Poster Reprint

ASMS 2018
TP-157

GC-MS Combined with Chemometric Method for Analysis of Rapid Aged White Tea Compared with Natural Aged and Fresh White Tea

Dandan Qi[1]; Wei Chen[1]; Chengying Ma[1]; Wenwen Wang[2]; Junxi Cao[1]; Aiqing Miao[1]; Shi Pang[1]

[1] Tea Research Institute, Guangdong Academy of Agricultural Sciences, Guangdong, China

[2] Agilent Technologies Company Ltd., Beijing, China

Introduction

It is known that volatile aroma compounds determine the aroma profile of tea. White tea, a kind of slightly fermented tea, is considered as the minimally-processed form of tea with only two main processing steps: withering and drying [1]. After a long aging period, white tea can develop unique aroma characteristics and then is named aged white tea. It is similar with the aging process of red wine and Pu-erh tea to some extent [2]. Solid phase micro-extraction coupled with gas chromatography-mass spectrometry (GC-MS) is proven to be an effective way to extract and analyze volatile compounds. In addition, chemometrics has advantages in extracting relevant information and discovering patterns in a large series of data. Herein, GC-MS coupled with chemometrics is applied to the analysis of the difference in volatile compounds among four treatments for white teas including fresh white tea (FWT), control group white tea (CKWT), rapid aged white tea (RAWT) and natural aged white tea (NAWT).

Method

The four white teas were analyzed by solid-phase micro-extraction and a triple quadrupole GC/MS/MS operated in MS scan mode. MassHunter Qualitative 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, hierarchical clustering analysis and Venn diagram to reveal the differences among samples.

Figure 1. 7000D GC-MS/MS system

Experimental

Tea Samples

White tea samples included four different treatments, namely fresh white tea (FWT), control group white tea (CKWT), rapid aged white tea (RAWT), natural aged white tea (NAWT) and each treatment had three replicates samples. Primary white tea of all four treatments were made of the same variety “YingHong NO.9” based on the same manufacturing process. FWT was produced by sealing and storing primary white tea in a refrigerator at -20℃ for 180 days. CKWT was obtained by sealing and storing primary white tea in a dry and well-ventilated storehouse for 180 days. RAWT was manufactured by storing unsealed primary white tea in an innovative rapid aging room for 180 days. NAWT was produced by sealing and storing primary white tea in a dry and well-ventilated storehouse for 12 years.

Figure 2. Four white teas with different treatments

SPME Conditions

3.5 g white 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 a 60 ℃ water bath 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 ℃.

Table 1. GC/QQQ Operational Conditions.

GC and MS Conditions	Value
GC system	Agilent 7890B
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

Results and Discussion

Data Extraction

Chromatographic peak extraction was done using the Masshunter Qualitative software by deconvolution. Cef files of each sample were obtained by Qualitative software and imported to MPP software for analysis. The total ion chromatograms of the four treatments of white tea are shown in Figure 3.

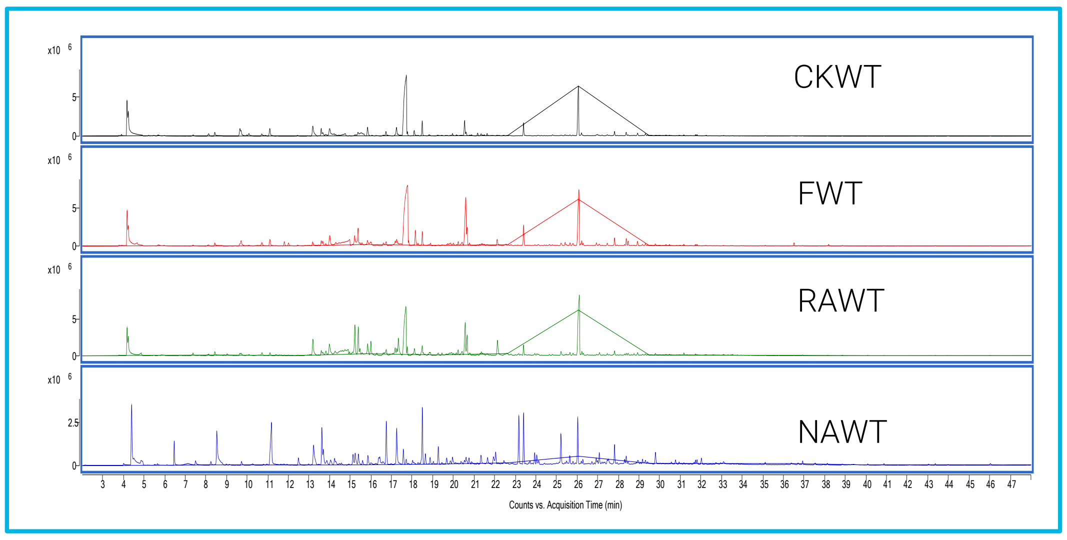

Figure 3. The total ion chromatograms of four white teas.

