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Gas Chromatography Mass Spectrometry Analysis Reveals the Differences in Volatile Metabolites of Royal Jelly from Different Honeybee Stocks

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Introduction

Royal jelly (RJ), secreted by young worker bees, is the decisive factor of sexual determination and longevity of queen bee [1,2]. To increase the production of RJ, a honeybee stock, royal jelly bees (RJBs) has now been selected, which could produce more than 5 ~ 10 times RJ than that of Italian bees (ITBs). Volatile metabolites secreted by honeybees function as important signals in honey bee colony, however, only very limited data is available on volatile metabolites in RJ and the differences between RJ produced from different honeybee stocks. Therefore, gas-chromatography-mass spectrometry analysis (GC-MS) coupled with chemometric analysis were applied on the study of differences in volatile metabolites of royal jelly from different honeybee stocks.

Method

The extraction of volatile metabolites in royal jelly was carried out by headspace-solid phase micro-extraction (HS-SPME) and analyzed by an Agilent 7890B gas chromatograph with a 7000D triple quadrupole mass spectrometry (GC/MS/MS) operated in MS scan mode. Raw data acquired by GC-MS system was first imported into Masshunter Qualitative software to find compounds by chromatography deconvolution and then exported as .cef documents, which was subsequently imported into Mass Profiler Professional (MPP) software for bioinformatics data mining and chemometric analysis. The remaining entities after data-mining by MPP were submitted to identification and labeled as differential metabolites which distinguish royal jelly from different stocks.

Figure 1. 7000D GC-MS/MS system

Experimental

Royal jelly samples

Three stocks of honeybees, high RJ bees (RJBs) from China, Italian bees from Italy, and American Italian bees from the USA were used to produce royal jelly. To minimize the environmental and human factors, three colonies of each bee strain with identical colony management, population and brood pattern. All the honeybee colonies were kept at the apiary of the Institute of Apicultural Research, Chinese Academy of Agricultural Sciences in Beijing. RJ obtained from RJBs, Italian bees, American Italian bees were designated as HRJ, ITRJ, and ARJ, respectively. All royal jelly samples were obtained in July, 2018 and stored at -80℃ before being analyzed. Each kind of RJ was sampled in triplicate with individual sampling.

Figure 2. Royal jelly in royal jelly producing frame

SPME Conditions

3 g of RJ was accurately weighed into a 150 mL vial, followed by adding 2 µL ethyl decanoate (0.5 µg/µL) into the vial as internal standard. The vial was sealed immediately and equilibrated at 70 ℃ for 5 min using water bath. Afterward, the SPME fiber was exposed to the royal jelly sample head space for 50 min at 70 ℃. Finally, the SPME fiber was desorbed for 4.5 min by maintaining the GC-MS injection port at 270 ℃.

GC Conditions

GC system: Agilent 7890B;
Column: DB-5MS (60 m×0.32 mm×0.25 µm);
Column temperature: 50 ℃ hold 3 min ,
at 5 ℃ /min to 250 ℃ hold 5 min;
Carrier gas: Helium; Flow rate: 1.0 mL/min;
Injection mode: Manual, SPME Fiber
Injection port temperature: 270 ℃

MS Conditions

MS system: Agilent 7000D GC/TQ;
Ion source: EI; Ionization voltage: 70 eV;
Quadrupole temperature 150 °C
Ion source temperature: 280℃;
Scan mode: full scan, 35-500 m/z.

Data extraction

The total ion chromatograms of three royal jelly samples were shown in Fig.1. Raw data acquired by GC-MS system was first deconvolved by Masshunter Qualitative Analysis software and then exported as .cef documents for further analysis.

