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STUDY ON GAS CHROMATOGRAPHY-MASS SPECTROMETER FINGERPRINT OF VOLATILE CONSTITUENTS FOR WATER SOLUBLE INGREDIENTS IN PU'ER TEA

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Abstract

The gas chromatography-mass spectrometry (GC-MS) fingerprint method of volatile constituents of the water soluble ingredients in Pu'er tea 7542 (106) was established in paper by comparing it with GC-MS chromatograms data to optimize and explore the volume of the tea extract, the ultrasound extracting time and the temperature and time of oscillation box in static head space (HS). Under the condition of optimization, volatile constituents of the water soluble ingredients in Pu'er tea for different ages and batches 7542 samples were detected by GC-MS. Their GC-MS fingerprint was set up and analyzed their cluster and principal component for reference fingerprint of Pu'er tea 7542 (106) by the traditional Chinese medicine (TCM) chromatographic fingerprint similarity evaluation system (A) 2004 edition software and the SPSS 19.0 software. The result of this experiment: Pu'er tea 7542 was extracted with 30 ml water and 30 min as the extraction by means of ultrasonication to volatile constituents. The extract were detected by HS and GC-MS. The temperature and time of oscillation box in static head space were 120ºC and 25min. There were 6 compounds in the volatile components of the water soluble ingredients in Pu'er tea for different ages and batches 7542 samples. These compounds belong to one main characteristic component, the contribution of that accounted for 99.74%. The ten samples for different ages and batches 7542 Pu'er tea had subdivided into four classes.

Introduction

Pu'er tea from Yunnan Province is the most special kind in Chinese tea, not only the long history of producing, with special shape, sweet, smooth, tasty and aged fragrant, but also an outstanding effect to human health. With warm tea stomach, reduce weight, cholesterol, prevent arteriosclerosis, prevent coronary heart disease, fall blood pressure, anti-aging, anti-cancer, fall blood sugar and wine. Usually the tea and drinks is a form human touch. So the contents of water extraction affected the quality of Pu'er tea directly such as tea polyphenol, catechin, caffeine, tea ammonia acid. It also includes polysaccharide biochemical components and the oxidation matrix of a series of reactions during the later ripening stages. The chemical composition of Pu'er tea, including chemical composition in machining process for late, were very complicated. Only one or several index components cannot fully and accurately described. Chemical fingerprinting is a methodology based on the holistic chemical profile obtained by various analytical techniques for the identification of authenticity, differentiation of origin, and evaluation of quality of herbal medicines and related products. Chemical fingerprint analysis views Pu'er tea as an interactive and indivisible whole, and it can be utilized to assess and control the quality of Pu'er tea systematically and comprehensively even in the absence of reference substances. This approach has attracted an increasing amount of interest in recent years, and it has been introduced and

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accepted by the World Health Organization (WHO), State Food and Drug Administration (SFDA) of China, and other authorities as a strategy for quality assessment and control. For it can be more comprehensive characterization of the distribution of volatile constituents for the water soluble ingredients in Pu'er tea. The method of GC-MS fingerprint about volatile constituents for the water soluble ingredients in Pu'er tea 7542(106) for reference samples was studied in paper. A combination of gas chromatography and mass spectrometry (GC-MS) allows the identification and quantification of volatile constituents of the water soluble ingredients in Pu'er tea, which were extracted with water as the extraction by means of ultrasonication. Sample pretreatment conditions and detection of GC-MS were optimized. Under the condition of optimization, volatile constituents of the water soluble ingredients in ten samples of different ages and batches about Pu'er tea 7542 were detected by GC-MS. Their GC-MS fingerprint was set up and analyzed.

Materials and Methods

The SHIMADZU GC-MS (QP2010, Japan), a ultrasound cleaner (Shanghai, China), a electronic balance (Switzerland, Germany), Rtx-5Ms(30.0 m × 0.25 μm × 0.25 mm), Rtx-35(30.0 m × 0.25 μm × 0.25 mm), Rtx-1(30.0 m × 0.25 μm × 0.25 mm) and a static Head space (HS) (Germany) were used.

