Chemometric Methods for the Analysis of Graftage-Related Black Tea Aroma Variation by Solid Phase Mirco-Extraction and Gas Chromatography-Mass Spectrometry

Application Note

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Abstract

A solid-phase micro-extraction (SPME) and gas chromatography/triple quadrupole mass spectrometry (GC/MS/MS) method was developed to analyze graftage-related black tea samples. Data extraction and statistical analysis were performed using Agilent MassHunter Profinder and Agilent Mass Profiler Professional (MPP) software. The characteristic volatile compounds, which were identified or tentatively identified, were subjected to principle component analysis and hierarchical clustering analysis to reveal the differences among tea samples.

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Introduction

Tea (*Camellia sinensis*) is a popular beverage worldwide, particularly in China. As one of the key indicators of sensory quality, tea aroma is the representation of volatile components. Grafting is a widely-used technique in tea propagation and cultivar change. Possible changes of the volatile components may occur after graftage due to potential secondary metabolite variation in the scion resulting from rootstock replacement [1,2]. Gas chromatography-mass spectrometry (GC/MS) coupled with chemometrics is an efficient technique to investigate and reveal variations in the complex mixtures of volatile and semivolatile compounds among tea samples.

In this study, to show the aroma profile difference induced by graftage, solid phase micro-extraction (SPME) combined with GC/MS/MS operated in scan mode and chemometrics was applied to extract and analyze the volatile components of black tea samples prepared from nongrafted and grafted YingHong No.9, a popular tea variety in Guangdong province in China. Agilent MassHunter Profinder software was applied to extract the compound information and export data in compound exchanged files (cef files). To obtain a data matrix of characteristic volatile compounds with good reproducibility, Agilent Mass Profiler Professional (MPP), a software for bioinformatics data mining and chemometric analysis, was used for sample alignment and data filtering [3,4]. The resulting compounds were subjected to principle component analysis (PCA) and hierarchical clustering analysis (HCA) to identify differences between various tea samples.

Experimental

Tea sample

Five groups of rotovane (cut-tear-curl type) black tea samples, including six biological replicates, were prepared from nongrafted YingHong No.9 (CK) and grafted YingHong No.9 on rootstocks of four different tea varieties including BaiMao No.2 (BM), HeiYe ShuiXian (HY), HuangZhiXiang DanCong (HZX), and WuLingHong (WLH) (Figure 1).

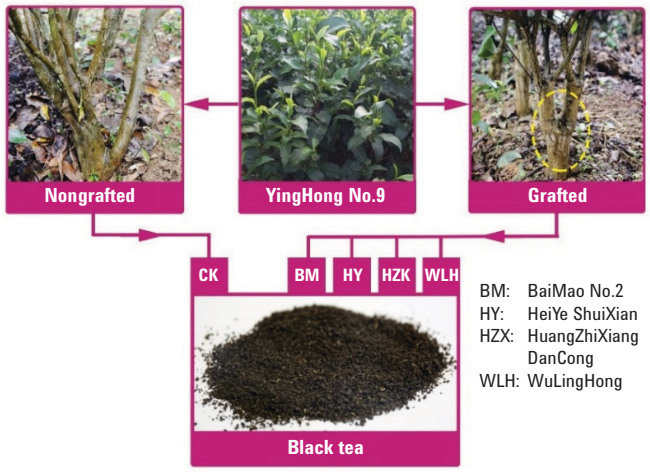


Figure 1. Sources of tea used in this study.

Instrument conditions

Table 1. GC/MS/MS Conditions

Parameter	Value
GC system	Agilent 7890B
Column	DB-5MS, 60 m × 0.32 mm, 0.25 µm (p/n 123-5562)
Oven program	50 °C hold 3 minutes, at 5 °C/min to 250 °C, hold 5 minutes
Carrier gas	Helium
Flow rate	1.0 mL/min
Injection mode	Manual, SPME fiber
Injection port temperature	270 °C
Interface temperature	280 °C
MS system	Agilent 7000D
Ion source	EI, 70 eV
Ion source temperature	230 °C
Quadrupole temperature	Q1 and Q2 = 150 °C
Spectral Acquisition	Full scan, 35–500 *m/z*