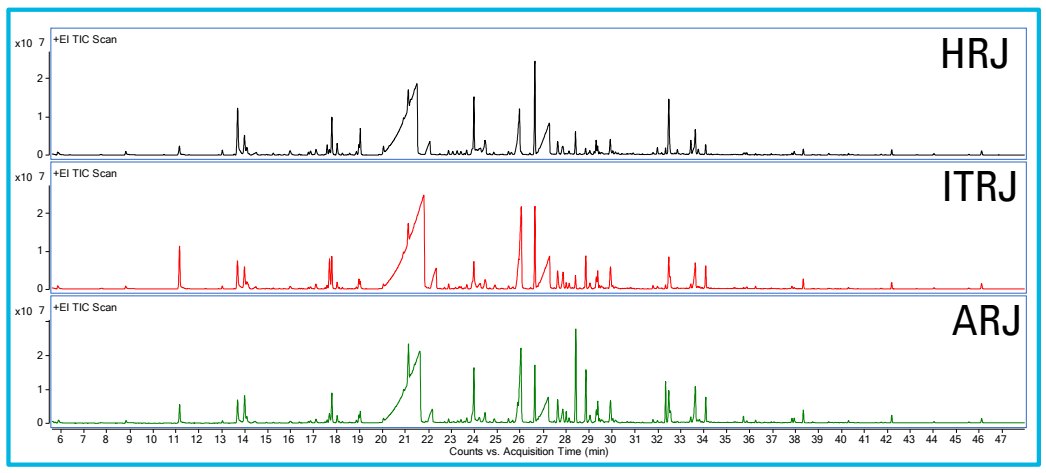

Figure 3. The total ion chromatograms of three royal jelly samples

Data Filtering

The .cef documents were imported into MPP for data mining and chemometric analysis. To exclude entities with poor repeatability and validity, frequency filtration (frequency > 60%) and coefficient of variability (CV < 25%) were applied as screening parameters. Analysis of variance (ANOVA) was performed to retain entities that displayed significant differences among groups. Eventually, 121 differential entities were found out as differential entities. The differential entities were identified by comparing mass spectra and retention index (RI) with that in NIST 14 Library and available standards. In total, 37 entities were identified, including 5 aldehydes, 5 esters, 6 alkanes, 14 alcohols and phenols, 4 ketones and 3 other metabolites.

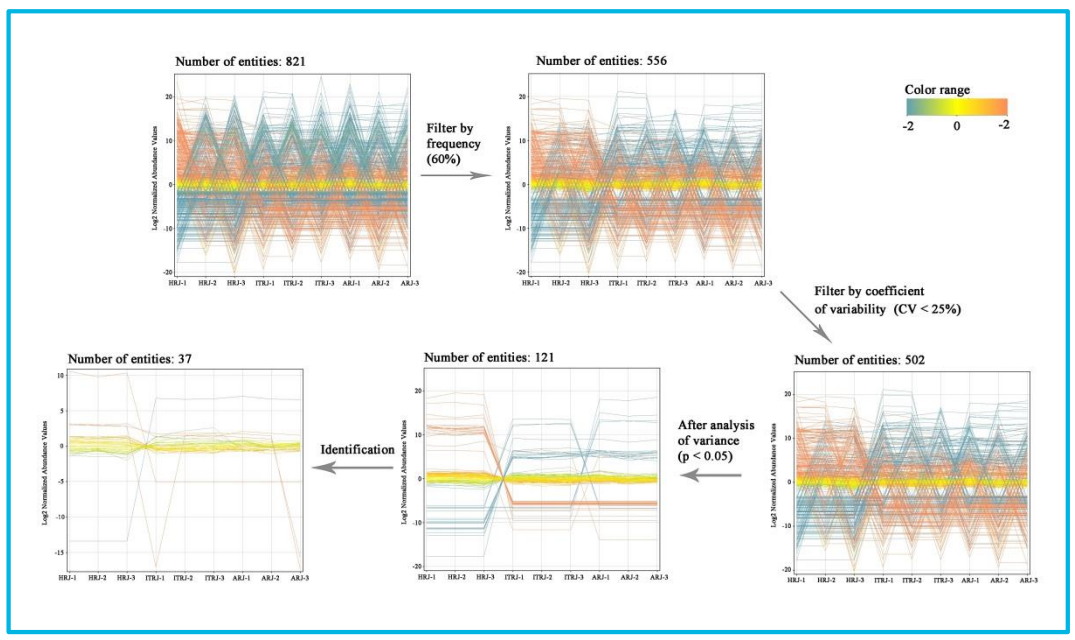

Figure 4. Data filtration to retain metabolites with significant differences in three RJ samples using MPP analysis

Principal Component Analysis (PCA)

PCA analysis was performed based on 37 differential metabolites using MPP. Excellent separation among three samples from different honeybee stocks were observed. As shown in Figure 5A, the first three components explained 91.33% of the total variance (67.36%, 19.81% and 4.16%, respectively), indicating significant differences among volatile metabolites in royal jelly from different honeybee stocks.