GC: Helium as carrier gas at a constant flow of 0.57 ml/min. The temperature programme was the following: initial temperature 15ºC, held for 1 min, 2ºC/min ramp to 230ºC, held for 10 min. The total analysis time was 55 min. The temperature of the injection port was 230ºC and sample was injected in splitless mode.

MS: The mass spectrometer was operated in electron ionization mode with an ionising energy of 0.88 kv, ion source temperature 200ºC, MS Quad temperature 230ºC. The scan was from m/z 45 to 800 and the solvent delay was 5 min.

HS: 1.0 ml volume was injected. The temperature and time of oscillation box in static head space were 120ºC and 25 min. The rate of oscillation was 250 rpm. Time of the blowing needle for air was 3 min. The rate of sample injection was 500 μl/s.

Table 1. Different years and batches of Pu'er tea 7542.

No.	Batch lot of sample	Information of batch Lot
1	504	The 4th batch of Pu'er raw tea 7542 in 2005
2	505	The 5th batch of Pu'er raw tea 7542 in 2005
3	606	The 6th batch of Pu'er raw tea 7542 in 2006
4	608	The 8th batch of Pu'er raw tea 7542 in 2006
5	703	The 3th batch of Pu'er raw tea 7542 in 2007
6	801	The first batch of Pu'er raw tea 7542 in 2008
7	810	The 10th batch of Pu'er raw tea 7542 in 2008
8	901	The first batch of Pu'er raw tea 7542 in 2009
9	912	The 12th batch of Pu'er raw tea 7542 in 2009
10	106	The 6th batch of Pu'er raw tea 7542 in 2001

Distilled water, prepared from demineralized water, was used throughout the experiment. Study involved 10 Pu'er tea 7542 which were purchased from Menghai Tea Factory's largest production quantity raw tea product, and is made from fat tender leaves and young tips blended to perfection. Pu'er tea 7542 is the courageous sea tea reprocessing factory production of a big group raw tea, i.e., the formula in 1975, produced by level 4 is ensured, the courageous sea tea

reprocessing factory production (Menghai factory code for 2) was born as the judge Pu'er tea market of tea (blue cake) quality standard products. List of varieties involved in the study is presented in Table 1.

Pu'er tea samples were homogenised. Then, 1.000 g of homogenized sample was accurately weighed into a 50 ml conical flask with stopper and added with 30 ml distilled water. The water solution extracted for 30 min with ultrasonic extraction. After filtration, the filtrate was transferred into a flat-bottomed flask and condensed to a small volume (5 - 10 ml) on a rotary evaporator at 40ºC under vacuum. Finally, the extract was filtered through a 0.45 μm filter for GC-MS analysis.

Results and Discussion

The selection of the different extracting solvent and ultrasound extracting time in sample pretreatment process with a proper polarity to match the analyte was beneficial to improve process efficiency and minimise potential interferences from the samples. Six samples had to be weighed accurately for pretreatment according to the experimental method . Two main factors including the extracting solvent volume and ultrasound extracting time were studied. Time and solvent volume can affect the extraction efficiency. The extracts were detected by GC-MS with HS after the extracting solvent of water with different volumes (20, 30 and 50 ml) and ultrasound extracting time (10, 20 and 30 min). The result showed that: when the efficiency of 30 ml the extracting solvent volume and 30 min ultrasound extracting time was tested, the obtained peak areas and peak number in their fingerprints were large and more. Therefore, 30 ml and 30 min had been adopted as the extracting solvent of boiling water and ultrasound extracting time in this method.

