A comprehensive analysis of $^{13}$C isotope ratios data of authentic honey in China by the EA-IRMS and LC-IRMS methods

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Honey samples

- **Total number:** 96

- **Honey species:** rape honey, acacia honey, Vitex honey, sunflower honey, cotton honey, Linden honey, Jujube honey, buckwheat honey

- **Geographical origin:** Henan, Sichuan, Hubei, Jiangsu, Jilin, Shanxi, Xinjiang Uygur Autonomous, and Inner Mongolia Autonomous
Authenticity and reliability of samples

- Sampled and sealed by SGS
- Gathered in full-bloom stage
- Stored at -20°C
Test items

- Physical and chemical properties
- Antibiotics
- $^{13}$C isotope ratios
- GMO
- Adulteration identification
Established a $^{13}\text{C}$ isotope ratios database of different honey species in China

To resolve the international trade dispute

Aimed to beat the adulteration of honey
$^{13}$C isotope ratios data of authentic honey in China

- Conventional $\delta^{13}$C-EA-IRMS method proposed by JW White
- $\delta^{13}$C-LC-IRMS method proposed by Intertek laboratory in Europe
Conventional $\delta^{13}\text{C-EA-IRMS}$ method \((\Delta \delta^{13}\text{C} (\text{‰})_{\text{P-H}} \geq -1.0\text{‰})\)

**Rape honey**

- $\delta^{13}\text{C}_\text{H}$ values: ranged from -26.55 to -29.76
- The $\delta^{13}\text{C}_\text{H}$ values that greater than -29.0 are found chiefly concentrated in the regions of Sichuan province.

- $\delta^{13}\text{C}_\text{P}$ values: ranged from -25.15 to -28.64
- About 70% samples have the $\Delta \delta^{13}\text{C}_{\text{P-H}}$ values that greater than 1.0
## Results from different Lab

<table>
<thead>
<tr>
<th>NO.</th>
<th>Lab 1</th>
<th></th>
<th>Lab 2</th>
<th></th>
<th>Lab 3</th>
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</table>
Acacia honey

- $\delta^{13}\text{C}_\text{H}$ values: ranged from -23.82 to -25.20
- $\delta^{13}\text{C}_\text{P}$ values: ranged from -22.79 to -24.75
- About 5% samples have the $\Delta\delta^{13}\text{C}_\text{P-H}$ values that greater than 1.0
Linden honey

- Produced from Jilin province
- $\delta^{13}C_H$ values: ranged from -24.20 to -24.52
- $\delta^{13}C_p$ values: ranged from -23.66 to -24.12
- The $\Delta\delta^{13}C_{p-H}$ values are less than 1.0
Vitex honey

- Produced from Hubei and Henan province
- $\delta^{13}C_H$ values: ranged from -25.38 to -26.56
- $\delta^{13}C_P$ values: ranged from -23.73 to -24.30
- About 27% samples have the $\Delta\delta^{13}C_{P-H}$ values that greater than 1.0
Cotten honey

- Originated from Xinjiang Uygur Autonomous Region
- $\delta^{13}C_H$ values: ranged from -24.28 to -25.14
- $\delta^{13}C_P$ values: ranged from -19.44 to -24.83
- More than 70% samples have the $\Delta\delta^{13}C_{P-H}$ values that greater than 1.0
- The maximum $\Delta\delta^{13}C_{P-H}$ values reached to 4.90
Sunflower honey

- Produced from Inner Mongolia Autonomous region and Xinjiang Uygur Autonomous region

- $\delta^{13}C_H$ values: ranged from -25.02 to -25.39

- $\delta^{13}C_P$ values: ranged from -23.39 to -24.55

- Honey produced from Xinjiang have the $\Delta\delta^{13}C_{P-H}$ values that greater than 1.0
Conclusion

- The C4 sugar of all honey samples are qualified, that is, fall within the theoretical ranges of JW White.

- Affected by climatic fluctuations, some samples have δ^{13}C_H values greater than -29.0.

- The Δδ^{13}C_{P-H} values of cotton honey and vitex honey are relatively larger than other honey samples.
The limits for $\Delta \delta^{13}C$ values of pure honey are as follows:

- $\Delta \delta^{13}C$ (%o) max. (maximum difference between all measured $\delta^{13}C$ values): $\leq \pm 2.1\%o$;

- $\Delta \delta^{13}C$ (%o) fru-glu (differences between fructose and glucose $\delta^{13}C$ values): $\leq \pm 1.0\%o$.

