamino)-benzoic acid-methyl ester;Peak 7: Caryophyllene.

Fig. 2 Chromatograms of different types of fibers

Table 2 Type and abundance of the volatile substance extracted at different temperatures（$n = 3$） 10^8

No.	Compound	Temperature / ℃							
		30	40	50	60	70	80	90	100
1	D-Limonene	35. 10	26. 70	23. 91	20. 46	13. 41	9. 26	6. 20	1. 31
2	γ-Terpinene	9. 18	7. 26	6. 45	5. 51	3. 97	2. 75	1. 72	0. 39
3	L-α-Terpineol	5. 11	3. 44	2. 75	3. 53	4. 42	3. 81	2. 66	1. 33
4	2-（Methylamino）-benzoic acid-methyl ester	17. 60	22. 58	31. 97	23. 30	24. 88	23. 75	21. 21	7. 36
5	Caryophyllene	0. 98	1. 01	1. 23	1. 22	1. 33	1. 11	0. 86	0. 26

Table 3 Extraction time resolved abundance of the individual volatile substance（$n = 3$） 10^8

No.	Compound	Extraction time / min				
		20	30	40	50	60
1	D- Limonene	19. 93	23. 91	17. 82	19. 19	21. 41
2	γ-Terpinene	5. 28	6. 45	4. 59	4. 90	4. 88
3	L-α-Terpineol	3. 55	2. 75	3. 19	3. 42	3. 55
4	2-（Methylamino）-benzoic acid-methyl ester	23. 84	31. 97	20. 55	22. 58	22. 79
5	Caryophyllene	1. 03	1. 23	0. 96	0. 99	0. 99

Table 4 The linear correlation coefficient R^2 and β value of each compound $[\ln A_i-(i-1)]$ ($n=3$)

No.	Compound	10 mg		20 mg		30 mg		40 mg		50 mg	
		R^2	β	R^2	β	R^2	β	R^2	β	R^2	β
1	D- Limonene	0. 995 2	0. 87	0. 995 0	0. 84	0. 949 5	0. 87	0. 988 4	0. 86	0. 994 2	0. 87
2	γ-Terpinene	0. 993 8	0. 85	0. 997 9	0. 82	0. 951 4	0. 86	0. 994 8	0. 86	0. 994 6	0. 87
3	L-α-Terpineol	0. 988 1	0. 66	0. 978 6	0. 76	0. 905 5	0. 88	0. 878 0	0. 92	0. 770 5	0. 95
4	2-(Methylamino)-benzoic acid-methyl ester	0. 994 0	0. 66	0. 983 8	0. 81	0. 839 9	0. 90	0. 900 1	0. 93	0. 801 4	0. 94
5	Caryophyllene	0. 993 3	0. 48	0. 990 7	0. 60	0. 905 0	0. 78	0. 978 8	0. 82	0. 946 6	0. 87

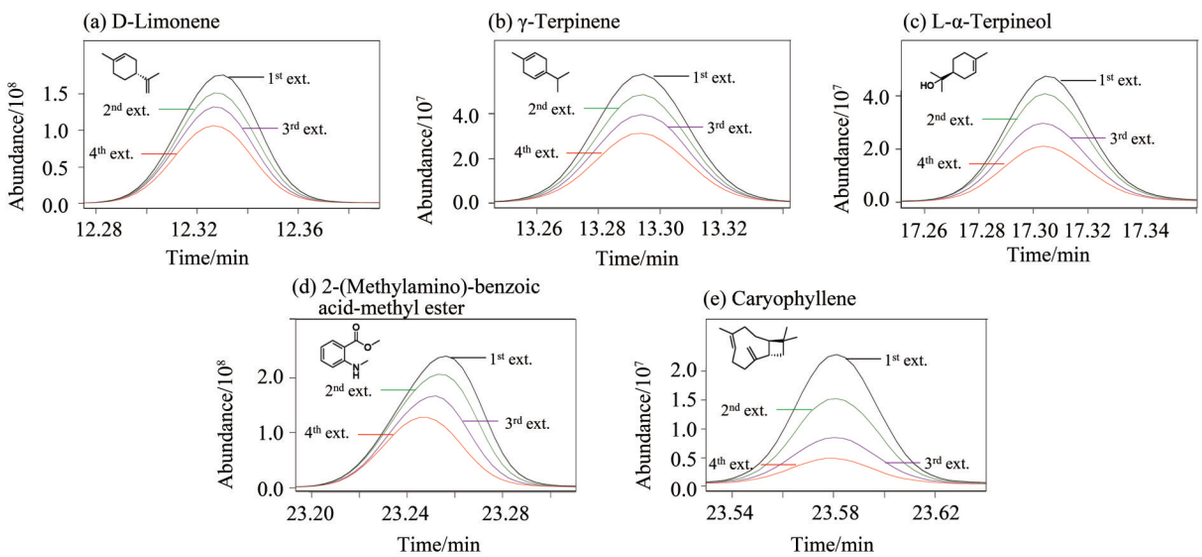


Fig. 3 Chromatograms of four consecutive extractions of GCP sample (Sample: 20 mg)