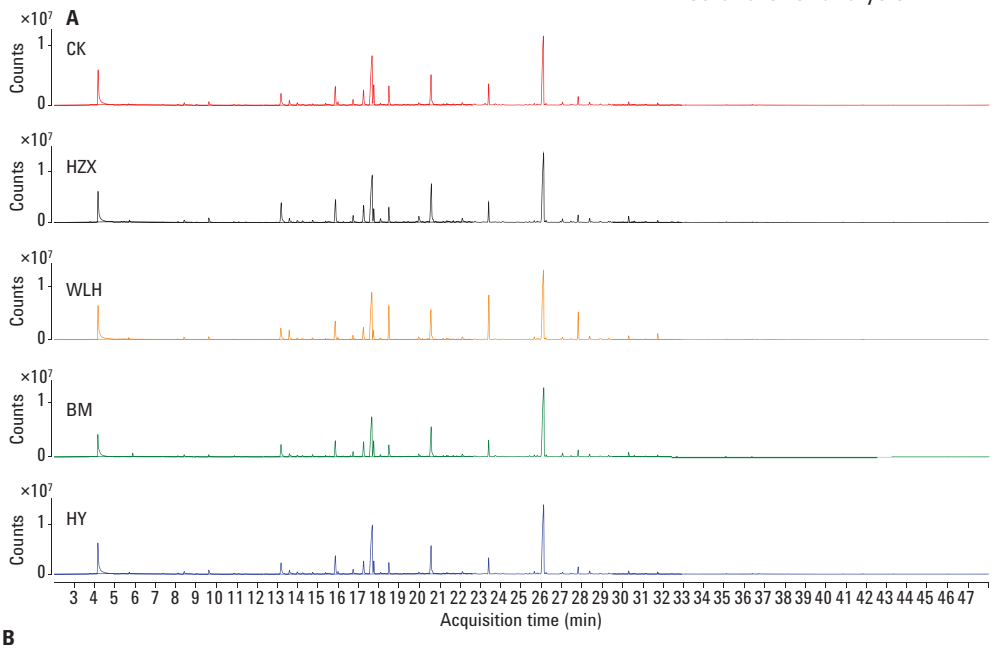
SPME Conditions

A 3.5 g sample of black tea was weighed in a glass vial, and 10 mL of boiling water was infused, followed by 10.0 µL of ethyl decanoate (0.2 µg/µL in ethyl ether) as an internal standard. The vial was sealed and transferred to a 60 °C water bath, and kept for 5 minutes. The extraction was carried out at 60 °C for 40 minutes with a DVB/CAR/PDMS-50/30 µm SPME fiber. The SPME fiber was desorbed for 4.5 minutes at 270 °C.

Results and Discussion

Data extraction

Figure 2A shows the total ion chromatograms of different graftage-related black tea samples. MassHunter Profinder software is a productivity tool for processing multiple samples in profiling analyses, allowing the user to visualize, review, and edit results by compound across many samples. Higher quality results can be obtained based on cross-sample processing. Chromatographic peak extraction was done using the Profinder software (version B. 08) using molecular feature extraction (MFE) (Figure 2B). Cef files of each sample were obtained in Profinder software, and imported into MPP software for analysis.