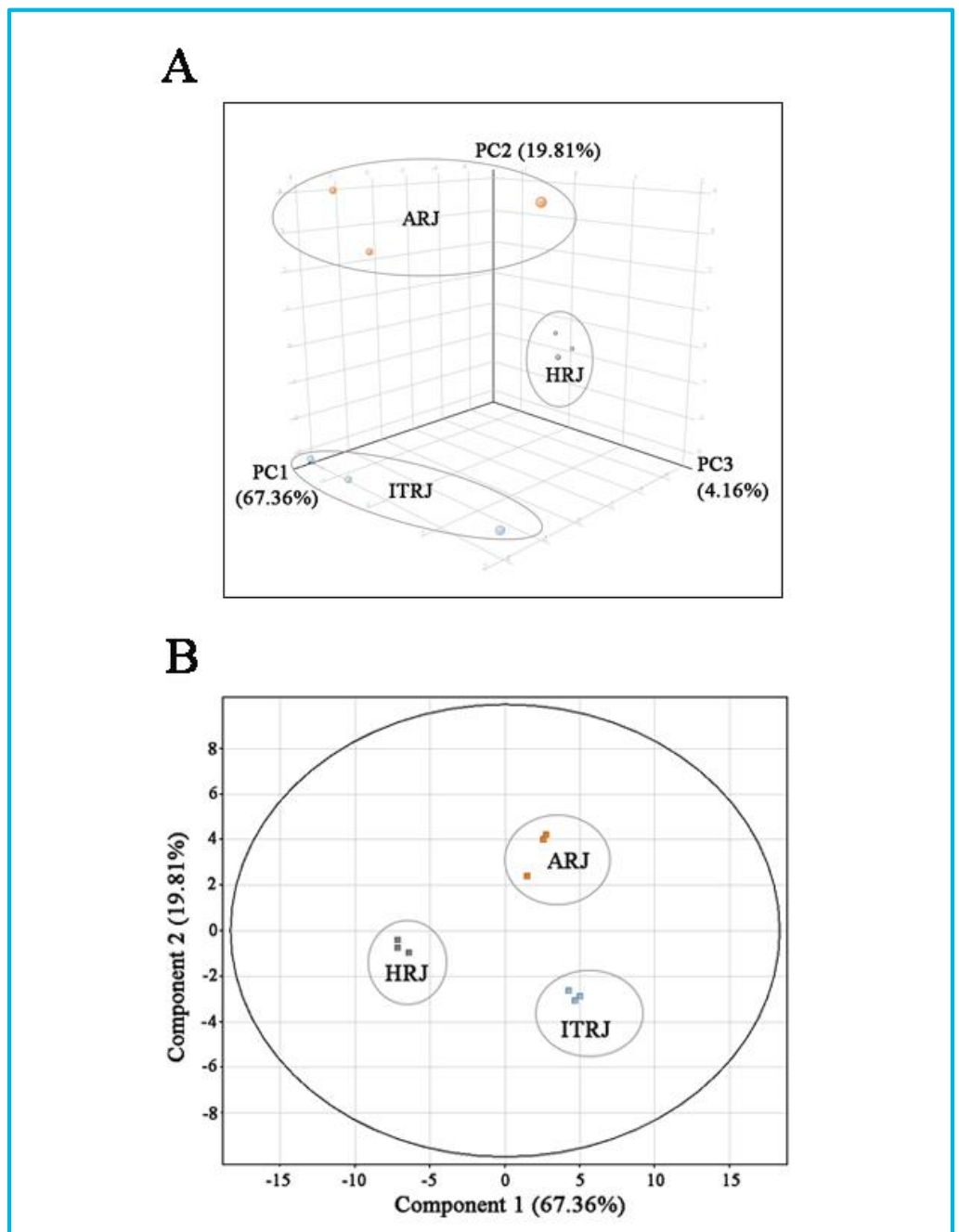

Figure 5. 2-D and 3-D principle component analysis(PCA) of three royal jelly samples

Hierarchical Clustering Analysis (HCA)

To visualize the abundance variance of volatile compounds among three royal jelly samples, clustering analysis was also performed using MPP software. Three royal jelly samples were divided into two groups, with ITRJ and ARJ in the same group, whereas HRJ was in an isolated one. This suggests that HRJ differ from the other two RJ samples on the level of volatile metabolites. This is in accordance with the PCA analysis.

