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

Introduction

As one of the key indicators of sensory quality, tea (Camellia sinensis) aroma is the representation of volatile components. Possible changes of the volatile components may occur after graftage due to potential secondary metabolite variation in the scion resulting from rootstock replacement. 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 semi-volatile compounds among tea samples. In this study, solid phase mirco-extraction (SPME) combined with GC-MS and chemometrics was applied to extract and analyze the volatile components of black tea samples prepared from non-grafted and grafted "YingHong No.9", a popular tea variety in Guangdong province, China, to show the aroma profile difference induced by graftage.

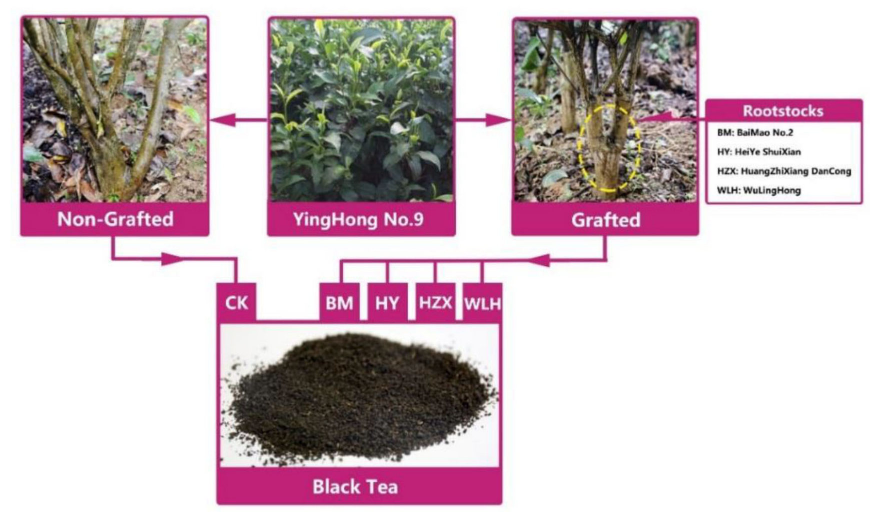
Method

The graftage-related black tea samples were analyzed by solid-phase micro-extraction and a triple quadrupole GC/MS/MS operated in MS scan mode. Masshunter Profinder software was applied to extract the compound information and export data in compound exchanged files (.cef). Mass Profiler Professional (MPP), a software for bioinformatics data mining and chemometric analysis, was used for sample alignment and data filtering to obtain a data matrix of characteristic volatile compounds with good reproducibility. The resulting compounds were subjected to univariate analysis, principle component analysis and hierarchical clustering analysis to reveal the differences among samples.

Experimental

Tea Sample

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



SPME Conditions

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

Instrument Conditions

GC and MS Conditions	Value
GC system	Agilent 7890A
Column	DB-5MS (60 m×0.32 mm×0.25 μm)
Oven program	50 °C hold 3 min , at 5 °C /min to 250 °C hold 5 min
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	150 °C
Spectral Acquisition	Full scan, 35-500 m/z

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Results and Discussion

Data Extraction

The total ion chromatograms of different graftage-related black tea samples are shown in Fig.1. 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) by molecular feature extraction features (MFE) (Fig. 2). Cef files of each sample were obtained by Profinder software and imported to MPP software for analysis.

