Study on Fingerprint Construction of Pu'er Raw Tea by GC-MS

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Abstract: Taking example for Pu'er raw tea 7542(106) in paper, sample pre-treatment conditions (Headspace) and detection of the gas chromatography mass spectrometry (GC-MS) were optimized. Under the condition of optimization, the chromatographic fingerprints of nine batches of Pu'er raw tea 7542 sample for different ages and batches were determined by GC-MS, followed by the analysis with the traditional chinese medicine (TCM) chromatographic fingerprint similarity evaluation system (A) 2004 edition software and the SPSS 19.0 software, to establish the mutual fingerprints of Pu'er raw tea. The results showed that GC-MS fingerprint of Pu'er raw tea had superioritied on the qualitative determination of multiple components at the same time, and good performance on repeatability, precision and stability. The chromatographic fingerprint provided scientists with useful tools in understanding the huge amounts of data generated by the analytical advances and proved to be valuable for quality control, classification and discrimination between Pu'er raw tea fingerprints.

Introduction

Pu'er tea from China, with a lubricous taste, has many health benefits and a strong cultural foundation, thus it becomes more and more popular among the consumers in worldwide. Pu'er tea is produced using the sun-dried leaves of large-leaf tea species in the Yunnan province of China.[1]The solid-state fermentation by microorganisms provides the specia characteristics of a mellow taste, a stable flavour and the brownish red colour of the Pu'er tea infusion. Pu'er tea can be divided into Pu'er raw tea and Pu'er ripen tea according to the processing technology and the quality characteristics. Pu'er raw tea has an even richer set of chemical substances than regular green tea[2,3], including the water-extractable substances and tea polyphenols, which provides a favourable material base for the transformation of chemical constituents during the post-fermentation process and the natural ageing process. Non-fermented Pu'er tea can reduce the serum of TG. However, the chemical constituents of these two forms are very similar to one another and to those of fresh tea leaves, which have been identified by high performance liquid chromatography (HPLC)[4], near infrared spectroscopy (NIRS)[5], fourier transform infrared spectroscopy (FTIR)[6]and metabolomic analysis. However, the levels of these constituents are quite different because heat during autoclaving may induce the degradation of some tea chemical compounds.To date, many compounds have been isolated and identified from the fresh leaves of C. sinensisvar.assamicaand Pu'er raw tea.[7]

The raw materials of Pu'er tea come from sun-dried green tea rather than from fresh tea leaves. There was a significant difference between ripened tea rare earth elements and raw tea's from the same sources, which affected some ripened tea quality at last. During the post-fermentation processing of Pu'er tea, the chemical constituents in the sun-dried green tea have changed dramatically, and therefore some new chemical constituents that provide the unique quality of Pu'er tea areformed. Consumer acceptability of tea depends mostly upon its aroma and taste, on the other hand aroma and taste depend upon spatiotemporal variability of the crop and manufacturing processes, which in turn highly influence its chemical composition, and are very critical in

determining its quality.[8] So the quality grade of tea and its market price are commonly determined by the aroma and taste, and the price of tea is variable according to the different quality grades, ranging from a few dozen to several hundred dollars per/kg. It is therefore very important to estimate the quality of tea. It was established that sensory property of tea, especially its specific aroma profile, is a key factor in determined its quality grade[9]. For this reason, conventional chemical methods are not sufficient for identification. Thus, there is significant interest in developing accurate methods to discriminate these varieties.[10] Over the past decade, major steps were taken not only to improve the chemical components of Pu'er tea but also to develop processing techniques ensuring their quality. Nowadays, chromatographic fingerprinting is the generally accepted technique for the assessment and quality control of Pu'er tea. In order to their current assessment and quality control, a chromatographic fingerprint analysis method was generated.

Experimental

Materials. Study involved 9 Pu'er raw tea 7542 which were purchased from Menghai Tea Factory. Different years and batches of Pu'err raw tea 7542 were 106,504,505,606,608,703,801,810,901,912. For example, 106 represented the 6th batch of Pu'er raw tea 7542 in 2010.

Sample Preparation. Dried tea leaves samples were homogenised. Then,1.0000g of homogenized sample was accurately weighed into the head space bottle.