Data Filtering

All of the .cef files were imported into MPP software for peak alignment and data filtering, followed by statistical analysis. To qualify the repeatability and validity of these entities, frequency (frequency$>$60%) and coefficient of variability (CV$<$25%) filters were applied to the target compounds. Next, ANOVA ($p<0.05$) and fold change (FC ≥2) were employed to pick out compounds that made significant differences on the aroma of these four white teas. Eventually, 164 entities were obtained as differential entities. By comparing mass spectra and RI with the information from NIST 14 library and standards, 40 entities were eventually identified as differential aroma compounds among the four white tea groups, including alcohols, aldehydes, ketones, esters, heterocylics and alkanes. The ID Browser function in MPP was used for compound identification by library searching (Figure 4).

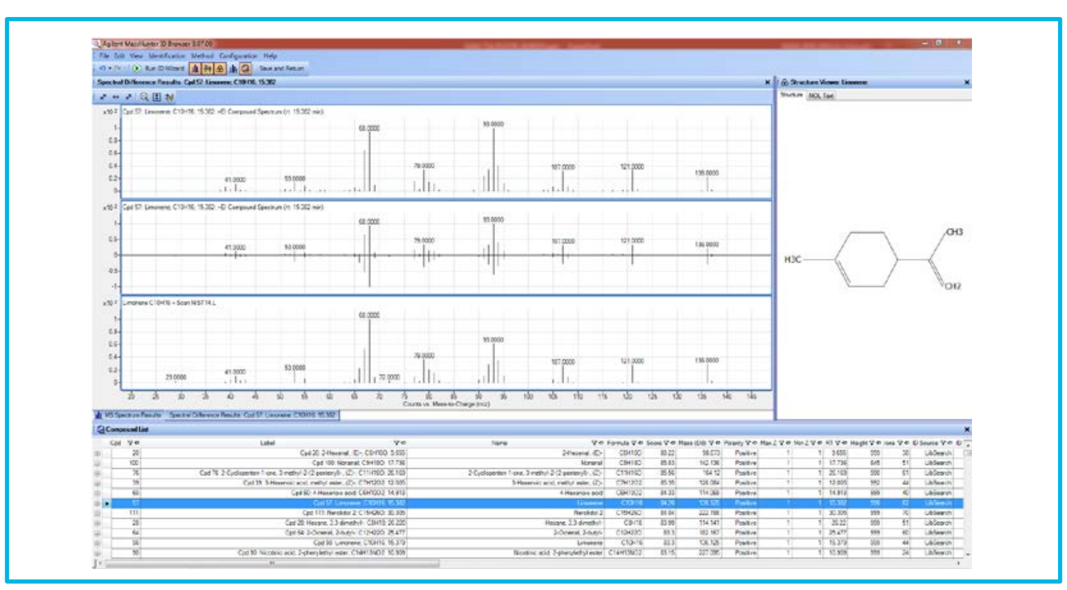

Figure 4. ID Browser function in MPP for compound identification

Principle Component Analysis (PCA)

PCA analysis was performed based on the 40 identified compounds to investigate the effects of rapid aging technology on white tea aroma compounds in comparison with NAWT and FWT. As shown in Figure 5A, the first three principal components account for 97.96% of the total variance (38.80%, 31.02% and 28.14%, respectively). Obviously, the clear separation of RAWT from CKWT based on PC2 and PC3 (Figure 5A) indicates some significant effects of rapid aging technology on aroma compounds of white tea, and FWT, CKWT and NAWT are separated mainly based on PC1 (Figure 5B).

