Sensitive Detection of Economically Motivated Adulteration of Honey by Bulk and Compound Specific ^{13}C IRMS using Liquid Chromatography and Elemental Analysis Inlet Devices.

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Overview

Purpose: Honey adulteration was investigated using 13C isotope ratio mass spectrometry coupled to elemental analysis (EA-IRMS) and liquid chromatography (LC-IRMS)

Methods: Using EA-IRMS $\delta^{13}C$ values of honey protein and bulk honey have been determined. Using LC-IRMS $\delta^{13}C$ values of glucose, fructose, di-, tri-, and oligosaccharides have been determined.

Results: Large differences between the individual values indicate addition of sugars other sources. Adulteration in the low single percent range can be detected.

Introduction

Honey is considered a value-added food of natural origin, yet it is simply structured regarding its composition, which makes it prone to economically motivated adulteration by addition of sugars of other sources. Testing for adulteration can be done using various methods, including melissopalynological pattern analysis, sensory analysis, amino acid profile analysis, and others. The limit of detection of these methods is generally in the double digit percent range, depending on the type of sugars added. The introduction of bulk ^{13}C isotope analysis by White and Doner in 1978 was a major step towards establishing better methods sensitivity, further improved by White and Winters in 1989 by using honey protein as an internal standard. Still, it is possible to adulterate honey undetected by bulk ^{13}C isotope analysis and sugar profile analysis by carefully selecting a mixture of sugars that mimic both, the bulk ^{13}C composition ($\delta^{13}C$) and the sugar profile of the natural product. Here we describe a multi-parametric method, looking at both, bulk and compound specific $\delta^{13}C$, identifying adulteration based on inconsistencies in the parameter set itself.

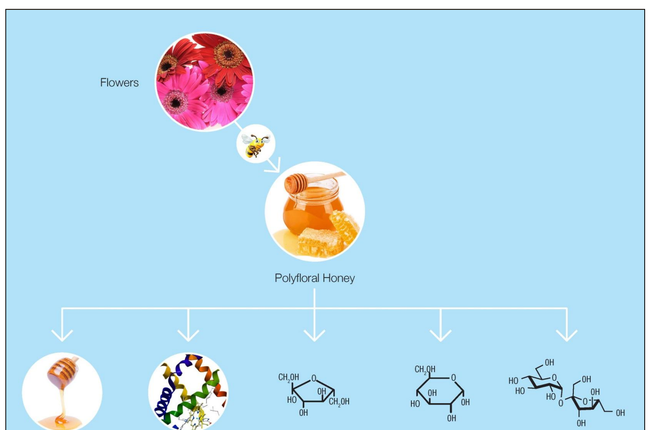

FIGURE 1a. Authentic Honey.

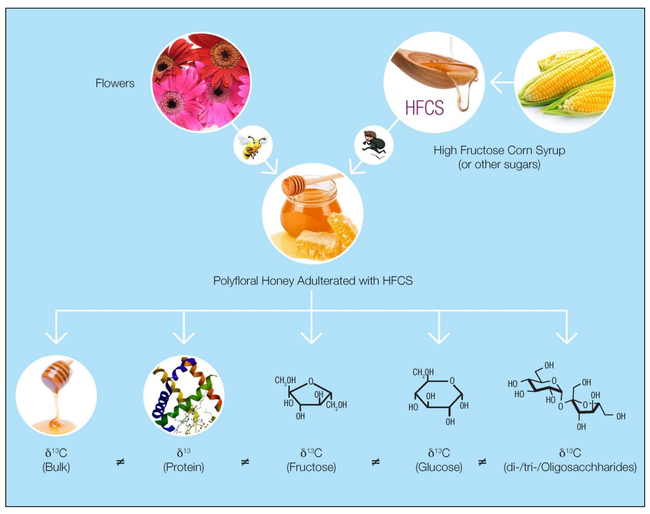

FIGURE 1b. Adulterated Honey.