Pu'er tea 7542(106) sample had to be weighed accurately for pretreatment according to the experimental method. The temperature and time of oscillation box were studied in GC-MS. They affected the extraction efficiency of volatile constituents for the water soluble ingredients in the extracts of Pu'er tea. The temperature of oscillation box was set respectively to 80, 90, 100, 110 and 120ºC. The time of oscillation box was set, respectively to 10, 15, 20 and 25 min. The results are given in Fig. 1. When the temperature and time of oscillation box were to set 120 and 25 min, respectively, the obtained peak areas and peak number in their fingerprints were large and more. Therefore, 120 and 25 min had been adopted as the temperature and time of oscillation box in this determination conditions.

Volatile constituents of the water soluble ingredients in different ages and batches Pu'er tea 7542 samples (504, 505, 606, 608, 703, 801, 810, 901 and 912) were extracted according to the experimental method and detected by GC-MS. Their GC-MS fingerprint were set up (Fig. 2).

The GC-MS fingerprint of volatile constituents of the water soluble ingredients in different ages and batches Pu'er tea 7542 samples were analyzed their matrix, similarity and principal component for reference fingerprint of Pu'er tea 7542 (106) by the traditional chinese medicine (TCM) chromatographic fingerprint similarity evaluation system (A) 2004 edition software and the SPSS 19.0 software.

(i) The similarity analysis: The results were analyzed by similarity grade calculate method to compare the fingerprint difference of samples. The matching result was measured by the relative coefficient. The results are shown in Table 2.

It is seen from Table 2, the similarity of samples between 0.800 and 0.900 in spectrum peaks were 606, 801, 810, 901 and 912. The similarity of samples to be lower than or equal to 0.800 in spectrum peaks were 504, 505, 608 and 703. Samples from 606, 801, 810, 901, 912 and 106 were of high similarity in spectrum peaks, most similarity ratio was at 0.9000 upwards, which indicated that the total quality was very stable. And yet,

samples from 504, 505, 608, 703 and 106 showed evident difference in spectrum peaks. The results showed clearly that the composition contents of different ages and batches Pu'er tea 7542 samples have apparent change.

