APPLICATION NOTE

EA-IRMS: Detection of Honey Adulteration

Oliver Kracht, Andreas Hilkert,
Thermo Fisher Scientific, Bremen,
Germany

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Introduction

Honey is subject to fraud by adulteration with low price invert sugar syrups. Saccharides in syrups derived from cane, corn or beet sugar are difficult to distinguish from those in pure honeys. In 1977, Doner & White established a method for detection of adulteration of honey with syrups using Isotope Ratio Mass Spectrometry (IRMS).[1]

Sugar cane and corn syrups, the most widely used adulterants, have distinctive isotopic ^{13}C signatures because both sugar cane and corn plants use the C4 photosynthetic pathway in contrast to most honey which is derived from plants that use the C3 photosynthetic pathway. These differences in ^{13}C isotopic composition allow detection of > 7% addition of such sugars.[2] Stable isotope analysis of honey, in order to detect adulteration is in widespread use and can be undertaken with an EA-IRMS System, such as the Thermo Scientific™ EA IsoLink™ IRMS System (Figure 1).

Figure 1. The Thermo Scientific EA IsoLink IRMS System.

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Analytical Method

The EA IsoLink IRMS System uses the principle of Dumas combustion for $\delta^{13}C$ analysis. Approximately 100-200 µg of honey are loaded into a tin capsule, which are dropped from the Thermo Scientific™ MAS Plus Autosampler into a reactor filled with chromium oxide and cobaltous/cobaltic oxide where it is combusted in the presence of pure oxygen to form CO_2 for analysis. A reactor can analyze 800-1000 honey and protein samples before a replacement of reactors and trap material is required.

The CO_2 produced from combustion of the bulk honey and of the protein fraction is analyzed for the $\delta^{13}C$ by IRMS. The elemental analyzer (EA) is interfaced to the IRMS via the Thermo Scientific™ ConFlo™ IV Universal Interface, which also performs automated referencing and dilution. The EA IsoLink System is operated through the Thermo Scientific™ Isodat™ Software Suite.

The proteins in the honey sample were extracted following the AOAC 998.12 guideline (Association of Analytical Communities Handbook), which specifies mixing 15 g of honey with 3 ml of water and heating to 80 °C.[3] The proteins precipitate after addition of acid and tungstic solution within about 2 minutes. The supernatant is repeatedly decanted after centrifuging and rinsing with water. After drying, 100-200 µg of the protein sample are loaded into a tin capsule for analysis.

Results

Figure 2 shows a typical CO_2 chromatogram. Average results and one standard deviation (1 sd) of three distinct honey samples and their extracted proteins are given in Table 1.

The values are well within the natural range of honeys as indicated in Figure 3. The difference between the $\delta^{13}C$ values of protein and honey is, in all three cases, less than 1‰, representing non-adulterated honey.

The excellent repeatability of the measurements helps to distinguish smallest adulteration by C4-sugars. Another case study is shown in Figure 3, in which $\delta^{13}C$ values of bulk honey and related protein are used to identify pure and adulterated honeys.

The data presented in Table 1 are application data and are not warranted because they exceed product specifications. The warranted product specification for $\delta^{13}C$ is ±0.1‰ (1 sd) for 50 µg carbon measured on Acetanilide or Urea.

Table 1. Average results and one Standard Deviation (1 sd) of $\delta^{13}C$ in ‰ of three honeys and their extracted proteins. Data kindly provided by SP Laboratorija A.D., Becej, Serbia.

	Honey-1	Protein-1	Honey-2	Protein-2	Honey-3	Protein-3
	-23.60	-24.08	-23.83	-24.01	-24.17	-24.49
	-23.68	-24.09	-23.81	-23.95	-24.06	-24.44
	-23.57	-24.09		-23.91	-24.07	-24.17
	-23.48	-24.09		-23.87	-24.11	-24.00
	-23.53	-24.01		-23.84		-24.29
	-23.60	-24.01				
	-23.61	-23.98				
	-23.60					
Average	-23.58	-24.05	-23.82	-23.91	-24.10	-24.28
1 sd	0.06	0.05	0.05	0.07	0.05	0.20