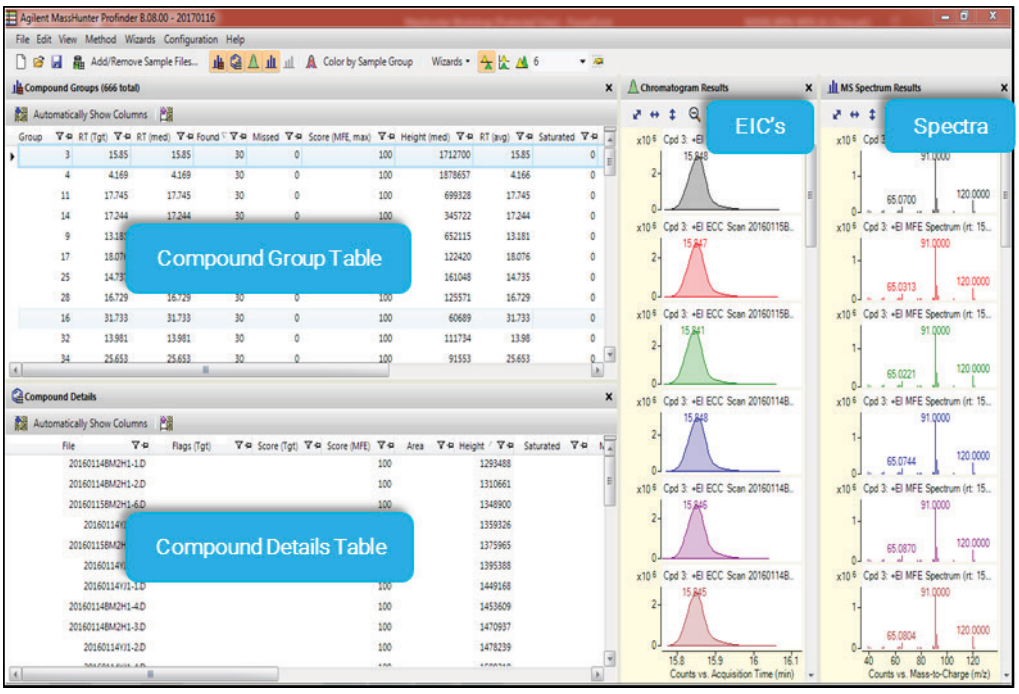


Figure 2. A) Total ion chromatograms of five groups of black tea samples. B) Main view of Agilent MassHunter Profinder software.

Data filtering and compound identification

Data filtering and chemometric analysis were carried out using MPP software (version B.14.5). All the cef files were subjected to data filtering. A total of 584 entities were obtained through data alignment across five sample groups. Step-wise data filtering was carried out based on filters of frequency-of-occurrence, sample variability, and one-way analysis of variance (one-way ANOVA), subsequently. One hundred two entities, which consistently existed within at least one sample group (frequency-of-occurrence filter), and demonstrated good reproducibility (coefficient of variation <25 %, sample variability filter) were obtained. Then, 44 entities were selected through one-way ANOVA with a p-value cutoff of 0.05 and a fold change threshold of 1.5 (FC ≥1.5) in reference to the CK (nongrafted group). Finally, 34 compounds were tentatively identified by ID Browser according to a library search based on the NIST14 database (Figure 3). Eight of the volatile compounds were confirmed with the reference standards, and are listed in Table 2. The 34 compounds, which primarily consisted of aldehydes, alcohols, ketones, and esters, were subjected to principle component analysis and hierarchical clustering analysis.