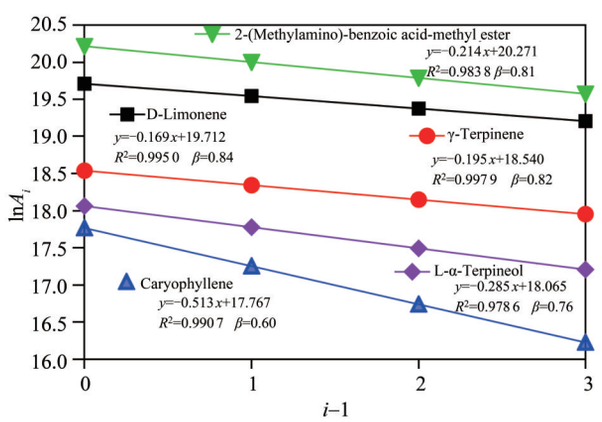


Fig. 4 $[\ln A_i-(i-1)]$ linear regression curves of 5 compounds

2. 3. 2 Repeatability test Data obtained from GC-MS analysis was processed to calculate the average contents and RSD of D-Limonene, γ-Terpinene, L-α-Terpineol, 2- (Methylamino) -benzoic acid-methyl ester and Caryophyllene (Table 6). Results showed that repeatability RSD of D-Limonene, γ -Terpinene, L- α -Terpineol and 2- (Methylamino) -benzoic acid-methyl ester were all below 10%, while repeatability RSD of Caryophyllene was over 30%. Therefore, Caryophyllene, given its low contents in GCP and poor repeatability, is excluded from further quantitative analysis.

2. 3. 3 Intermediate precision Data obtained from GC-MS analysis was processed to calculate the average contents and RAD of D-Limonene, γ-Terpinene, L- α -Terpineol and 2- (Methylamino) -benzoic acid-methyl ester (Table 6). Results showed that the approach had a good intermediate precision.

2. 3. 4 Recovery Based on the recovery result of D-Limonene, L- α -Terpineol, γ -Terpinene and 2- (Methylamino) -benzoic acid-methyl ester ranged from 83. 17% – 105. 33%, 81. 40% – 96. 92%, 84. 3% – 102. 65%, and 81. 58% – 107. 16%, respectively (Table 6). The RSD for all analytes ranged from 5. 96% to 8. 09%, correspondingly.

2. 4 Analysis of the GCP sample

Extraction is a crucial procedure in the analysis of volatile GCP compounds. Compared with the extrac-

Table 5 Linear relationship and range of each compound [1)]

No.	Compound	Linear interval / ng	Standard curve equation	Correlation coefficient (R)
1	D-Limonene	1. 80−115. 20	$A_T=(2.923\times10^7)m+(28.07\times10^7)$	0. 989 9
2	γ-Terpinene	0. 16−80. 75	$A_T=(4.097\times10^7)m+(18.41\times10^7)$	0. 994 9
3	L-α-Terpineol	0. 04−18. 68	$A_T=(11.09\times10^7)m+(3.404\times10^7)$	0. 984 9
4	2-(Methylamino)-benzoic acid-methyl ester	0. 04−22. 52	$A_T=(12.95\times10^7)m+(14.73\times10^7)$	0. 994 9
5	Caryophyllene	0. 03−17. 64	$A_T=(13.55\times10^7)m+(5.743\times10^7)$	0. 995 0

1) "m" represents the mass of the compound.

Table 6 The repeatability, precision, and recovery of the content of GCP [1)] %

No.	Compound	Repeatability (RSD) ($n=6$)	Precision (RAD) ($n=2$)	Recovery ($n=6$)	Recoveries (RSD)
1	D-Limonene	5. 33	4. 55	83. 17−105. 33	7. 22
2	γ-Terpinene	7. 81	3. 48	81. 40−96. 92	5. 96
3	L-α-Terpineol	4. 11	3. 87	84. 30−102. 65	6. 37
4	2-(Methylamino)-benzoic acid-methyl ester	4. 33	3. 44	81. 58−107. 16	8. 09
5	Caryophyllene	37. 80	—	—	—

1) "—" represents no data.

tion methods such as hydro-distillation, carbon dioxide supercritical fluid extraction (Yi et al. , 2015) that are time- and sample-consuming, the Headspace solid-phase microextraction (HS-SPME), with the advantage of simplicity, high speed, high sensitivity, high throughput, less samples and solvent-free (Zheng et al. , 2018). In this study, six different aged GCP samples were analyzed using the MHS-SPME method under the optimal conditions for the subsequent GC-MS analysis (Fig. 5). The extracted abundance of the volatile compositions drops with the increase of the GCP age. The D-Limonene and the γ-Terpinene showed the largest abundance drop while the L-α-Terpineol and the 2-(Methylamino)-benzoic acid-methyl ester showed a slowing rate of decline and leveled off gradually. This agrees with the findings of Kashiwagi et al. (2010). Total monoterpene hydrocarbons decreased markedly, with major losses of limonene and γ-Terpinene.

