DLLME-Gas Chromatography-Quadrupole Time-of-Flight Mass Spectrometry for Classification of Botanical Origin of Chinese Honey

Hui Chen[a], Linghe Jin[a] Chunlin Fan[a]*, Guofang Pang[a]*, Shan Zhou[b], Wenwen Wang[b]
[a]Chinese Academy of Inspection and Quarantine, Beijing Economic-Technological Development Area; [b]Agilent Technologies (China) Company, Ltd.

ASMS 2016
Poster TP236

Agilent Technologies

Introduction

Honey is well known for its valuable nutritional and medical properties.[1] The quality and prices of honey are highly related to its botanical origins. Conventionally, researchers classify the botanical origin of honey by target parameters, such as physicochemical properties, nutrient elements, amino acids, and others. Mass spectrometry as a tool to fingerprint the volatile profile of honey has been proven to be a powerful alternative method to distinguish botanical origin. In this study, dispersive liquid-liquid micro-extraction (DLLME) combined with gas chromatography quadrupole time-of-flight mass spectrometry (GC/Q-TOF MS) methodology was developed to determine the volatile and semi-volatile compound profile for the discrimination of honey from various botanical origins.

Method

Honey samples were analyzed by GC/Q-TOF MS in TOF-only (MS) mode. Compounds were then extracted based on deconvolution by Unknowns Analysis software. The extracted compounds were exported as a compound exchange format (.cef) file and subsequently imported into Mass Profiler Professional (MPP) software, a chemometric software for data alignment and filtering, followed by statistical and clustering analysis. The remaining compounds were analyzed to identify significant differences among groups of honey which were visualized using principles component analysis. A predictive model was built to analyze samples with specific biomarkers.

Experimental

Honey Sample

24 honey samples, including rape flower, acacia, linden and vitex honey, were collected directly from beekeepers with guaranteed origin in China. All samples were unprocessed and stored in plastic containers at 4 °C until analysis.

DLLME Conditions

Honey (2.0 g) was diluted with 3 mL of ultrapure water, followed by 3 mL acetonitrile. The mixture solution was blended by vortex for 2 min, centrifuged at 3500 rpm for 5 min and the upper organic solution was transferred into a 15 mL conical-shaped bottom tube. Ultrapure water (6 mL) and 0.5 g NaCl were added to the tube followed by 0.5 mL acetonitrile and 0.08 mL CCl_4. The tube was centrifuged at 3500 rpm for 5 min, and the CCl_4 fraction was recovered for analysis.

Instrument conditions

System: Agilent 7200 GC/Q-TOF
Column: HP-5ms UI (30 m×0.25 mm×0.25 µm)
Oven temperature program: 50 °C hold 2 min , at 5 °C /min to 180 °C hold 2 min , at 10 °C /min to 250 °C hold 5 min;
Carrier gas: Helium; Flow rate: 1.0 mL/min;
Injection port temperature: 280 °C; Injection volume: 1 µL;
Injection mode: Splitless, purge on after 1.5 min
Ion source: EI; Source temperature: 280 °C;
Quadruple temperature: 150 °C;
MS Acquisition: Full Scan 40-650 *m/z*; 5 Hz

Results and Discussion

Data Extraction

Unknowns Analysis Software Workflow:

1. Analyze and perform deconvolution to find components
2. CEF files of each sample exported for MPP analysis

By using the GC/Q-TOF with high sensitivity and high mass accuracy under TOF-only (MS) mode, the data analyzed can provide rich information on both volatile and semi-volatile compounds in honey. The total ion chromatogram (TIC) of honey is shown in fig.1. Chromatographic peak deconvolution using the Unknowns Analysis tool was able to find more than 300 components in each sample. Chromatogram deconvolution was able to extract clean spectra from background noise based on both retention time and peak shape.