Results and Discussion

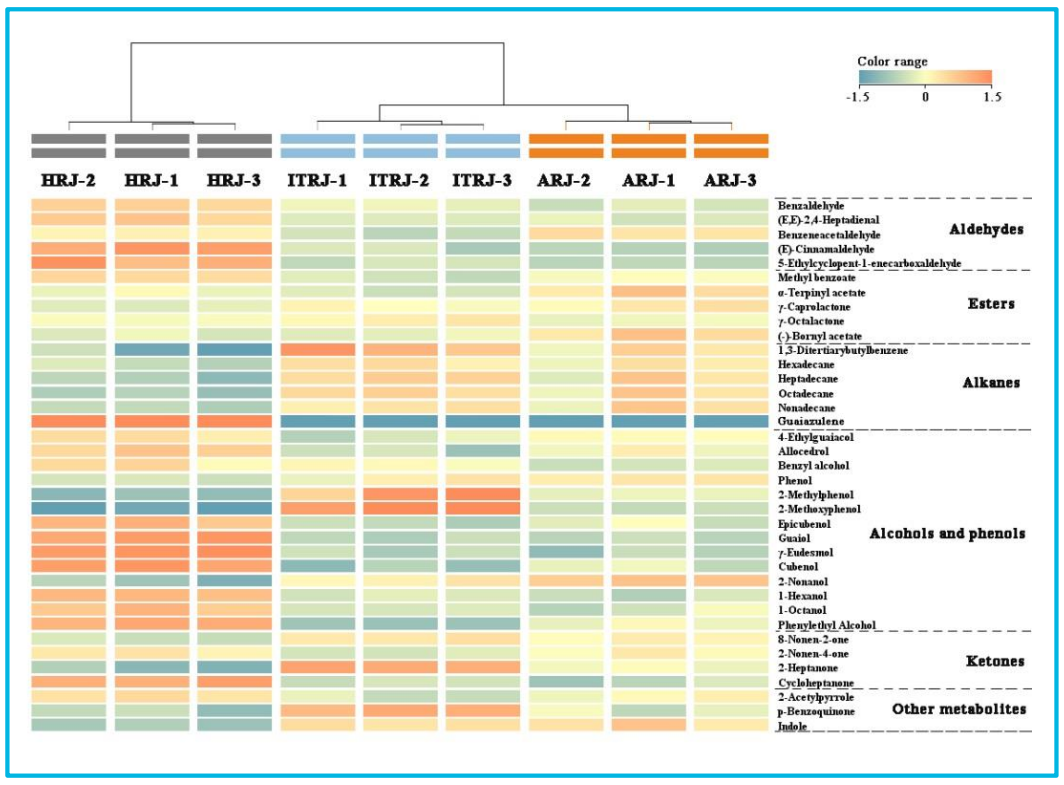

Figure 6. Clustering analysis of differential volatile metabolites in three royal jelly samples