- $\Delta \delta^{13}C$ (%o) _p-H_ (differences between protein and honey $\delta^{13}C$ values): $\geq -1.0\%o$
21 among 33 pure honey samples from Qinhuangdao CIQ were failed to pass the test and the qualification yield is 36.4%. The $\Delta \delta^{13}\text{C} (\%o)$ max. value is 4.12.

11 among 32 samples from Jiangsu CIQ were failed to pass the test and the qualification yield is 65.6%. The $\Delta \delta^{13}\text{C} (\%o)$ max. value is 4.36.

The difference values between $\delta^{13}\text{C}_{ds}$ and $\delta^{13}\text{C}_P$ of all rape honey samples are much larger.
### Results from Qinhuangdao CIQ

<table>
<thead>
<tr>
<th>Location number</th>
<th>δ^{13}C (%o) protein</th>
<th>δ^{13}C (%o) honey</th>
<th>δ^{13}C (%o) fuu</th>
<th>δ^{13}C (%o) gru</th>
<th>δ^{13}C (%o) ds</th>
<th>δ^{13}C (%o) ts</th>
<th>fru-gru δ^{13}C (%o)</th>
<th>Δδ^{13}C (%o) max.</th>
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</table>
Acacia honey

- 24 among 29 pure honey samples from Qinhuangdao CIQ were failed to pass the test and the qualification yield is 17.2%. The $\Delta \delta^{13}C$ (‰) max. value is 4.39.

- 24 among 28 samples from Jiangsu CIQ were failed to pass the test and the qualification yield is 14.3%. The $\Delta \delta^{13}C$ (‰) max. value is 4.20.

- The difference values between $\delta^{13}C_{ds}$ and $\delta^{13}C_{mono}$ of all rape honey samples except for those originated from Henan province are much larger.

- Trisaccharide was detected from all these samples
## Results from Qinhuangdao CIQ

<table>
<thead>
<tr>
<th>Location number</th>
<th>$\delta^{13}$C (‰) protein</th>
<th>$\delta^{13}$C (‰) honey</th>
<th>$\delta^{13}$C (‰) fuu</th>
<th>$\delta^{13}$C (‰) gru</th>
<th>$\delta^{13}$C (‰) ds</th>
<th>$\delta^{13}$C (‰) ts</th>
<th>fru-gru $\Delta\delta^{13}$C (‰) max.</th>
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</table>
11 among 11 pure honey samples from Qinhuangdao CIQ were failed to pass the test and the qualification yield is 0%. The $\Delta \delta^{13}C$ (‰) max. value is 4.34.

10 among 11 samples from Jiangsu CIQ were failed to pass the test and the unqualification yield is 9.1%. The $\Delta \delta^{13}C$ (‰) max. value is 4.16.

About 63.6% of these samples have large difference values between $\delta^{13}C_{ds}$ and $\delta^{13}C_{mono}$ while the left 36.4% have large difference values between $\delta^{13}C_{H}$ and $\delta^{13}C_{P}$.

Trisaccharide was detected from 80% of these samples.
3 among 10 pure honey samples from Qinhuangdao CIQ were failed to pass the test and the qualification yield is 70%. The $\Delta \delta^{13}C$ (‰) max. value is 5.24.

3 among 10 samples from Jiangsu CIQ were failed to pass the test and the unqualification yield is 70%. The $\Delta \delta^{13}C$ (‰) max. value is 5.13.

All samples have large difference values between $\delta^{13}C_H$ and $\delta^{13}C_P$

No trisaccharide was detected from all samples.
Linden honey

- 4 among 4 pure honey samples from Qinhuangdao CIQ were failed to pass the test and the qualification yield is 0%. The $\Delta\delta^{13}C$ (%) max. value is 2.81.
- 3 among 4 samples from Jiangsu CIQ were failed to pass the test and the unqualification yield is 25%. The $\Delta\delta^{13}C$ (%) max. value is 2.37.
- The difference values between $\delta^{13}C_{\text{mono}}$ and $\delta^{13}C_P$ are relatively larger.
- Trisaccharide was detected from Qinhuangdao CIQ
Sunflower honey

- 0 among 4 pure honey samples from Qinhuangdao CIQ were failed to pass the test and the qualification yield is 100%.

- 1 among 4 samples from Jiangsu CIQ were failed to pass the test and the unqualification yield is 75%. The $\Delta \delta^{13}C$ (‰) max. value is 2.41.
Conclusion

- All samples have $\Delta \delta^{13}C (\%o)$ fru-glu values less than or equal to ±1.0‰

- A large number of pure honey samples have $\Delta \delta^{13}C (\%o)$ max. values larger than ±2.1‰

- According to the method of the LC-IRMS, about 70% pure honey samples in China were considered to be adulterated, although those are 100% natural honeys.