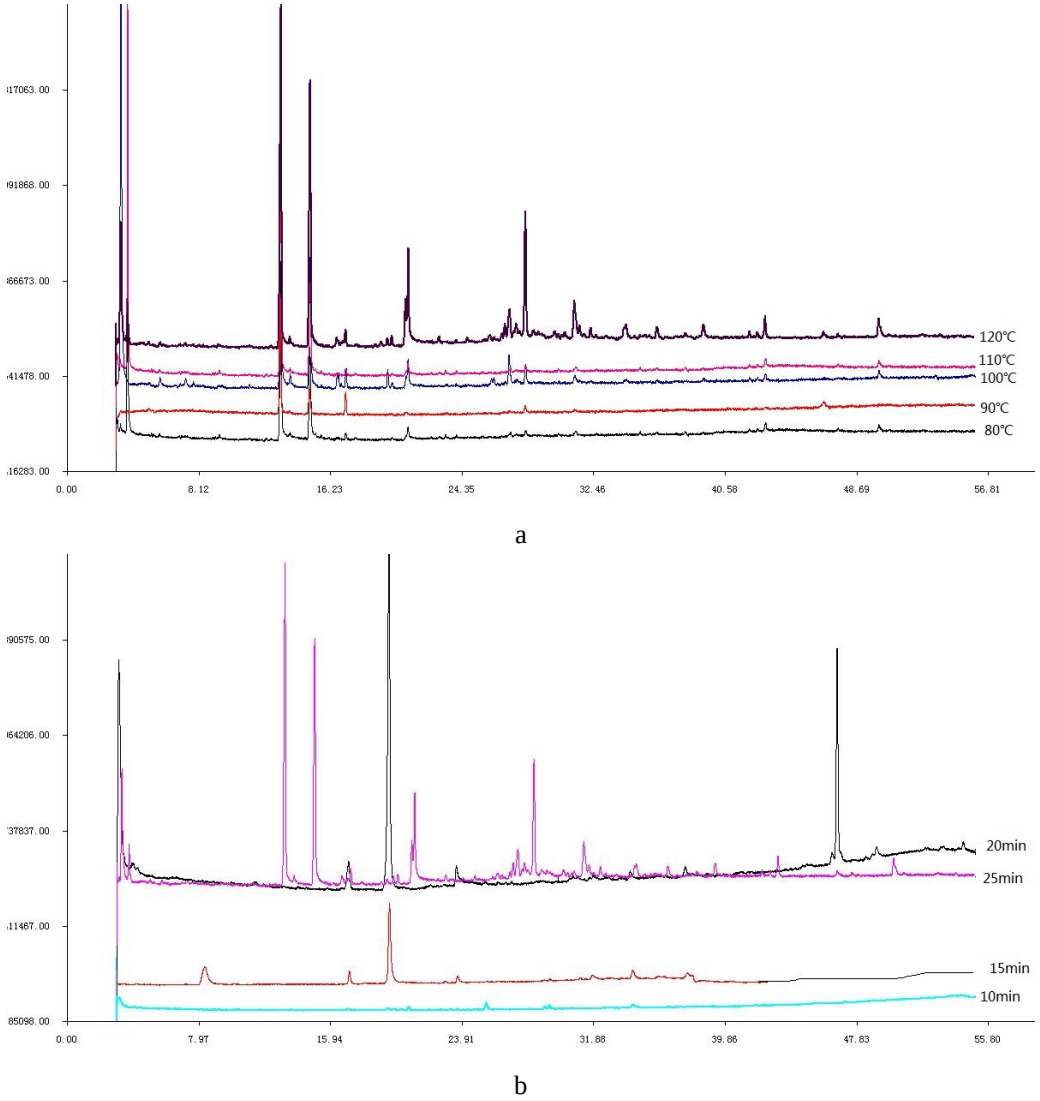

Fig. 1. GC-MS fingerprints of the various temperature and time of oscillation box (a. GC-MS fingerprints of the various temperature of oscillation box; b. GC-MS fingerprints of the various time of oscillation box).

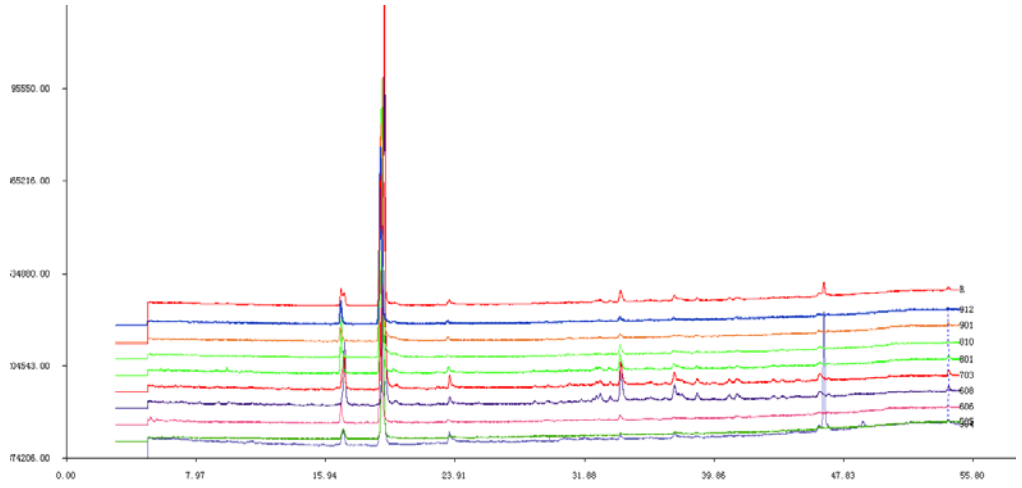

Fig. 2. The GC-MS fingerprint of volatile components for the water soluble ingredients of Pu'er tea for different ages and batches 7542 samples (bottom-up volumes followed by 504, 505, 606, 608, 703, 801, 810, 901, 912, R and 106).

Table 2. Similarity calculation results of volatile constituent fingerprints for the water soluble ingredients in different ages and batches 7542 Pu'er tea samples.