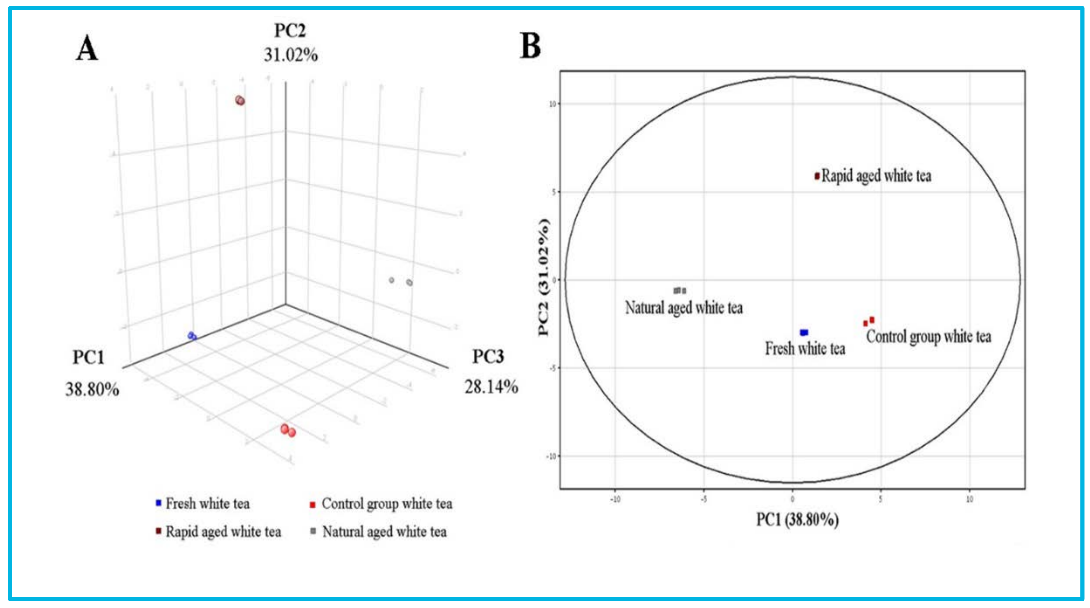

Figure 5. Principle component analysis of differential aroma compounds in four white teas. A. Scatter plots of first three principal components; B. Scatter plots of the first two principal components.

Hierarchical Clustering Analysis (HCA)

The dendrogram of HCA was applied to visualize differences of aroma compounds among four white teas based on the 40 identified compounds, as shown in Figure 6. Four white teas were divided into two groups and three white teas including FWT, CKWT and RAWT were in the first group. The first group was further divided into two groups by distance, and two white teas including FWT and CKWT were in the same group; RAWT was in an isolated group. Hence, it is shown that rapid aging technology did influence the aroma compounds by comparing RAWT with CKWT. Results of HCA were consistent with those of PCA, suggesting that the analysis methods and data processing in this study are reliable. To summarize, clear separation of these samples in PCA and uniform clustering results in HCA both suggest that GC-MS coupled with chemometrics analysis is a valid and accurate approach to discriminate different white teas produced by different aging methods.

