Discovery of the Potential Marker Compounds for Stored White Tea by Metabolomics Approach

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Introduction

The traditional Chinese white tea has been demonstrated to have potential beneficial health effects[1], thus promoting the consumption of white tea in China[2]. White teas stored for long periods are considered to have high health-protective effect and thus high commercial value. Currently, only some known common high-abundant substances have been investigated during the storage. In order to further the understanding of white tea quality change during storage and prevent artificial adulteration and mislabeling of tea ages, metabolomics approach was applied to study the non-volatile components in white tea during storage and seek the potential markers relevant with the tea quality change[3].

Experimental

Sample Preparation Procedure

Fig.1 Schematic diagram showing the procedure for sample preparation

Instruments Conditions

LC Conditions:

Agilent 1290 Infinity II UHPLC with built-in degasser

Autosampler with temperature control

Column temperature control compartment.

Column: Zorbax Eclipse Plus C18, 150 × 3.0 mm, 1.8 µm; Column Temperature: 40 °C

Mobile Phase:

Solvent A– 0.1% formic acid in H_2O;

Solvent B–MeOH; Flow rate: 0.4 mL/min;

Injection volume: 3.0 µL;

Needle backflush: 5 sec with pure methanol;

Gradient elution profiles:

0-4 min: 10-15% B; 4-7 min: 15-25% B;

7-9 min: 25-32% B; 9-16 min: 32-40% B;

16-22 min: 40-55% B; 22-28 min: 55-95%B

28-30 min: 95% B; 30-31 min: 95%-10% B

31-35 min: 10% B

MS/MS Conditions:

Agilent 6540/6545 QTOF with Dual JetStream ESI;

Polarity: positive ionization;

Drying gas temperature: 300°C;

Drying gas flow rate: 8 L/min;

Nebulizer gas pressure: 35 psi;

Sheath gas temperature: 300°C;

Sheath gas flow rate: 11 L/min;

Capillary voltage: 3500 V ;

MS scan range: m/z 100-1100;

MS/MS scan range: m/z 50-1100;

Reference ions: 121.0509/922.009

Results and Discussion

Data Quality Validation

An optimized UHPLC gradient elution was applied for separation of thousands of compounds in the tea extract followed by detection using accurate QTOF/MS under the scanning mode. Pooled quality control samples were also analyzed through the sample set to evaluate the data quality. Using the molecular feature extraction algorithm, the extracted compound results were imported into MPP software for data alignment and statistical analysis. Initial data alignment of the total data set resulted in 2584 compound features. Non-supervised PCA analysis demonstrated that the QC samples are tightly clustered in the center of the score plot (Fig.2), demonstrating the good reproducibility of the tea sample extraction and the UHPLC-QTOF/MS analysis during the metabolomics investigation.

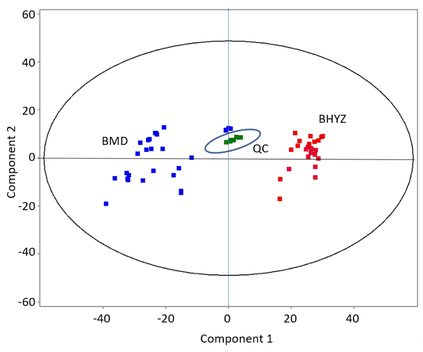

Fig. 2. The tight clustering of QC samples in the non-supervised PCA score plot of white tea samples including pooled QC samples indicating the reliability of the analytical methods

Influence of Storage on the White Tea Compounds

Both PCA and PLS-DA models were constructed to investigate the influence of the storage duration on the white tea compounds. The patterns in Fig.3 demonstrates that the white tea compounds have changed significantly over the extended storage time.