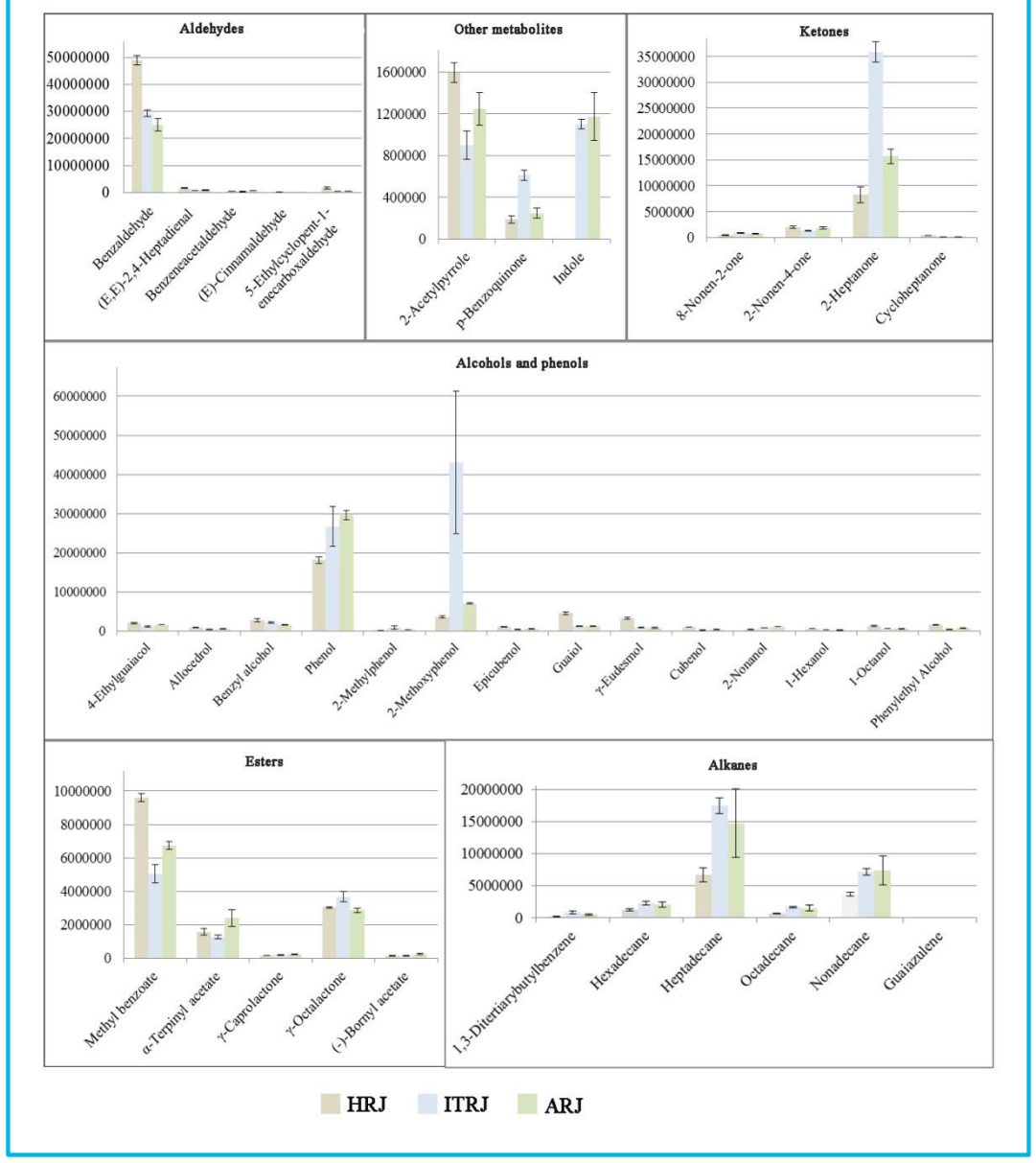

Figure 7. Histogram of 37 differential volatile metabolites in three royal jelly samples

Histogram

Figure 7 shows the differences of 37 volatile metabolites in three royal jelly samples using histogram. In brief, differences among three royal jelly samples show mostly in content rather than components. In particular, guaiazulene was unique to HRJ, and indole was exclusive to ITRJ and ARJ. Phenol-scented 2-methylphenol and sweet 2-methoxyphenol shows extremely high content in ITRJ than HRJ and ARJ.

In summary, it is the first report about the difference among royal jelly samples of high yield and low yield. Thirty seven differential volatile metabolites significantly expand the coverage of volatile metabolites in royal jelly.

Conclusions

• SPME combined with GC-MS analysis for profiling of volatile metabolites in royal jelly samples has been developed.

•37 differential volatile metabolites were identified to distinguish three royal jelly samples produced by different honeybee stocks. It is the first report about the difference among royal jelly samples of high yield and low yield honeybee stocks.

•A lot of volatile metabolites with unpleasant odor show lower content in HRJ than ITRJ and ARJ, while metabolites with sweet, flower, fruit aroma are highly abundant in HRJ compared with ITRJ and ARJ, suggesting they may contribute a more pleasant aroma of HRJ comparing with the two other RJ samples.

•This findings significantly extend our novel insight into the distinction and potential variation among royal jelly samples secreted by RJBs and unselected Italian bees on the level of volatile metabolites.

References

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