Batch	504	505	606	608	703	801	810	901	912	R
504	1.000	0.757	0.117	0.797	0.089	0.008	0.028	0.046	0.062	0.490
505	0.757	1.000	0.150	0.797	0.148	0.010	0.055	0.079	0.114	0.493
606	0.117	0.150	1.000	0.046	0.024	0.881	0.893	0.904	0.898	0.843
608	0.797	0.797	0.046	1.000	0.087	0.003	0.008	0.013	0.019	0.481
703	0.089	0.148	0.024	0.087	1.000	0.008	0.007	0.007	0.013	0.101
801	0.008	0.010	0.881	0.003	0.008	1.000	0.974	0.963	0.924	0.841
810	0.028	0.055	0.893	0.008	0.007	0.974	1.000	0.972	0.935	0.854
901	0.046	0.079	0.904	0.013	0.007	0.963	0.972	1.000	0.937	0.859
912	0.062	0.114	0.898	0.019	0.013	0.924	0.935	0.937	1.000	0.849
R	0.490	0.493	0.843	0.481	0.101	0.841	0.854	0.859	0.849	1.000

R- reference fingerprint of Pu'er tea 7542 (106).

(ii) The matrix analysis: The relative peak area ratio of each common peak in the fingerprints of different ages and batches Pu'er tea 7542 samples was determined by calculation. The relative peak area for the key indicators import the SPSS 19.0 software. It applies weighted K-means clustering and nearest neighbor element for measures to gain the tree. The as seen in the tree, the nine samples for different ages and batches 7542 Pu'er tea had subdivided into 4 classes: the samples from 901, 912, 505 and 606 belonged to type 1; the samples from 608 and 810 belonged to type 2; the samples from 703 and 801 belonged to type 3; the samples from 504 belonged to type 4. Methods for chemical pattern recognition was performed on Pu'er tea 7542 collected from various ages and batches by the clustering analysis method.

(iii) The principal component analysis (PCA): I had integrated the chromatogram on different ages and batches 7542 samples. The relative contents of the components were determinated by area normalization. Compounds that the relative contents of the components were bigger than 0.1, match NIST05.LIB installed in GC-MS. According to compatible with 90 per cent and compounds of Pu'er tea reported in literature, there were 6 compounds in volatile components for the water soluble ingredients of Pu'er tea for different ages and batches 7542 samples. They were linalool, N-butylbenzenesulfonamide, caffeine, 9,12-octadecadienoate,11,14,17-eicosatrienoicacid methyl ester and epoxy linalool. The molecular mass and fragmented ions of 6 components are shown in Table 3. Then we had comparison with the ten samples for different ages and batches 7542 Pu'er tea. The results showed that the content of N-butylbenzenesulfonamide, caffeine, 11, 14, 17-eicosatrienoic acid methyl ester was much higher. It was also the substantial part of the content of caffeine, which was more than 50%. The content of linalool and epoxy linalool were relatively low. The older and the longer of Pu'er tea, the higher rate of the content of 11,14,17-eicosatrienoic acid methyl ester. The older and the longer of Pu'er tea, the lower rate of the content of caffeine. All of these compounds had different amounts in various teas. Calculated the percentage compounds according to peak area normalization method. The percentage compounds import the SPSS 19.0 software. The application of multifactor dimensionality reduction (MDR) method and maximum deviation method were extensively applied to analyze data. The results from calculation and analysis show that these compounds belong to one main characteristic component, the contribution of that accounted for 99.74%. These principal components could identify different ages and batches of Pu'er tea 7542.

Table 3. The names, molecular mass and fragmented ions of 6 components.