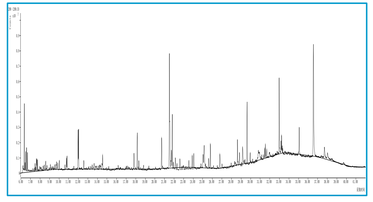

Fig.1 TIC of honey sample by GC/Q-TOF

Mass Profiler Professional (MPP)

Basic statistical and multivariate analyses were carried out using MPP software (version B.13.1.1, Agilent Technologies, Santa Clara, CA, USA). Chemometric approaches such as principal component analysis (PCA), partial least squares-discriminant analysis (PLS-DA), and support vector machine (SVM) were performed for all of the data to discriminate and classify honey samples according to their botanical origin. MPP provides an easy-to-follow guided workflow that helps the user decide how best to evaluate the data, while expert users can go directly to the data processing they wish to use.

Data Filtering

The entities were further filtered by analysis of variance (ANOVA, P=0.05) and fold change (FC>=2.0). 165 entities remained with significant differences among the four botanical origins.

Principles Component Analysis (PCA)

By PCA of the 165 entities mentioned above, an excellent separation of honey samples from four botanical origins could be observed (Fig. 2).

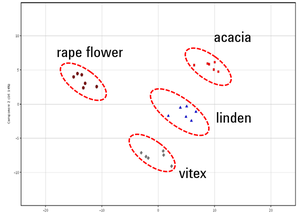

Fig. 2 2-D principle component analysis (PCA) of four honey botanical origins

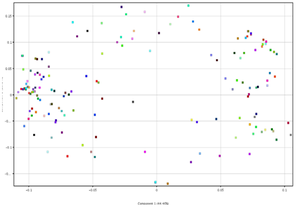

Fig. 3 Loading plot of principle component analysis (PCA) of four honey botanical origins

Results and Discussion

PLS-DA Predictive Model

The prediction ability was represented by the percentage of the honey samples correctly classified using a typical N-fold cross-validation procedure.
The whole process was repeated 10 times with 3 folds. It can be found from Tab 1 that all of the samples were classified according to their own botanical origin with an overall accuracy of 100%.

SVM Predictive Model

The prediction ability was represented by the percentage of the honey samples correctly classified using a typical leave-one-out cross-validation procedure.
It can be found from Tab 1 (the same results of PLS-DA model) that all of the samples were classified according to their own botanical origin with overall accuracy 100%.

	Acacia	Linden	Rape	Vitex	Accuracy (%)
Model training					
Acacia	6	0	0	0	100.0
Linden	0	6	0	0	100.0
Rape Flower	0	0	6	0	100.0
Vitex	0	0	0	6	100.0
Recognition ability (%)					**100.0**
Model prediction					
Acacia	1	0	0	0	100.0
Linden	0	1	0	0	100.0
Rape Flower	0	0	1	0	100.0
Vitex	0	0	0	1	100.0
Prediction ability (%)					**100.0**

Tab. 1 The results of PLS-DA and SVM model

Compound Identification

The selected compounds were subjected to identification through the integrated ID Browser in MPP for searching against the NIST14 library. Compound identification was further confirmed using accurate mass information. The Fragment Formula Annotation tool (FFA, Fig 4) confirmed compounds using accurate mass information,

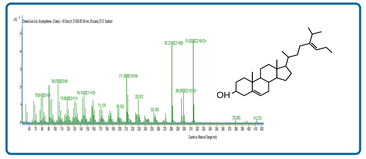

Fig 4. Annotated spectrum of one of the compounds (cholest-5-en-3-ol,24-propylidene,(3.beta)) using FFA in MassHunter Qualitative Analysis - Fragment formulas are assigned based on the empirical formula of the library hit and accurate mass spectral data.

Conclusions

- A DLLME combined with GC/Q-TOF method for the profiling of Chinese honey from various botanical origins has been developed;
- The acquired data have been subjected to chemometric analysis using MPP, and principle components analysis has shown excellent separation among four groups of honey samples;
- The data analyzed by GC/Q-TOF can provide rich information on both volatile and semi-volatile compounds in honey;
- It is a potential and promising method for the discrimination and classification of Chinese honey from various botanical origins.

[1]Mandal, M. D., & Mandal, S. (2011). Honey: its medicinal property and antibacterial activity. *Asian Pacific Journal of Tropical Biomedicine*, *1*(2), 154-160.