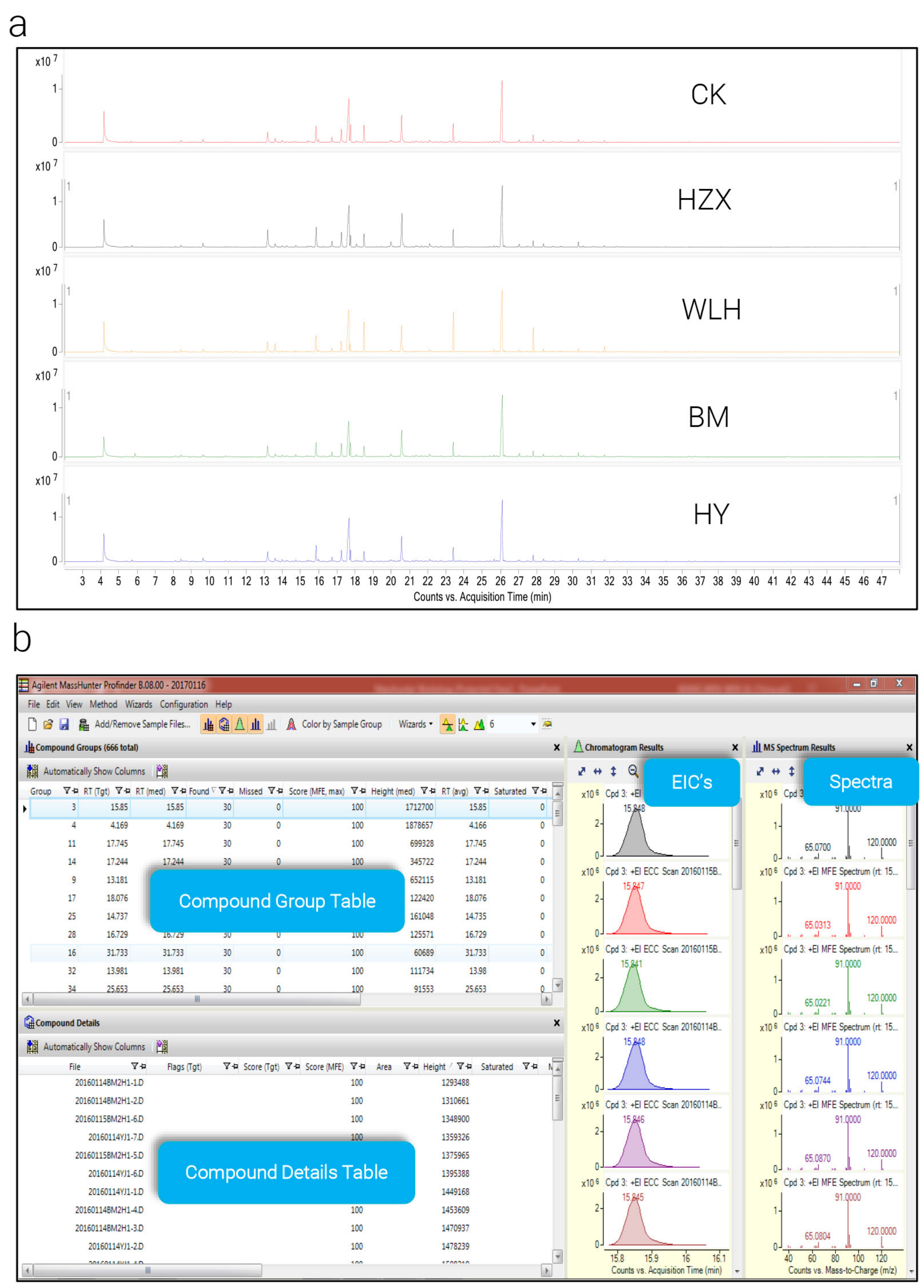

Fig.1 (a) The total ion chromatograms of five groups of black tea samples;

(b) The main view of the MassHunter Profinder software.

Mass Profiler Professional (MPP)

Data filtering and chemometric analyses were carried out via MPP software (version B. 14.5, Agilent Technologies). All the collected cef files were subjected to data filtering. The identified compounds underwent the chemometric analyses of principal component analysis and hierarchical clustering analysis for sample classification.