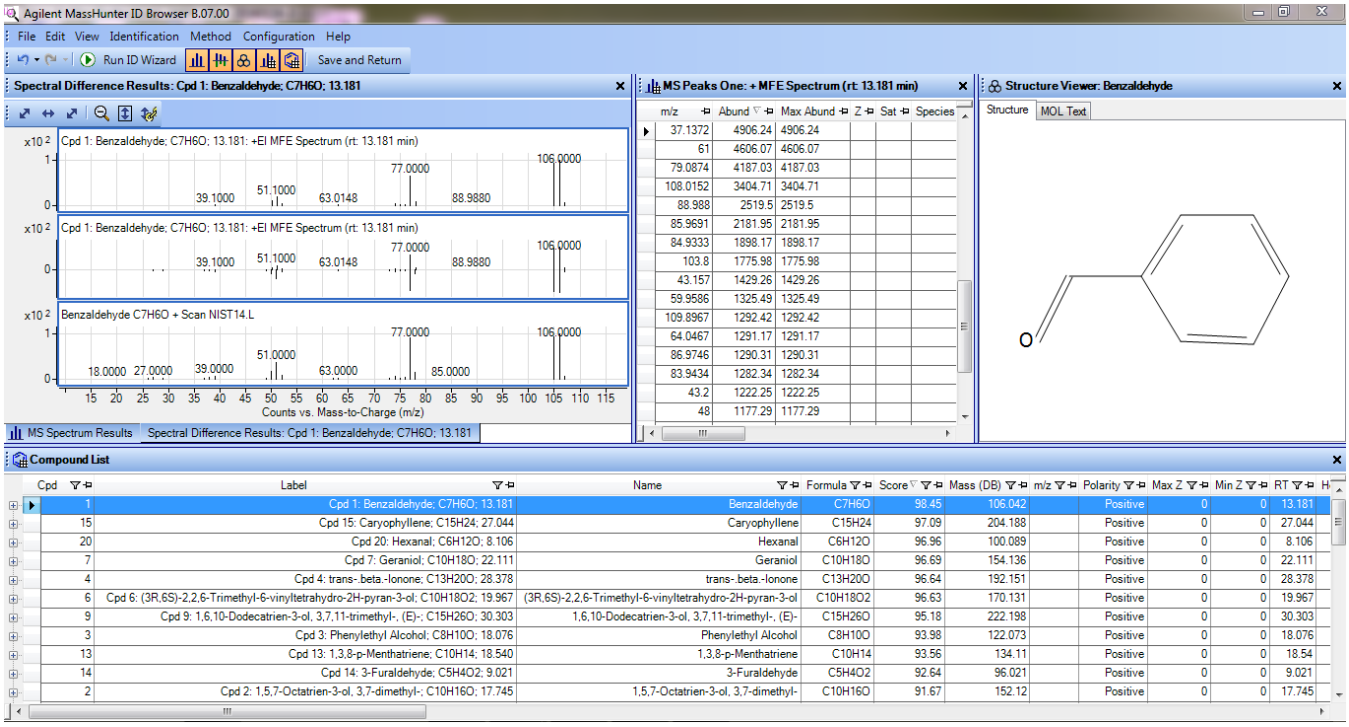


Figure 3. ID Browser function in Agilent Mass Profiler Professional for compound identification.

Table 2. Identification of Compounds by Reference Standards

No.	RT (min)	Compound	CAS Number
1	8.10	Hexanal	66-25-1
2	13.18	Benzaldehyde	100-52-7
3	14.74	(E,E)-2,4-Heptadienal	4313-03-5
4	16.24	(E)-2-Octenal	2548-87-0
5	19.44	(E)-2-Nonenal	18829-56-6
6	20.81	Decanal	112-31-2
7	22.11	Geraniol	106-24-1
8	28.38	(E)-β-Ionone	79-77-6

Principle component analysis (PCA)

PCA is a commonly used unsupervised statistical method to reduce the dimensionality of large data sets to reveal differences among samples. The 34 selected compounds were subjected to PCA. The first three principle components explained approximately 90 % of the variance in the original data. The 3D score plot presented clear separation among CK and the four grafted sample groups, indicating that the selected compounds were characteristic for nongrafted and grafted sample discrimination (Figure 4). PC1 explained 47.6 % of the variance; separation of HZX, BM, and the rest of the groups was achieved along this coordinate. PC2 explained 25.4 % of the variance; samples of CK, HY, and WLH were separated from each other along this coordinate.

Hierarchical clustering analysis (HCA)

HCA is a powerful method to uncover subgroups within a dataset, permitting observations with similar abundance profiles to merge into clusters. The HCA was conducted with the 34 selected compounds. The result is displayed as a dendrogram (Figure 5). Tea samples were classified into five clusters in accordance with their graftage treatment. Samples from grafted groups of BM shared high similarity of compound abundance with those from CK, while the abundance profile of HZX was distinctive toward both CK and the rest of the grafted groups.