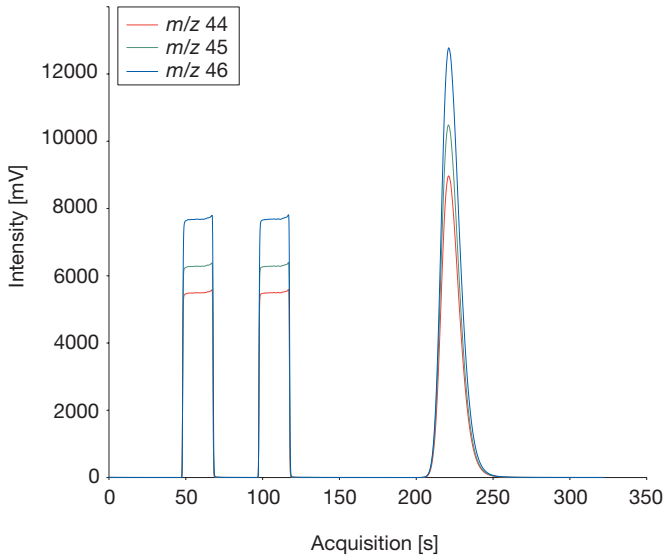

Figure 2. Typical chromatogram of an analysis of protein or honey. Two reference gas injections of sample CO_2 are followed by the sample CO_2 peak.

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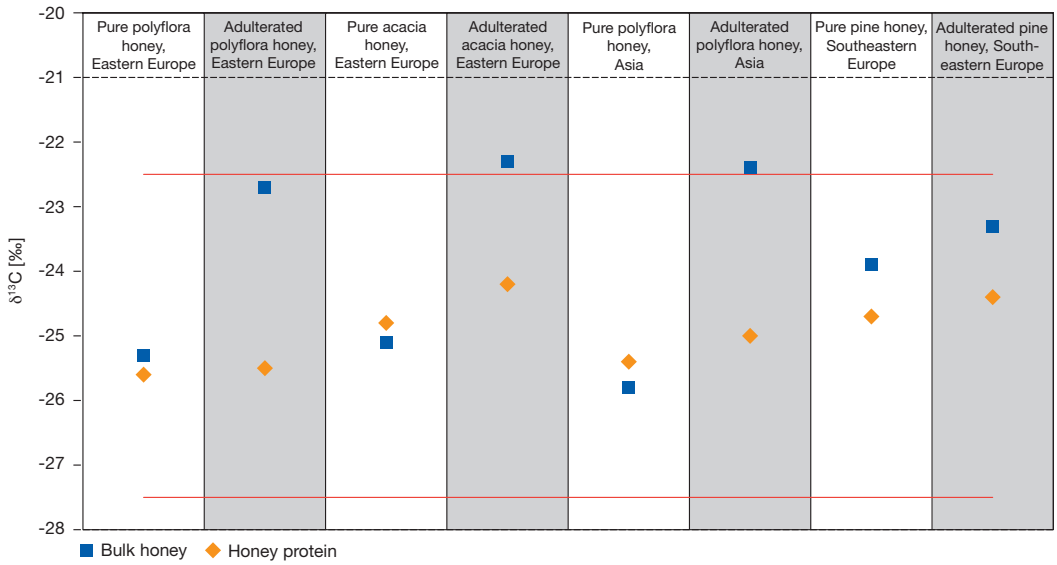

Figure 3. $\delta^{13}C$ values of honey and related proteins. Limit of detection due to natural variation: 7% C4 sugar (agreed value). The red lines show the natural variation of $\delta^{13}C$ in honey.[5] Data kindly provided by Applica GmbH, Bremen, Germany.

Additional Analytical Methods

The same analytical setup can also be used for isotopic analysis of sugar in fruit juices and ethanol in wine.[4] In addition, the EA IsoLink System incorporates a dedicated reactor for combustion EA-IRMS for $^{13}C/^{12}C$ and $^{15}N/^{14}N$ and a reactor for quantitative hightemperature reduction for D/H and $^{18}O/^{16}O$ isotope ratio determination.

Much lower limits of detection of fraudulent addition of invert sugar syrups can be obtained using the Thermo Scientific™ LC IsoLink™ Interface. This method allows the separation and isotopic analysis of individual sugars in honey, including fructose, glucose, di- and trisaccharides. The compound specific isotope analysis of these sugars combined with the bulk honey analysis of the EA IsoLink System enables the detection of adulteration with C3-sugar, e.g. rice syrup.[5, 6, 7]

Conclusion

The EA IsoLink IRMS System ensures reproducible $\delta^{13}C$ analyses for the detection of honey adulteration with C4-syrups according to the AOAC 998.12 guideline.

Acknowledgement

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