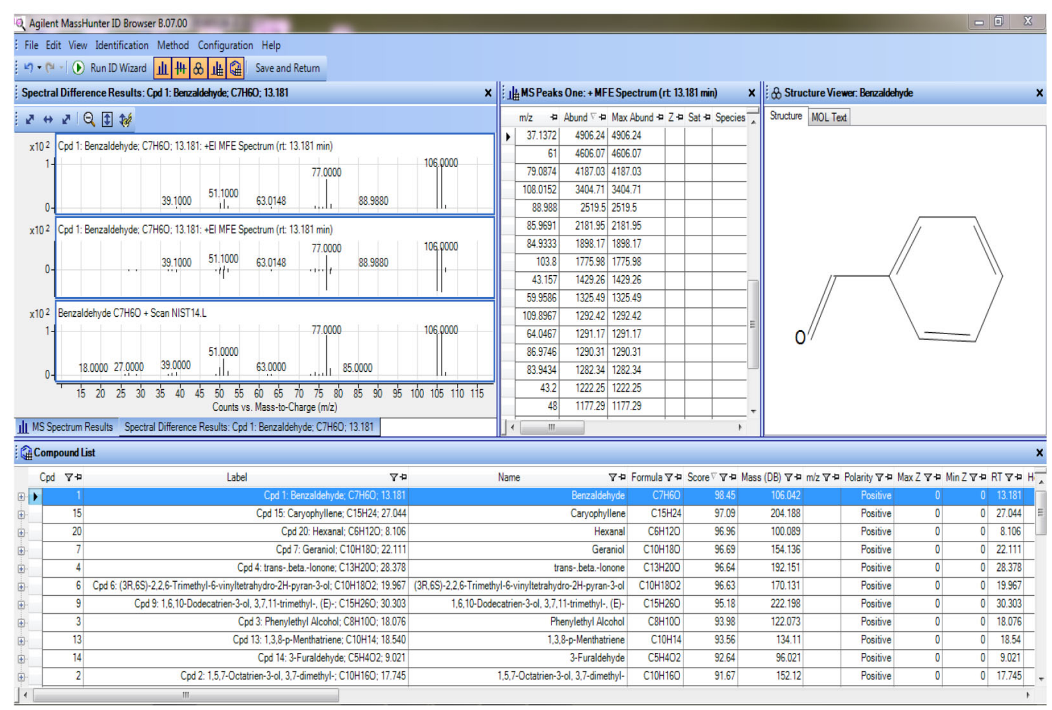

Fig.2 ID Browser function in MPP for compound identification

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). 102 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 (non-grafted group). Finally, 34 compounds were identified by ID Browser according to the library searching based on NIST14 standard database (Fig.2). The 34 identified tea volatile compounds mainly consisted of alcohols, ketones, organic acids, esters and etc.

Results and Discussion

Principle Component Analysis (PCA)

PCA is a commonly used unsupervised statistical method to reduce the dimensionality of large data sets to reveal the differences among samples. The 34 identified compounds were subjected to PCA. The first 3 principle components explained approximately 90% of the variance in the original data. The 3-D score plot presented clear separation among CK and the four grafted sample groups indicating that the selected compounds were characteristic for non-grafted and grafted sample discrimination (Fig. 3). 47.6% of the variance was explained in PC1; 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.

