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Chemometric Methods for Botanical Classification of Chinese Honey by Solid-Phase Microextraction and GC/MS

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Introduction

As a natural product, honey is among the most appreciable ones in the world for its nutritional and medicinal properties. At the present, consumers are more concerned about the botanical and geographical origins of honey than its adulteration. The price of honey is usually different based its botanical/geographical origins. Therefore, it's essential to develop a fast and powerful method for the identification of honey from different origins. Mass spectrometry fingerprinting of volatile profile of honey has proven to be an effective method to track the honey botanical origin. However, this method was rarely used for Chinese honey.

In this study, a headspace solid-phase micro-extraction (SPME) coupled with gas chromatography-mass spectrometry (GC-MS) method has been developed and applied to the analysis and profiling of volatile compounds in Chinese honey.

Method

Honey samples were analyzed by GC-MS in MS scan mode. Compounds were then extracted based on deconvolution using unknowns analysis software. The extracted compounds were exported as a compound exchanged file(.cef) and subsequently imported into Mass Profiler Professional (MPP) software, a chemometic 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 and were displayed using principal component analysis. And then the predictive model was built to analyze the real samples.

Experimental

Honey Sample

30 honey samples, including rape, acacia and linden 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.

SPME Conditions

The volatile compounds of three botanical origins of Chinese honey, linden, rape and acacia were extracted under the following optimized SPME parameters: 3g honey , DVB/CAR/PDMS fiber, 80 °C, 30 min, 1.5 ml water and 0.5g NaCl. A CTC-PAL was installed for headspace SPME sample treatment.

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GC Conditions

GC system: Agilent 7890A;
Column: HP-5MS (30 m×0.25 mm×0.25 μm);
Column temperature: 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: 1.0mL/min;
Injection mode: manual, SPME Fiber
Injection port temperature: 280 °C;

MS Conditions

MS system: Agilent 5975C;
Ion source: EI; Ionization voltage: 70 eV;
Ion source temperature: 230°C;
Quad. temperature 150 °C
Interface temperature: 280°C;
Scan mode: full scan, 30-600 m/z

Results and Discussion

Data Extraction

Unknowns Analysis Software Workflow

(1) Analyze and perform deconvolution to find components.
(2) *. cef files of each sample could be obtained.

Chromatographic peak deconvolution using the Unknowns Analysis tool was able to find more than 200 components in each samples (Fig. 1).Chromatogram deconvolution was able to extract clean spectra from background noise based on both retention time and peak shape.

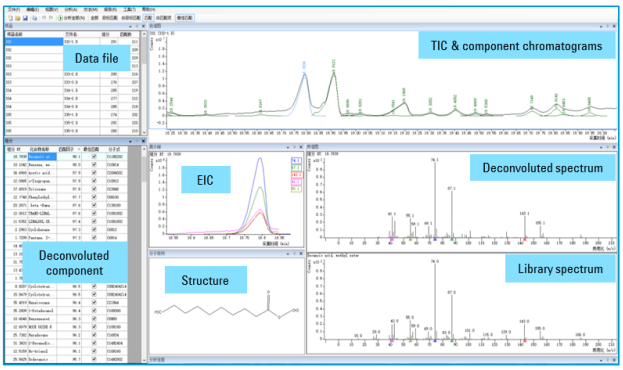

Fig.1 Unknown Analysis in honey samples via trasfered data of GC-MS

Mass Profiler Professional(MPP)

Basic statistical and multivariate analyses were carried out using MPP software (version B. 12.05, Agilent Technologies, Santa Clara, CA, USA). Chemometric approaches such as principal component analysis (PCA), PLS-DA, and BP-ANN were performed for all of the data to discriminate and classify honey samples according to their botanical origin.

Data Filtering

The entities (compounds) were further filtered by analysis of variance (ANOVA, P=0.05) and fold change (FC>=2.0). 146 entities remained with significant differences among the three botanical origins.

Principles Component Analysis (PCA)

By PCA analysis of the 146 entities mentioned above, an excellent separation of honey samples from three botanical origins could be observed (Fig. 2).