Methods

Determination of $\delta^{13}C$ (bulk)

- Approx. 100 µg sample weighed into tin capsule.
- Directly analyzed using a Thermo Scientific™ FLASH 2000 Elemental Analyzer coupled to a Thermo Scientific™ Delta V™ IRMS (EA-IRMS) (Figure 2a).
- Figure 4 shows a functional diagram of the EA-IRMS technique.

Determination of $\delta^{13}C$ (protein)

- Protein fraction is prepared by Na_2WO_4/H_2SO_4 precipitation from aqueous sample solution, washed and dried.
- Analyzed using EA-IRMS as above (Figures 2a and 4).

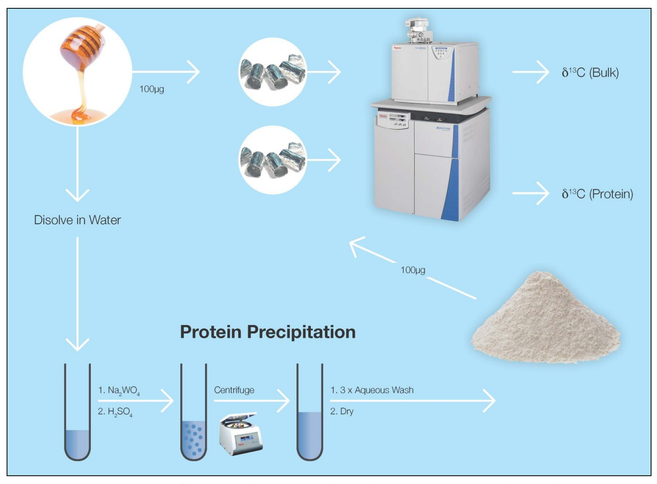

FIGURE 2. Bulk and Protein $\delta^{13}C$ Determination.

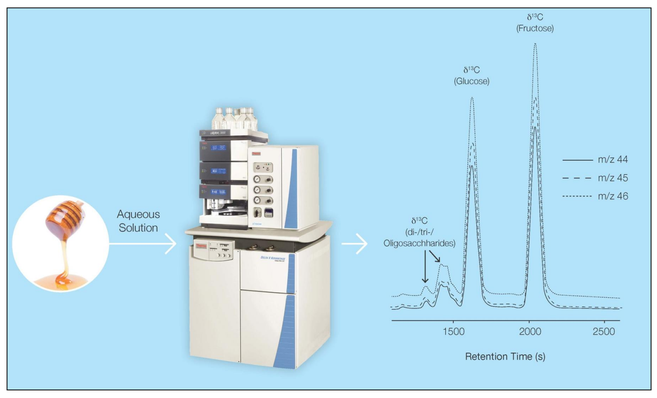

FIGURE 3. Individual sugars and sugar-fraction d^{13}C Determination

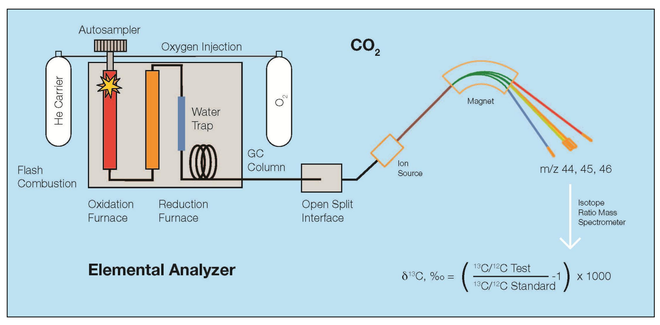

FIGURE 4. EA-IRMS Operating Principle.