Such dramatic declines implied not only loss of the odor but also the active transformation of the volatile substances during aging that might involve the hydrolysis and oxidation of some chemical components, etc. Wedler et al. (2015) suggested that limonene in wine formed α-terpineol through hydration. In the acidic environment, limonene can be dehydrated to

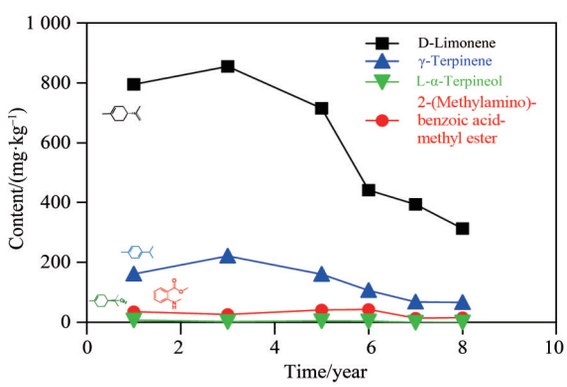


Fig. 5 Comparison of volatile components content of GCP in different years

form the 1, 8-terpinene with double bonds, and then dehydrated and cyclized to form the 1, 8-cineole. Njoroge et al. (1996) have shown that the concentration of terpenes such as limonene would be significantly reduced after 12 months of aging. Nguyen et al. (2009) also proposed that the limonene is oxidized and degraded to limonene oxide and carvone. The changes of terpenoids such as D-Limonene and γ-Terpinene during storage may be closely related to tautomerism, oxidation, and esterification of terpenoids. For example, during drying, citrus aroma substances such as Limonene would form α-terpineol through hydration (Wedler et al. , 2015), and terpineol would

be further oxidized into ketones, aldehydes, and other substances (Duetz et al. ,2001; Tai et al. ,2016). Terpenes would decrease significantly under aerobic conditions (Nguyen et al. ,2009).

Different volatile compositions are linked to the smelling signatures. Empirically, GCP smells better and effects greater when the age period is prolonged, as the proverb said "older is better" (Wang et al. , 2016). As the old saying goes, the 3-year-old GCP can stimulate fragrance and tastes sour. 5－10 years old smells wooden fragrance; 10－15 years old smells mellow sweet of old medicine. It was suggested that D-Limonene in GCP might be converted into α-terpineol and other substances during aging, thereby changing the proportion of terpenes in GCP and affecting its fragrance.

3 Conclusion

An extraction method for volatile substances from GCP was developed using MHS-SPME and GC-MS, identifying 50 components. We found that extraction was easier near component boiling points, which may explain aged GCP's distinct smell and enhanced effects.

This method, which was reliable for repeatability and recovery, analyzed GCP samples stored for different years. Results showed fruity - smelling olefinic terpenes decreased faster, while woody-scented components rose slightly then declined slowly, revealing GCP's composition changes over years and age identification. The MHS-SPME method, effective in reducing matrix effects and eco-friendly like HS-SPME, offers insights for analyzing volatiles in other Chinese herbs.

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多次顶空固相微萃取在不同年份广陈皮挥发性化合物定量中的应用

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摘要：采用多次顶空固相微萃取（MHS-SPME，multiple headspace solid-phase microextraction）结合气相色谱−质谱联用（GC-MS，gas chromatography-mass spectrometry）技术，定量分析了不同年份广陈皮中的挥发性成分。通过考察萃取时间、萃取温度、样品量以及不同萃取头涂层，建立了采用多次顶空固相微萃取（MHS-SPME）对广陈皮中D-柠檬烯、γ-萜品烯、L-α-萜品醇和2-甲氨基苯甲酸甲酯含量测定方法。定性出50种挥发性化合物，包括萜类、醇类、醛类和酯类。结果显示，广陈皮中的萜类化合物随着陈化年份增加含量下降快于酯类和醇类。该发现有助于揭示广陈皮在陈化过程中挥发性化合物的变化。

关键词：MHS-SPME；GC-MS；广陈皮；挥发性化合物

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