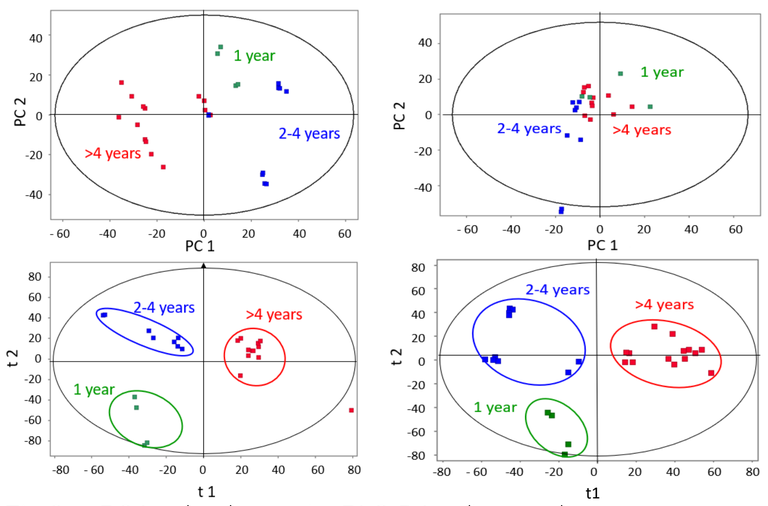

Fig.3. PCA (top) and PLS-DA (bottom) score plots demonstrating the change of the two sub-class of white tea over storage time. A. BMD; B: BHYZ.