Name	Retention times (t/min)	Molecular mass	Fragmented ions
Linalool	3.445	154	59,93,121,136
N-butylbenzenesulfonamide	17.03	213	77,141,170,213
Caffeine	19.48	194	55,67,109,194
9,12-octadecadienoate	31.87	294	41,57,71,97
11,14,17-Eicosatrienoicacidmethyl ester	35.24	320	91,119,163,230
Epoxy linalool	46.30	170	43,81,123,166

From the perspective of similarity calculation and hierarchical clustering analysis, this article explores that the volatile constituents of the water soluble ingredients in different ages and batches Pu'er tea 7542 samples were influenced by various chromatogram column (Rtx-5Ms, Rtx-35 and Rtx-1). The experimental results showed that the effect was relatively obvious and the span was larger in similarity calculation and hierarchical clustering analysis. The effects of Rtx-5Ms were better for detecting many compounds. According to cluster analysis on the nine different ages and batches Pu'er tea 7542 samples, they with Rtx-5Ms had subdivided into 4 classes (the samples from 901, 912, 505 and 606 belonged to type 1; the samples from 608 and 810 belonged to type 2; the samples from 703 and 801 belonged to type 3; the samples from 504 belonged to type 4), they with Rtx-35 had subdivided into 3 classes (the samples from 505 and 912 belonged to type 1; the samples from 901, 606, 504 and 608 belonged to type 2; the samples from 703, 801 and 810 belonged to type 3), they with Rtx-1 had subdivided into 4 classes (the samples from 606 and 912 belonged to type 1; the samples from 608 and 810 belonged to type 2; the samples from 505, 703 and 801 belonged to type 3; the samples from 504 and 901 belonged to type 4).

The determined method on gas chromatography mass spectrometer fingerprint of volatile constituents for the water soluble ingredients in Pu'er tea was established. GC-MS fingerprint of Pu'er tea for different ages and batches 7542 samples were set up and analyzed their cluster and principal component by the traditional Chinese medicine (TCM) chromatographic fingerprint similarity evaluation system (A) 2004 edition software and the SPSS 19.0 software. The results can be applied to identify different ages and batches of Pu'er tea 7542. The contents of the reports can lay the foundation for evaluating quality of Pu'er tea 7542 .

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References

Deng JW and Yan YY 2013. Chemical fingerprint analysis for quality assessment and control of Bansha herbal tea using paper spray mass spectrometry. Anal. Chim. Acta, **785**:82-90.

Lu HP, Zhang YJ, Lin Z and Liang YR 2013. Processing and chemical constituents of Pu-erh tea: A Rev. Food Res. Int. **53**: 608-618.

Lu HP, Gu JP, Lin Z, Guo L, Tan JF and Peng QH 2007. Advance in the study on the chemical composition and biological activity of Pu-erh tea . J. Tea Sci. **27**(1):8-18.

Lin J, Zhang P, Pan ZQ, Xu HR, Luo YP and Wang XC 2013. Discrimination of Oolong tea (*Camellia sinensis*) varieties based on feature extraction and selection from aromatic profiles analysed by HS-SPME/GC-MS. Food Chem. **141**: 259-265.

Tistaert C, Dejaegher B and Heyden YV 2011. Chromatographic separation techniques and data handling methods for herbal fingerprints: A review. Anal. Chim. Acta **690**: 148-161.

Zhao L, Huang C and Shan Z 2005. Fingerprint analysis of Psoralea corylifolial by HPLC and LC-MS. J. Chromatogr Banalyt Technol. Biomed. Life Sci. **821**(1):67.

Wang YF, Xian JH,Xi XG and Wei XL 2013. Multifingerprint and quality control analysis of tea polysaccharides. Carbohydrate Polymers. **92**:583-590.

Wu G, Bao XX, Zhao SH, Wu JJ, Han A and Ye QF 2011. Analysis of multipesticide residues in the foods of animal origin by GC-MS coupled with accelerated solvent extraction and gel permeation chromatography cleanup. Food Chemistry **126**(2): 646-654.

Xu L, Han X, Qi Y, Xu YW, Yin LH, Peng JY, Liu KX and Sun CK 2009. Multiple compounds determination and fingerprint analysis of Lidanpaishi tablet and keli by high-performance liquid chromatography. Analytica Chimica Acta **633**(1): 136-148.

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