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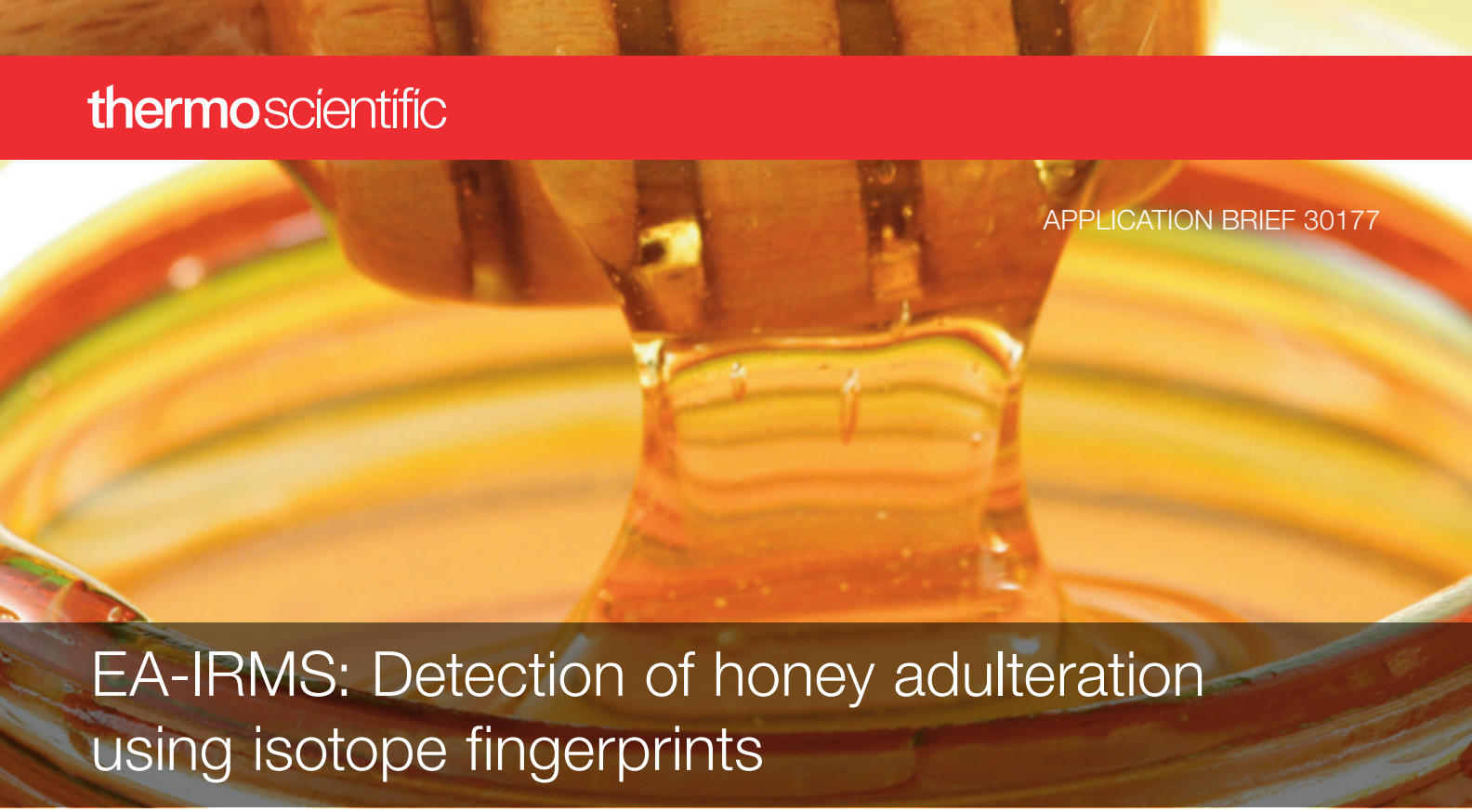
APPLICATION BRIEF 30177



EA-IRMS: Detection of honey adulteration using isotope fingerprints

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Keywords

EA-IRMS, EA IsoLink IRMS System, Food authenticity, Honey, Isotope Ratio MS

Goal

Demonstrate the use of the EA-IRMS for isotope fingerprint analysis of $\delta^{13}C$ analyses for the detection of honey adulteration with C4-syrups according to the AOAC 998.12 guideline.

Introduction

Honey is subject to fraud by adulteration with low price 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).