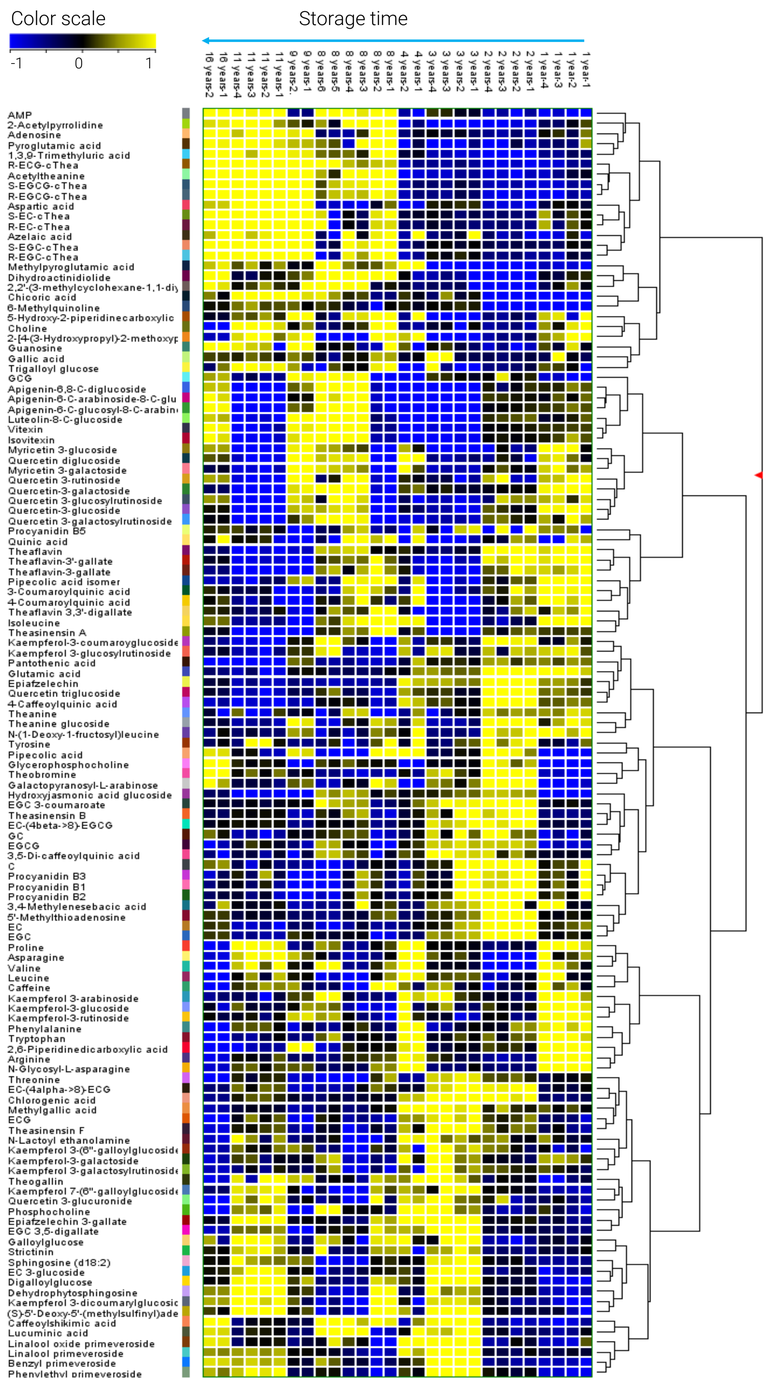

Fig.4. Heat map demonstrating the identified compounds abundance change over the storage time for BMD, one sub-class of white tea.

Results and Discussion

Identified Metabolites Change Over Storage

The metabolites features (125) were identified based on database searching and accurate MS/MS spectra, and further confirmed using the authentic standards. Heat map in Fig.4 demonstrates the obvious change for the identified compounds in the sub-class of white tea.

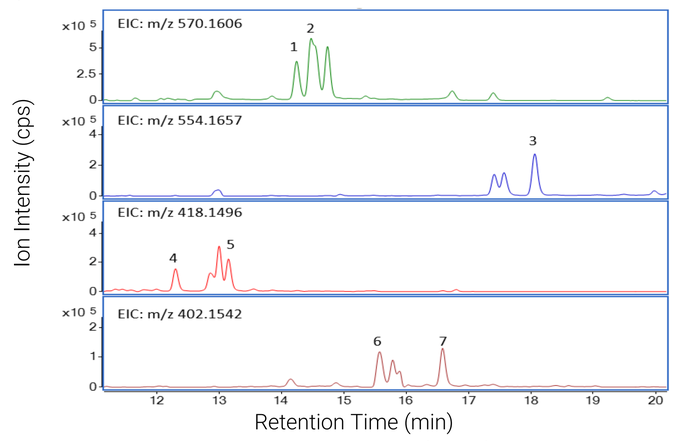

Fig. 5. Extracted ion chromatograms (EICs) of EPSFs in stored white teas

EPSFs Identification and Their Presence in Aged White Tea

Seven novel compounds were found in stored white tea obviously (Fig.4&5). They were identified as 8-C N-ethyl-2-pyrrolidinone substituted flavan-3-ols (Fig.6 &Table1).