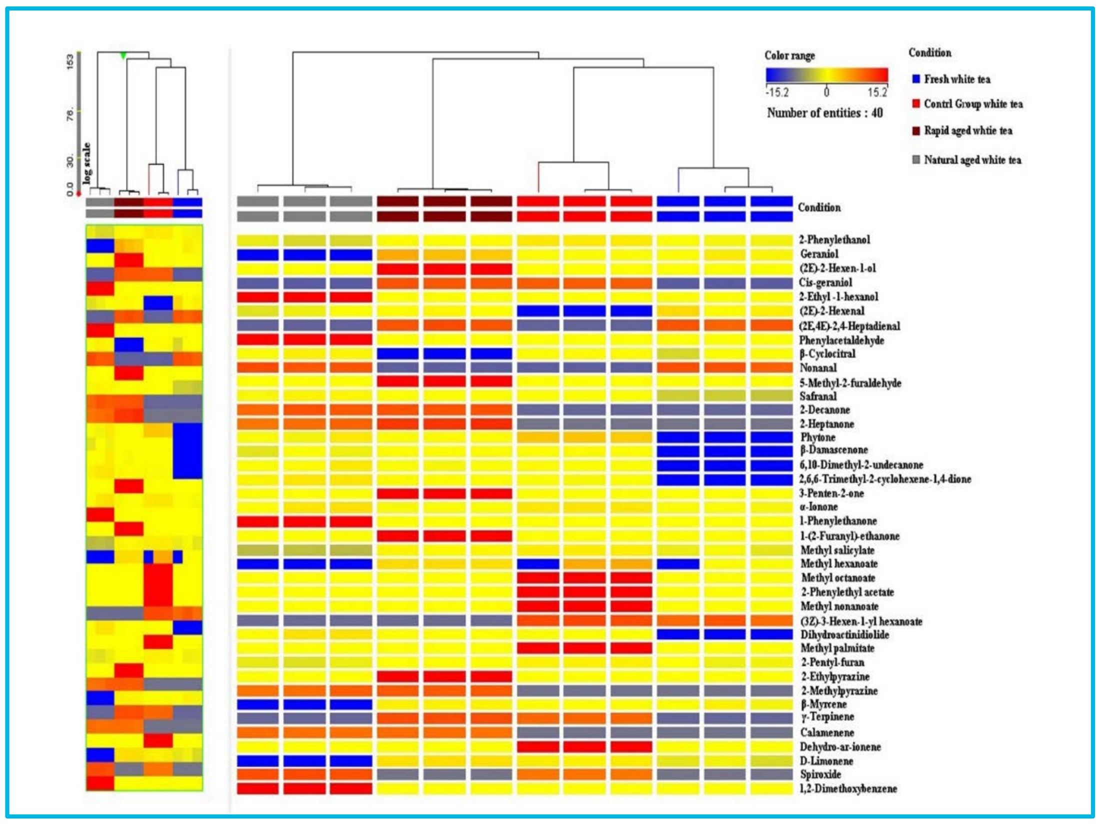

Figure 6. Hierarchical cluster analysis of four white teas.

Venn diagram

To visualize the possible logical relations among four white teas, a Venn diagram was created based on the 40 identified compounds (Figure 7). FWT and RAWT shared 10 aroma compounds; CKWT and RAWT shared 16 aroma compounds ; NAWT and RAWT shared 15 aroma compounds. In short, the Venn diagram showed significant difference in the constitution of volatile compounds among the four white teas. Circle **a** shows that there are 4 differential aroma compounds that exist only in CKWT. Figure 8 displays detailed information about these 4 differential aroma compounds. Circle **b** shows there are 5 compounds that exist only in RAWT, circle **c** that there are 4 compounds that exist only in NAWT, and circle **d** that there are 5 compounds that exist in all four white teas.

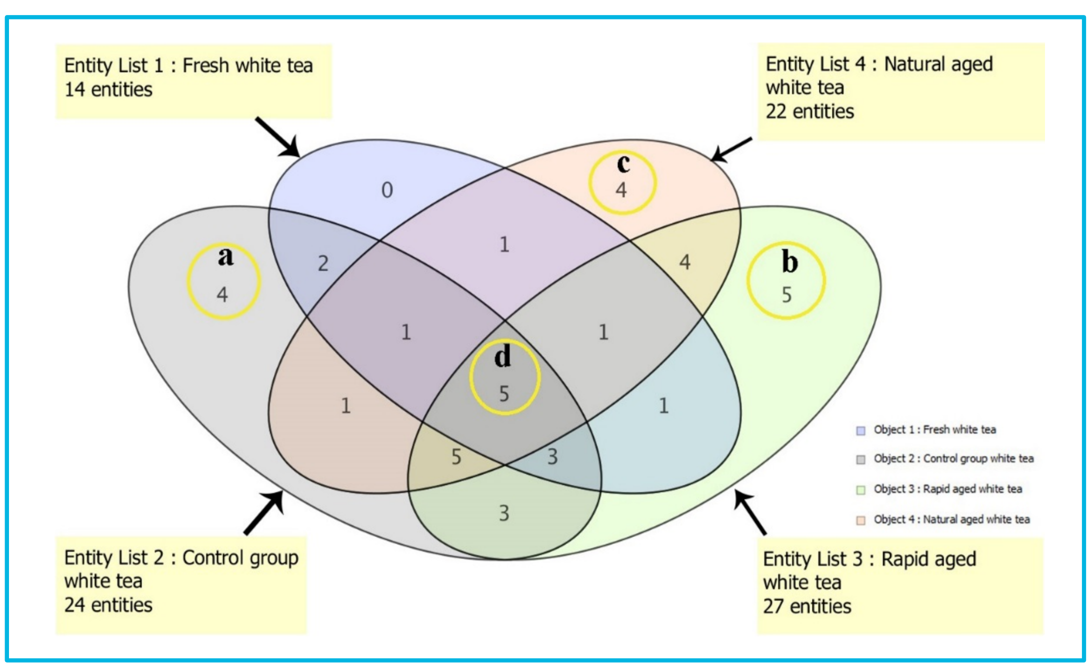

Figure 7. Venn diagram of 40 differential aroma compounds in four white teas.

