

Detection of honey adulteration with ^{13}C isotope ratio mass spectrometry of single sugar fractions

Klaus Beckmann, Gudrun Beckh, Cord Lüllmann

Quality Services International GmbH
Flughafendamm 9a, D-28199 Bremen
Germany
info@qsi-q3.de



Honey can be adulterated by foreign sugars for cost saving reasons. Such adulterations are easy to implement. Often starch hydrolyzates, whose water content is adjusted to a viscosity similar to honey, are used.

With the help of ^{13}C isotope ratio mass spectrometry (IRMS), which is established as AOAC Official Method 988.12 [1, 2], honey blends containing C_4 sugar products can be detected. Bees usually use C_3 plants for honey production. Because the natural $^{13}\text{C}/^{12}\text{C}$ isotope ratios of C_3 and C_4 sugars are different [Fig. 1], sugar admixtures of C_4 plants to honeys can be detected when compared. Sugars of C_3 plants can only be detected in large admixtures because the nectar sources for the bees belong to the same class of plants.

The coupling of high-pressure liquid chromatography (HPLC) and IRMS allows isotope measurements of single honey sugar fractions, first presented by Cabanero et al. (2006) [3]. LC-IRMS measurements of unadulterated honeys as well as adulterated honey, containing admixtures of sugar syrups, show new possibilities of the detection of honey adulterations

Method

Honey is diluted in pure water (1:1) and filtered. The solution is separated on a *Bio Rad-Aminex HPX-87C* column with ultrapure water as eluent at 85 °C (flow rate: 0.6 ml/min; detection: RI).

Plant Origin	Examples	range $\delta^{13}\text{C}$ values [‰]VPDB
C_4 -Plants	sugar cane corn	-10 up to -12
C_3 -Plants	beet sugar rice	-22 up to -27

Fig. 1: natural ranges of $\delta^{13}\text{C}$ values of C_3 - and C_4 -plants

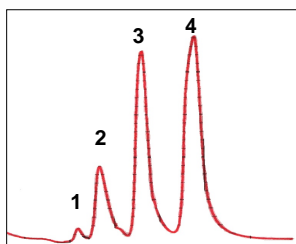


Fig. 2: HPLC chromatogram of an unadulterated honey sample (1: trisaccharides, 2: disaccharides, 3: glucose, 4: fructose)

Sugar Fraction	Range $\delta^{13}\text{C}$ Values
Fructose - Glucose	+/- 1.0
Fructose, Glucose - Disacch.	+/- 3.0
Fructose, Glucose - Trisacch.	+/- 3.0
Disacch. - Trisacch.	+/- 3.0

Fig. 3: natural ranges between honey sugar fractions

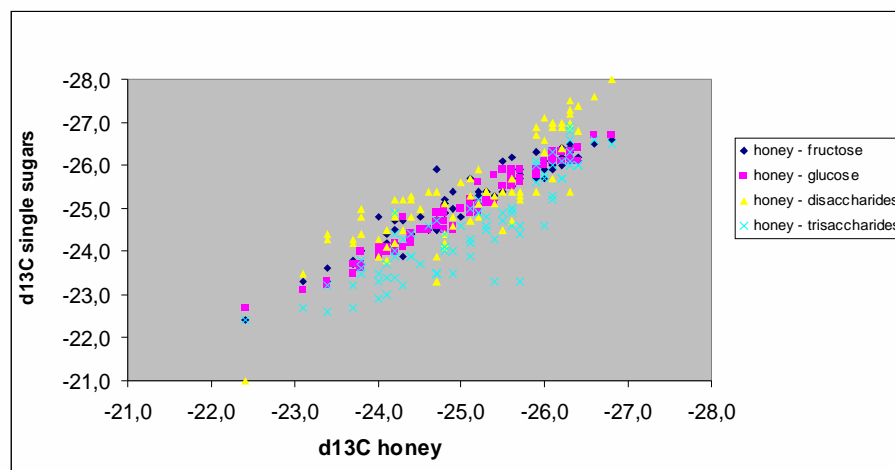


Fig. 4: natural distribution of $\delta^{13}\text{C}$ values of single sugar fractions of unadulterated honeys related to respective $\delta^{13}\text{C}$ values of the pure honeys

Conclusions

1. The coupling of HPLC and ^{13}C -IRMS extends the analysis of honey adulterations.
2. The amount of admixed sugars can not be determined yet.