Determination of $\delta^{13}C$ (glucose, fructose, di-, tri-, oligosaccharides)

- Aqueous sample solution, 10 µl loop injection, 0.8g/L for determination of fructose and glucose and 4g/L for di-, tri- and oligosaccharides.
- Phenomenex™ Rezek™ RCM (Ca^{2+}) 300 × 8 mm column.
- Eluent: water.
- Analyzed using a Thermo Scientific™ LC-Isolink™ interface coupled to a Thermo Scientific Delta V mass spectrometer (LC-IRMS) (see figure 3).
- $\delta^{13}C$ values for glucose, fructose, di-, tri-, and oligosaccharides are obtained by integrating the respective peaks on m/z 44 and 45 and calculation of the ratio of the peak areas.
- Figure 5. shows a functional diagram of the LC-IRMS technique.

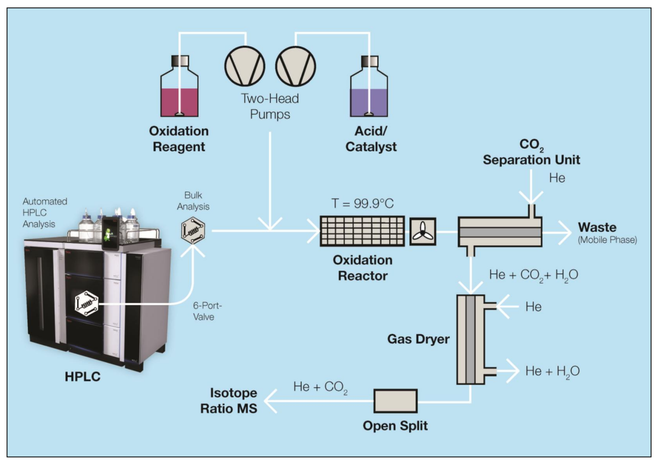

FIGURE 5. LC-IRMS Operating Principle.

Samples
11 samples of different type (1:acacia, 2:polyfloral, 3:lime, 4:honeydew) were analyzed. Samples 1a, 2a, 3a, 4a were authentic, all others were adulterated.

Results

- Figure 6a. and 6b. show example chromatograms obtained from authentic and adulterated polyfloral honey.
- Isotope ratios determined for individual sugars and sugar fractions as well as the bulk and protein fraction carbon isotope ratios are listed in table 1.
- The method based on EA-IRMS alone[3,4] could not detect adulteration in any of the cases. The differences between $\delta^{13}C$ (bulk) and $\delta^{13}C$ (protein) of all adulterated samples are well within 1.0‰ and don't give rise to suspicion.
- Using the combined method, adulterated samples show particularly large $\Delta\delta^{13}C$ (max-min), i.e. the difference between the highest and the lowest reported individual $\delta^{13}C$ value, values or large $\Delta\delta^{13}C$ (fructose-glucos) values and should be investigated further.
- The method show sensitivity levels of approx. 1% for adulteration with sugars from C4 plants and 5-8% for sugars from C3 plants.

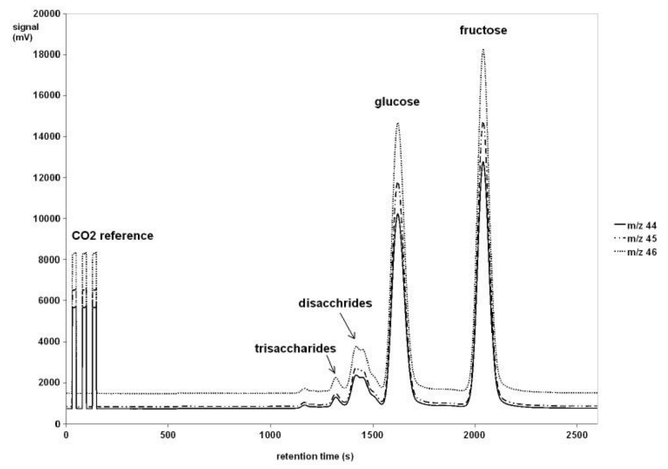

FIGURE 6a. Chromatogram of Authentic Polyfloral Honey. LC-IRMS chromatogram of authentic polyfloral honey (sample 2a).