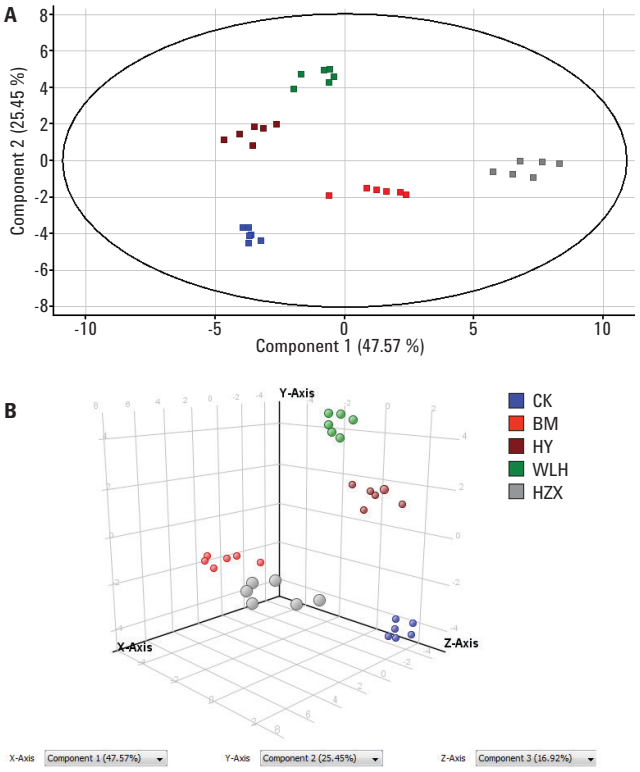


Figure 4. 2D and 3D PCA of five groups of black tea samples.

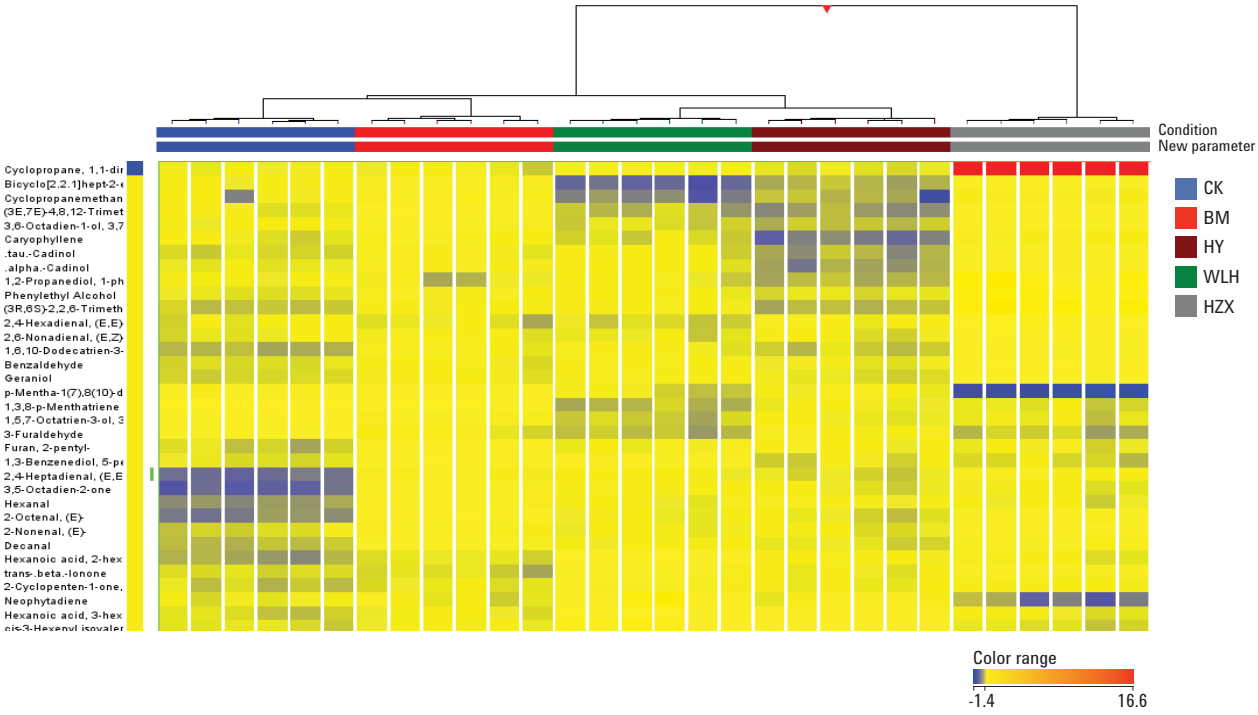


Figure 5. HCA heat map for association of compounds detected in various black tea samples.

Conclusions

Untargeted GC/MS analysis using the Agilent 7000D GC/MS/MS can provide the information-rich data required to classify and differentiate graftage-related black tea. Agilent MSD Productivity MassHunter and Agilent Mass Profiler Professional software enabled the automatic mining and processing of the data to find the characteristic compounds. Clear separation was achieved among the five groups using PCA and HCA based on the identified compounds. The finding showed that grafting influenced the volatile organic compound profile of the black tea, and is potentially beneficial for guidance of rootstock selection in tea propagation.

References

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