Sugar cane and corn syrups, the most widely used adulterants, have distinctive ^{13}C isotope fingerprints 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. In this application brief, we report carbon isotope fingerprints of honey and proteins extracted from honey and illustrate how the addition of exogenous sugars can be successfully tracked and identified. This enables the evaluation of honey authenticity in terms of original sugar content.

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Analytical configuration

For $\delta^{13}C$ analysis, approximately 100-200 µg of honey were loaded into a tin capsule and introduced to the combustion reactor from the Thermo Scientific™ MAS Series Autosampler, where they were combusted in the presence of oxygen to produce CO_2 gas. 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 an Elemental Analyzer (EA) interfaced to the IRMS via the Thermo Scientific™ ConFlo™ IV Universal Interface, which is automated using 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)[1], which specifies mixing 15 g of honey with 3 ml of water and heating to 80 ˚C. The proteins precipitate after addition of acid and tungstic solution within 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[2].

The analysis can be readily undertaken on the latest Thermo Scientific™ EA-IRMS system, the EA IsoLink™ IRMS System.

The isotope fingerprint of honey

Carbon isotope fingerprints can be used to identify adulteration of honey that results form the addition of exogenous sugars.

The carbon isotope fingerprint ($\delta^{13}C$) of plants are different because of photosynthetic processes and broadly grouped as C3, C4 and CAM plant types. C3 plants utilize the Calvin photosynthetic pathway to fix CO_2. C4 plants utilize the Hatch-Slack photosynthetic pathway and CAM by Crassulacean Acid Metabolism.

Therefore, C3 plants have a carbon isotope fingerprint between -33‰ to -22‰, C4 plants a carbon isotope fingerprint between-16‰ to -8‰. And CAM plants between -20‰ to -10‰.

Results

Data for three distinct honey samples and their extracted proteins are given in Table 1.

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

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

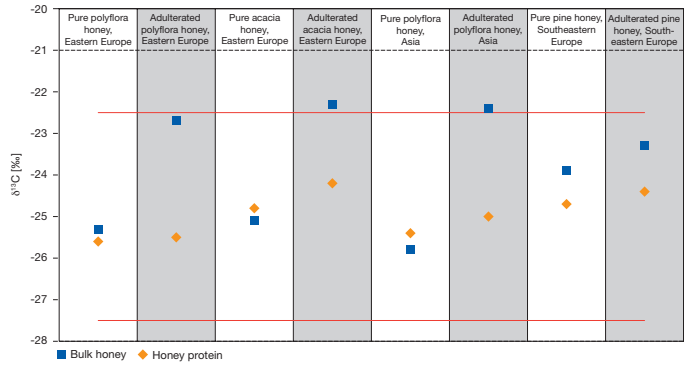

Figure 1. $\delta^{13}C$ values of honey and related proteins. Limit of detection due to natural variation: 7% C4 sugar (agreed value). The red lines show the natural variation of $\delta^{13}C$ in honey. Data kindly provided by Applica GmbH, Bremen, Germany.

The repeatability of the measurements allows one to distinguish smallest adulteration by C4-sugars. Another case study is shown in Figure 1, in which $\delta^{13}C$ values of bulk honey and related protein are used to identify pure and adulterated honeys.

Summary

Adulteration of honey affects producer and consumer value and food safety. Laboratories require an analytical technique providing conclusive answers on origin and authenticity of primary ingredients. The carbon isotope fingerprint of sugar and plants from which honey is derived, allows the identification of sugar addition in commercial honey, i.e. C4-syrups adulteration. This helps protect producer reputation and consumer confidence. With the EA IsoLink IRMS System laboratories can achieve reproducible $\delta^{13}C$ analyses for the detection of honey adulteration with C4-syrups according to the AOAC 998.12 guideline.

References

1. Association of Analytical Communities (AOAC) official methods of analysis method 998.12: C-4 plant sugars in honey, internal standard stable carbon isotope ratio method. *AOAC Int. Gaithersburg MD (USA).* **1999**, Chap. 44, 27–30.
2. Kracht, O., Racz-Fazakas, T. Hilkert, A.: ^{13}C, and simultaneous ^{18}O and ^{2}H Isotope Analysis in Ethanol with DELTA V Mass Spectrometers. *Thermo Fisher Scientific Application Note No. 30147.* **2008**, pp 4.

For more details please refer to AN30177.

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