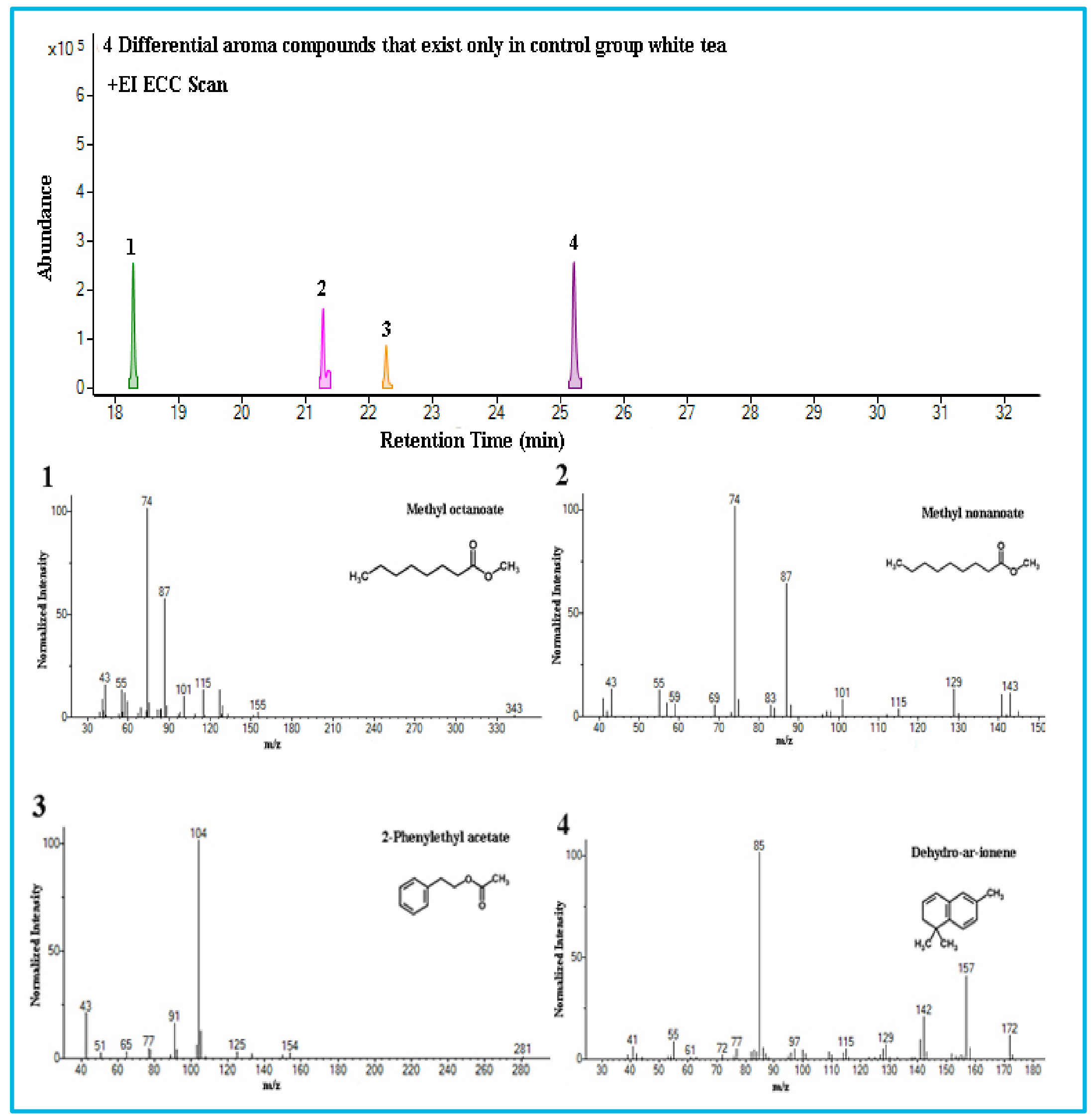

Figure 8. Four differential aroma compounds that existed only in control group white tea.

Conclusions

- A SPME and GC-MS method for profiling of four different treatment white teas has been developed.
- Clear separation was achieved among the four groups with PCA and HCA based on the 40 identified compounds via MPP.
- As rapid aging technology continues to get better at producing the effects of natural aging conditions, we speculate that it is a feasible way to produce aged white tea with similar aroma quality compared with natural aged white tea in a relatively short period.

References

[1] Dai, W., Xie, D., Lu, M., Li, P., Lv, H., Yang, C., Peng, Q., Zhu, Y., Guo, L., Zhang, Y., Tan, J., & Lin, Z. (2017). Food Research International, 96, 40-45.

[2] Lorenzo, C., Garde-Cerdán, T., Pedroza, M. A., Alonso, G. L., & Salinas, M. R. (2009). Food Research International, 42(9), 1281-1286.