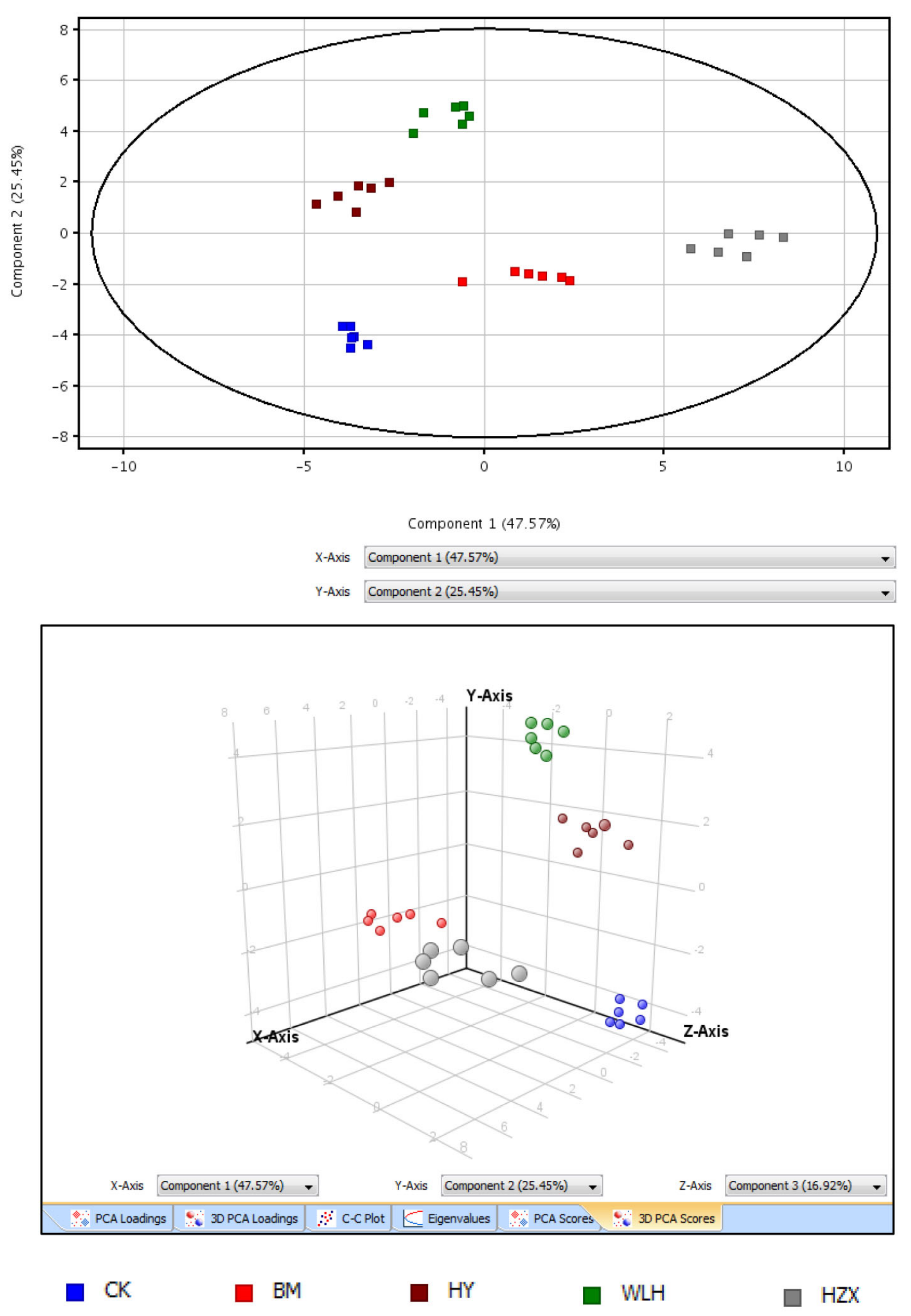

Fig. 3 2-D and 3-D principle component analysis(PCA) of five groups of black tea samples

Hierarchical Clustering Analysis (HCA)

HCA is a powerful method to uncover subgroups within a dataset. The method allows observations with similar abundance profiles to merge into clusters. The HCA was conducted with the 34 identified compounds. The result is displayed as a dendrogram (Fig. 4). 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 grafted groups.

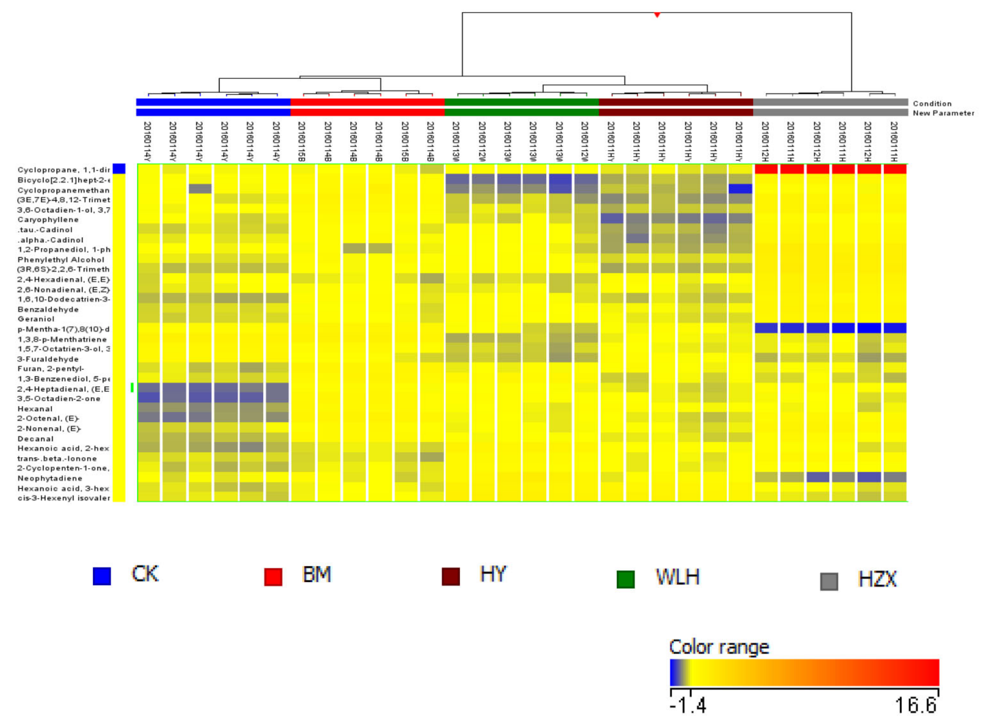

Fig. 4 Hierarchical clustering analysis (HCA) heat map for association of compounds detected in various black tea samples.

Conclusions

- A SPME and GC-MS method for profiling of black tea samples prepared from non-grafted and grafted "YingHong No.9" has been developed;
- 34 volatile compounds with significant variation among non-grafted and grafted sample groups were identified;
- Clear separation was achieved among the 5 groups with PCA and HCA based on the 34 identified compounds via MPP;
- The finding is potentially beneficial for guidance of rootstock selection in tea propagation.