GC-MS condition. GC: Helium as carrier gas, it was a constant flow of 0.57mL.min^{-1}.The temperature programme was the following: initial temperature 150˚C, held for 1 min, 2˚C.min^{-1} ramp to 230˚C, held for 10min. The total analysis time was 55 min. The temperature of the injection port was 230˚C. MS: The mass spectrometer was operated in electron ionization mode with an ionising energy of 0.88kv, ion source temperature 200˚C, MS Quad temperature 230 ˚C. The scan was from 45 to 800m/z and the solvent delay was 5 min.HS: 1.0mL volume was injected.The temperature and time of oscillation box in static headspace were 180˚C and 30min. The rate of oscillation was 250rpm..Time of the blowingneedle for air was 3 min.The rate of sample injection was 500μL/s.

Data analysis. The traditional chinese medicine (TCM) chromatographic fingerprint similarity evaluation system (A) 2004 edition software and the SPSS 19.0 software.

Results and discussion

Selection of the temperature and time of oscillation box. It had to be weighed accurately Pu'er tea 7542 (106) sample according to the experimental method in the paper. The temperature and time of oscillation box were studied in GC-MS. The temperature of oscillation box were set respectively to 80˚C, 90˚C, 100˚C, 110˚C, 120˚C, 130˚C, 140˚C, 150˚C, 160˚C, 170˚C, 175˚C and 180˚C. The time of oscillation box were set respectively to 10min、20min、30min、35min、40min. The result that when the temperature and time of oscillation box were 180˚C and 30 min respectively, the obtained peak areas and peak number in their fingerprints were large and more. Therefore, 180˚C and 30 min have been adopted as the temperature and time of oscillation box in this determination conditions.

The precision experiment of the laboratoryinstruments. It had to be weighed accurately 1 Pu'er tea 7542 (106) sample according to the experimental method in the paper to continuous sample Introduction about 5 in the same instrument conditions. The common peaks as well as their retention times and peak areas on GC-MS fingerprint can be counted. The experimental results provided that RSD of the relativeretention time for each chromatographic peak was less than 0.15% and RSD of relative peak area was less than 3.90%. The results show that the instrument is precise.

The repetitive experiments of the method. It had to be weighed accurately 5 Pu'er tea 7542 (106) samples according to the experimental method in the paper. The 5 sample are determined in the same instrument conditions. The common peaks as well as their retention times and peak areas on GC-MS fingerprint can be counted. The experimental results provided that RSD of the relativeretention time

for each chromatographic peak was less than 0.20% and RSD of relative peak area was less than 2.50%. The results show that the reproducibility of the method was good.

The establishment of fingerprints and analysis. The 9 Pu'er raw tea 7542 samples in different ages and batches detectded by GC-MS. Their GC-MS fingerprint were set up. The result see figure 1. The contents of the compounds were determined using the square peak to normalization in the GC-MS chromatogram of 9 Pu'er raw tea 7542 samples. 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 percent and compounds of Pu'er tea reported in literature, there were 26 compounds in volatile components of Pu'er raw tea for different ages and batches 7542 samples.