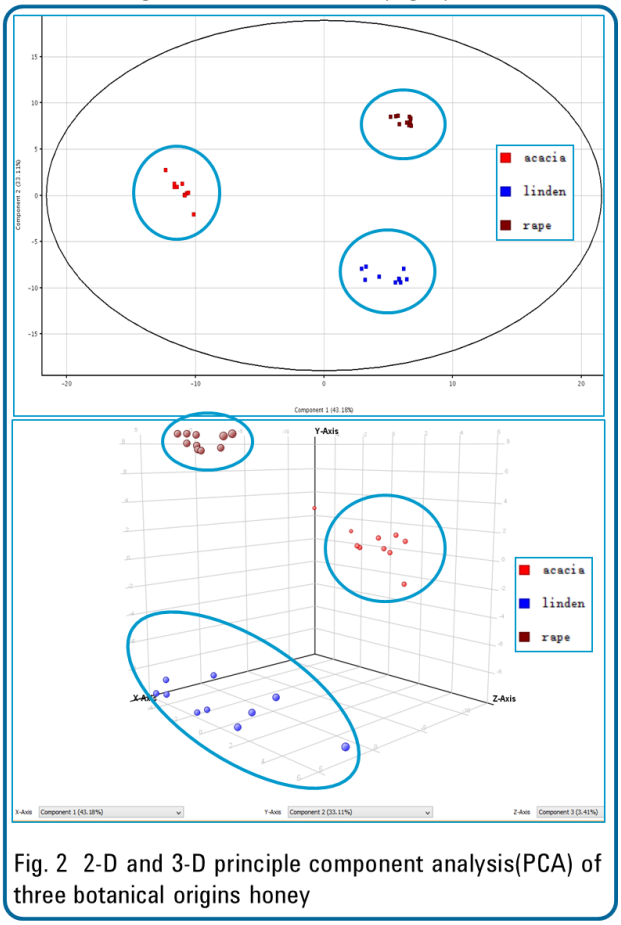

Fig. 2 2-D and 3-D principle component analysis(PCA) of three botanical origins honey

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 Fig. 3 that all of the samples were classified according to their own botanical origin with overall accuracy 100%.

Results and Discussion

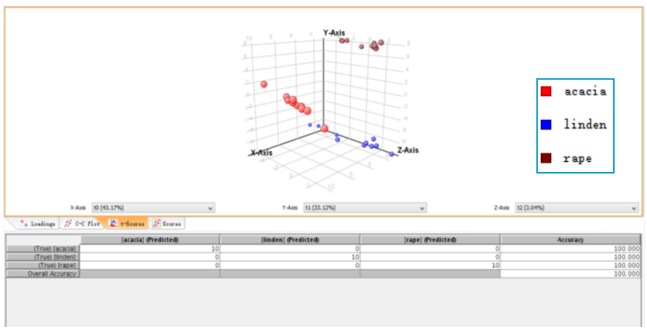

Fig. 3 Results of PLS-DA predictive model

Tab. 1 Results of model prediction by PLS-DA

Sample Name	Predicted	Confidence Measure
284-3-AllComponents	[rape]	0.74441534
285-3-AllComponents	[rape]	0.7743422
286-3-AllComponents	[rape]	0.7887382
287-3-AllComponents	[rape]	0.7773185
288-3-AllComponents	[rape]	0.8107677
303-3-AllComponents	[acacia]	0.96634156
304-3-AllComponents	[acacia]	0.9158132
305-3-AllComponents	[acacia]	0.8510345
306-3-AllComponents	[acacia]	0.969828
307-1-AllComponents	[acacia]	0.8586234
332-3-AllComponents	[linden]	0.7658745
333-3-AllComponents	[linden]	0.78289235
334-3-AllComponents	[linden]	0.8318631
335-3-AllComponents	[linden]	0.7905407
353-4-AllComponents	[linden]	0.80369425

BP-ANN Predictive Model

Both the input and output layer have one neuron, whereas the number of neurons in each hidden layer is 15. The learning process is continued with a learning rate of 0.7 and the number of iterations (epochs) of 100. The model was also evaluated in terms of recognition and prediction ability.

All honey samples can be successfully classified into three groups representing corresponding botanical origins during model training process with accuracy 100% (Fig. 4).

	[acacia](Predicted)	[linden](Predicted)	[rape](Predicted)	Accuracy
(True) [acacia]	10	0	0	100.00
(True) [linden]	0	10	0	100.00
(True) [rape]	0	0	10	100.00
Overall Accuracy				100.00

Fig. 4 Results of BP-ANN predictive model

Tab. 2 Results of model prediction by BP-ANN

Sample Name	Predicted	Confidence Measure
284-3-AllComponents	[rape]	0.8805758
285-3-AllComponents	[rape]	0.9386789
286-3-AllComponents	[rape]	0.9643812
287-3-AllComponents	[rape]	0.91923743
288-3-AllComponents	[rape]	0.96265805
303-3-AllComponents	[acacia]	0.96382976
304-3-AllComponents	[acacia]	0.9646205
305-3-AllComponents	[acacia]	0.86872536
306-3-AllComponents	[acacia]	0.96462536
307-1-AllComponents	[acacia]	0.971173
332-3-AllComponents	[linden]	0.78388005
333-3-AllComponents	[linden]	0.8280019
334-3-AllComponents	[linden]	0.83167315
335-3-AllComponents	[linden]	0.8827004
353-4-AllComponents	[linden]	0.82896984

Model Prediction

The duplicate determinations of each sample were considered as the independent test set for prediction. Linden, rape and acacia honey samples were predicted 100% with the confidence level between 0.74 to 0.97 by PLS-DA (Tab. 1) and 0.78 to 0.97 by BP-ANN (Tab. 2) .

Conclusions

It can be concluded that the volatile compound profiling of honey by SPME-GC-MS coupled with chemometric methods could be considered as a potential and promising tool for discrimination and classification of Chinese honey from various botanical origins.

Ref.1: Mei Wang, Bharathi Avula, Yan-Hong Wang, et al. Food Chemistry, 2014, 152: 391-398.