In Fig. 2 a chromatogram of an unadulterated honey is shown. The four peaks are results from fructose, glucose, di- and trisaccharides. The fractions are collected manually, concentrated to a volume of 20 μl and transferred into tin capsules. The measurement of $^{13}\text{C}/^{12}\text{C}$ isotope ratios takes place on a *Finnigan MAT Delta Plus* mass spectrometer (element analyser: *Carlo Erba NC 2500*). The isotope ratios of the single sugars are compared.

Results

More than 200 unadulterated honey samples of different botanical and geographical origin were measured with IRMS and LC-IRMS. The $\delta^{13}\text{C}$ values of glucose and fructose indicate only small variations from the honey values whereas the dispersions of di- and trisaccharides were larger [Fig. 3]. The determined natural ranges of the sugar fractions are shown in Fig. 4.

Afterwards, honeys were mixed with original sugar syrups, which are used for honey adulterations in some countries, in different grades. In Fig. 5 and 6 results of IRMS, LC-IRMS and fructose/glucose-ratios of these admixtures are presented. It can be observed that it was only possible to detect these mixtures by means of LC-IRMS. Additions of 30 % can be recognized, depending on the type of syrup. Due to varying compositions of the syrups, the precise amounts of added sugars however can not be determined yet.

$\delta^{13}\text{C}$	Acacia Honey	30 % Syrup I	50 % Syrup I	Sugar Syrup I
Fructose	-25.4	-23.4	-22.8	-20.4
Glucose	-25.5	-25.5	-25.3	-25.6
Disacch.	-25.5	-25.3	-25.3	-
Trisacch.	-25.5	-25.3	-25.6	-
Adulteration	no	yes	yes	-
Honey	-25.4	-24.4	-24.1	-22.5
Protein	-25.1	-24.9	-24.9	-
Deviation	+0.3	-0.5	-0.8	-
Adulteration	no	no	no	-
F/G	1.50	1.49	1.52	1.51
Adulteration	no	no	no	-

Fig. 5: admixtures of sugar syrup type I to acacia honey and results of LC-IRMS, IRMS and fructose/glucose ratios (F/G)

$\delta^{13}\text{C}$	Polyflora Honey	30 % Syrup II	50 % Syrup II	Sugar Syrup II
Fructose	-25.5	-26.0	-26.3	-27.2
Glucose	-25.6	-25.8	-26.0	-26.4
Disacch.	-25.0	-24.2	-23.7	-22.4
Trisacch.	-25.8	-25.8	-25.6	-
Adulteration	no	no	yes	-
Honey	-25.4	-25.9	-26.1	-25.9
Protein	-25.6	-25.6	-25.7	-
Deviation	-0.2	+0.3	+0.4	-
Adulteration	no	no	no	-
F/G	1.31	1.29	1.28	1.25
Adulteration	no	no	no	-

Fig. 6: admixtures of sugar syrup type II to acacia honey and results of LC-IRMS, IRMS and fructose/glucose ratios (F/G)

References

- [1] White, J. W., Winters, K., Honey Protein as Internal Standard for Stable Carbon Isotope Ratio Detection of Adulteration of Honey, *J. Assoc. Off. Anal. Chem.* 72 (6) (1989), 907-911
- [2] AOAC Official Method 998.12: C-4 Plant Sugars in Honey
- [3] Cabanero, A. I., Recio, J. L., Rupérez, M., Liquid Chromatography Coupled to Isotope Ratio Mass Spectrometry: A New Perspective on Honey Adulteration Detection, *J. Agric. Food Chem.* 54 (2006), 9719-9727