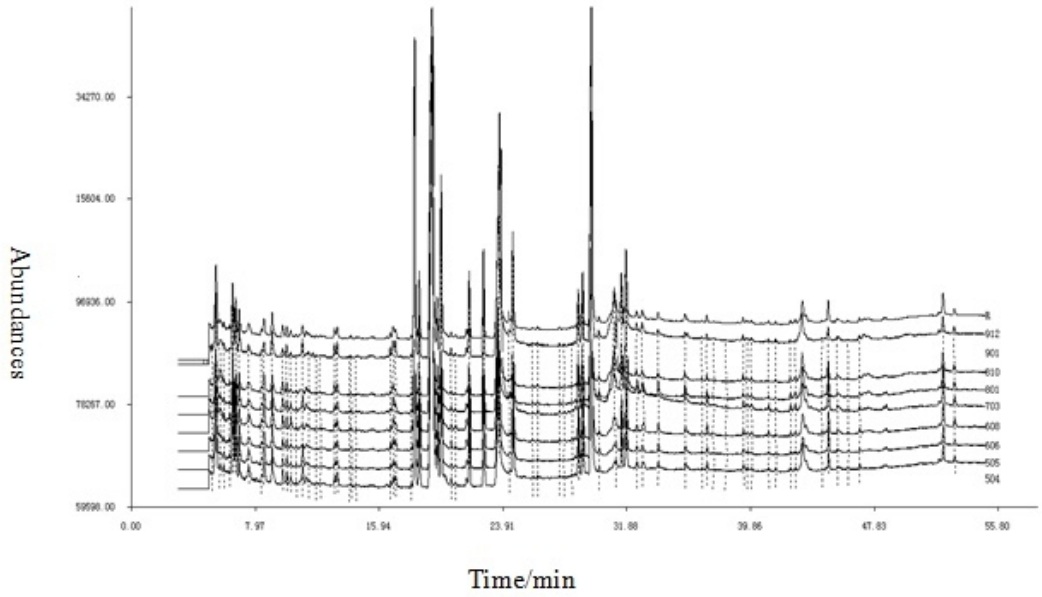

Fig. 1 The GC-MS fingerprint of volatile components of Pu'er raw tea for different ages and batches 7542 samples (Note: bottom-up volumes followed by 504,505,606,608,703,801,810,901,912,R（106）)

The GC-MS fingerprint of volatile constituents in different ages and batches Pu'er raw tea 7542 samples were analyzed their similarity for reference fingerprint of Pu'er raw 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 results were analyzed by similarity grade calculate method to compare the fingerprint difference of samples. The matching result is measured by the relative coefficient. The results are shown in table 1.

Table 1 Similarity calculation results of volatile constituents fingerprints in different ages and batches 7542 Pu'er raw tea samples

No.	504	505	606	608	703	801	810	901	912	R
504	1.000	0.623	0.854	0.877	0.217	0.449	0.558	0.602	0.776	0.734
505	0.623	1.000	0.728	0.735	0.660	0.763	0.937	0.962	0.702	0.905
606	0.854	0.728	1.000	0.984	0.414	0.502	0.686	0.720	0.917	0.880
608	0.877	0.735	0.984	1.000	0.447	0.555	0.729	0.743	0.938	0.904
703	0.217	0.660	0.414	0.447	1.000	0.752	0.693	0.603	0.575	0.728
801	0.449	0.763	0.502	0.555	0.752	1.000	0.818	0.770	0.671	0.810
810	0.558	0.937	0.686	0.729	0.693	0.818	1.000	0.963	0.757	0.918
901	0.602	0.962	0.720	0.743	0.603	0.770	0.963	1.000	0.720	0.903
912	0.776	0.702	0.917	0.938	0.575	0.671	0.757	0.720	1.000	0.924
R	0.734	0.905	0.880	0.904	0.728	0.810	0.918	0.903	0.924	1.000

Note:R- reference fingerprint of Pu'er raw tea 7542 (106)

We can see from table 1, the similarity of samples between 0.800 and 0.900 in spectrum peaks were 606,801. The similarity of samples to be lower than or equal to 0.800 in spectrum peaks were 504,703. The similarity of samples to be more than or equal to 0.900 in spectrum peaks, were 505,608,810,901,912. Samples from 505,608,810,901,912 and 106 were high similarity in spectrum peaks. And yet, difference of similarity in samples from 504,703 and 106 were small. Samples from 606,801 and 106, showed evident difference in spectrum peaks.The results show clearly that the composition contents of different ages and batches Pu'er raw tea 7542 samples have not apparent change.

Conclusions

The advantage on the establishment of fingerprint by GC-MS is componential certainty. Qualitative ability of this method is more reliable compared with GC method. GC-MS fingerprints of common peaks in different ages and batches Pu'er raw tea 7542 samples have not apparent change.The method of GC-MS fingerprint about Pu'er tea 7542 samples was established in paper to perfect quality control and achieve the standardization andmodernization. So, the fingerprint of Pu'er tea is the trend of quality control technology and is an importantmeasure in enterprise managerial modernization of Pu'er tea.

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