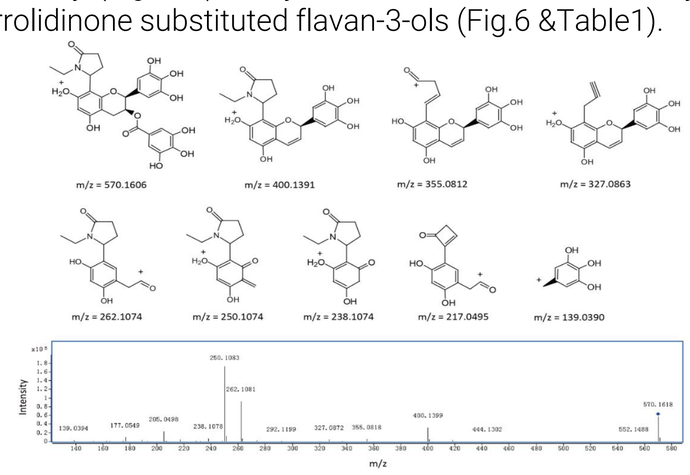

Fig. 6. Identification of peak1 in stored tea by MS/MS spectra

Table 1. List the identified ESPF compounds

No.	Identification	RT (min)	Formula	$[M+H]^+$ $(m/z)_{exp}$	Error (ppm)	MS/MS fragments
1	S- EGCG-cThea	14.24	$C_{28}H_{28}O_{12}N$	570.1617	1.93	400.1391,355.0812, 327.0863,262.1074, 250.1074, 238.1074, 217.0495,139.0390
2	R- EGCG-cThea	14.45	$C_{28}H_{28}O_{12}N$	570.1613	1.23	
3	R-ECG-cThea	18.06	$C_{28}H_{28}O_{11}N$	554.1657	1.80	384.1229,339.0863, 311.0914,262.1065, 250.0490, 177.0514, 123.0441
4	S-ECG-cThea	12.30	$C_{21}H_{23}O_8N$	418.1506	2.39	400.1391, 355.0812,327.0863, 262.1074, 250.0490, 177.0546, 139.0390
5	R-EGC-cThea	13.15	$C_{21}H_{23}O_8N$	418.1505	2.15	
6	S-EC-cThea	15.55	$C_{21}H_{23}O_7N$	402.1551	2.24	384.1447, 311.0914, 262.1074, 250.1074, 205.0490, 177.0546, 123.0411
7	R-EC-cThea	16.58	$C_{21}H_{23}O_7N$	402.1553	2.74	

Variation of ESPFs During Storage and Their Correlation with the Precursor Compounds in the White Tea

The content of 7 EPSFs increase obviously with the increase of storage duration for both sub-class of white tea (Fig.7A&B), and the increasing abundance of these compounds are correlated with the decreasing abundance of theanine and flavan-3-ols, suggesting both as the precursor compounds for EPSFs in the white tea. It is further confirmed by controlled aging experiments.

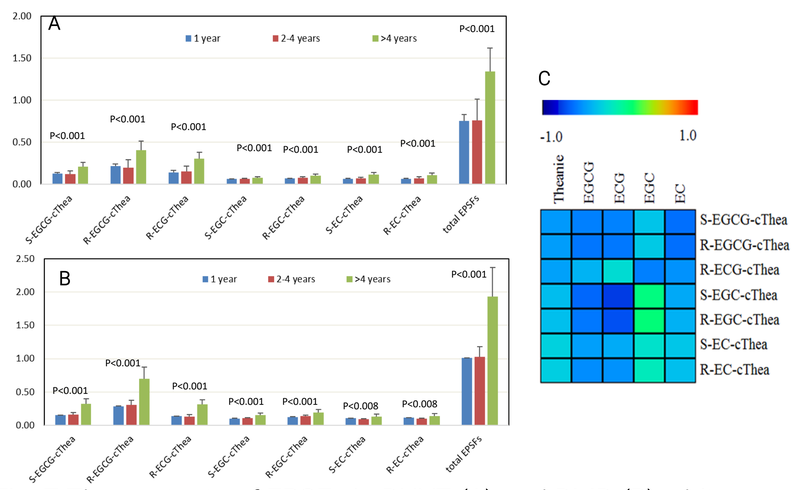

Fig. 7 The contents of EPSFs in BHYZ (A) and BMD (B) white teas. The significance of the compound difference among groups was tested using ANOVA; (C) correlation coefficients between EPSFs and theanine and between EPSFs and flavan-3-ols in white teas.

Conclusions

- Non-targeted metabolomic analysis was successfully performed for study of the white tea during extended storage duration.
- Up to 125 differential metabolites among the white tea samples were identified.
- EPSFs (8-C N-ethyl-2-pyrrolidinone substituted flavan-3-ols) are newly identified compounds in aged white tea samples, and their abundance is positively correlated with the storage duration, and also correlated with the decrease of their precursor compounds, theanine and flavan-3-ols in the white tea, suggesting that EPSFs can be promising markers for discriminating the long-term stored white tea.

References: 1. Mao et al., Cancer Prev. Res., 2010, 3, 1132-40; 2. Ning et al. Eu. Food Res. Technol., 2016, 242, 2093-104; 3. Dai et al. J. Agric. Food Chem., 2018, 66 (27), 7209-18.