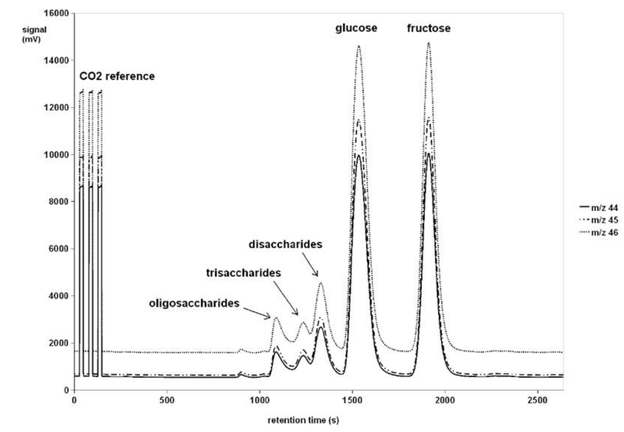

FIGURE 6b. Chromatogram of Adulterated Polyfloral Honey. LC-IRMS chromatogram of adulterated polyfloral honey (sample 2d).

Sample	1(a)	1(b)	1(c)	2(a)	2(b)	2(c)	2(d)	3(a)	3(b)	4(a)	4(b)
Type	authentic	adulterated	adulterated	authentic	adulterated	adulterated	adulterated	authentic	adulterated	authentic	adulterated
Measured values											
$\delta^{13}C$ (bulk), ‰	-24.7	-24.6	-23.9	-26.0	-25.0	-23.8	-27.0	-25.6	-25.7	-25.8	-25.6
$\delta^{13}C$ (protein), ‰	-24.6	-25.2	-24.3	-25.6	-25.2	-24.6	-26.4	-25.7	-25.6	-25.6	-25.3
$\delta^{13}C$ (fructose), ‰	-24.7	-23.8	-25.0	-26.0	-24.4	-24.7	-27.4	-25.5	-26.0	-25.7	-26.1
$\delta^{13}C$ (glucose), ‰	-24.8	-26.0	-24.6	-25.9	-25.7	-24.2	-27.0	-25.5	-25.7	-25.7	-25.7
$\delta^{13}C$ (disaccharides), ‰	-24.8	-25.7	-22.6	-26.8	-24.8	-17.3	-26.4	-25.8	-23.9	-26.5	-23.8
$\delta^{13}C$ (trisaccharides), ‰	-23.9	-25.0	-18.7	-26.1	-24.5	-16.2	-24.3	-25.5	-24.4	-26.1	-23.8
$\delta^{13}C$ (oligosaccharides),	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	-26.7	n.d.	n.d.	n.d.	n.d.
$\Delta\delta^{13}C$ (protein-bulk), ‰	0.1	-0.6	-0.4	0.4	-0.2	-0.8	0.6	-0.1	0.1	0.2	0.3
$\Delta\delta^{13}C$ (fructose-glucose	0.1	2.2	-0.4	-0.1	1.3	-0.5	-0.4	0.0	-0.3	0.0	-0.4
$\delta^{13}C$ min, ‰	-24.8	-26.0	-25.0	-26.8	-25.7	-24.7	-27.4	-25.8	-26.0	-26.5	-26.1
$\delta^{13}C$ max, ‰	-23.9	-23.8	-18.7	-25.6	-24.4	-16.2	-24.3	-25.5	-23.9	-25.6	-23.8
$\Delta\delta^{13}C$ (max-min), ‰	0.9	2.2	6.3	1.2	1.3	8.5	3.1	0.3	2.1	0.9	2.3

FIGURE 1. Results of EA-IRMS and LC-IRMS measurements. Values giving rise to suspecting adulteration are circled in red.

Conclusion

LC-IRMS combined with EA-IRMS is a sensitive method for detecting adulteration in honey.

References

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