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Picarro's Combustion Module-CRDS Provides Excellent Data Using the Approved AOAC Internal Standard Isotope Ratio Analysis (ISCIRA) Method for Honey (AOAC 998.12[1]).

This note provides details about a Picarro CRDS-based fast screening method to test for adulteration with corn or cane sugar

Keywords:

Material: Honey, high-fructose corn syrup
Process: ISCIRA, stable isotopes, $\delta^{13}C$, CM-CRDS

Summary and Relevance:

Honey is one of a number of natural products that are regularly tested for adulteration with lower cost sweeteners such as High Fructose Corn Syrup (HFCS) and cane sugar. Such frequent adulteration poses a problem for scrupulous honey producers and importers who end up operating at a cost disadvantage. The problem is significant enough that U.S. Customs and Border Protection agents regularly test for adulteration in honey shipments.

Previous work (AN022) has shown that the faster and less costly Picarro Combustion Module-Cavity Ring-Down Spectroscopy system (CM-CRDS) provides $\delta^{13}C$ values equivalent or better than values obtained using Isotope Ratio Mass Spectroscopy (IRMS) for honey samples. This study has been extended to cover the published ISCIRA method[2]. The data again shows excellent precision. The results further validate the use of the Picarro CM-CRDS as a screening tool for food products.

Natural products may have divergent stable isotope compositions based on a variety of factors. These stable isotopic compositions create a measurable isotopic signature for a certain botanical class. Carbon isotope ratio analysis is a well-known tool used to detect food adulteration by comparing botanical isotopic signatures. The stable carbon isotope value, $\delta^{13}C$, of plant material or plant-derived products is the metric identifying botanical origin. Scientists have not used this value to its full extent to detect food adulteration due to the considerable difficulty, time and cost of obtaining $\delta^{13}C$ data using traditional IRMS instrumentation. In contrast, Picarro's (CM-CRDS) platform can quickly test for fraudulent adulteration of honey by measuring both the $^{13}C/^{12}C$ isotope ratio of the honey sample itself and that of the protein content isolated from honey. The protein originates from the bee so the $\delta^{13}C$ of the protein will be unchanged even if corn or cane sugar is added to the honey. This makes it the internal standard of choice. The difference

 · 480 Oakmead Parkway, Sunnyvale, CA 94085 · T 408.962.3900 · E info@picarro.com · W www.picarro.com

between the $\delta^{13}C$ values of the honey compared to the protein therefore provides a significantly greater degree of certainty in fraud detection.

Picarro's table-top CM-CRDS system can replace a far larger and more costly EA-IRMS system and doesn't require a highly skilled lab technician to operate. Up to 147 samples of honey can be analyzed in one automated sequence with no human intervention over the course of 24 hours. The automation capabilities and ease-of-use can save significant man-hours in a lab setting. This feature set and reliability can translate into annualized cost savings of 50% or greater, including instrument depreciation, instrument downtime, labor, and consumables. The combination of reduced cost, system portability, and higher throughput means that Picarro CM-CRDS can be used both in a laboratory and a field setting without sacrificing precision.

In this application note we tabulate the $\delta^{13}C$ of a set of honey samples and compare against the $\delta^{13}C$ for the protein fraction isolated from each sample. The values indicate that honey adulteration is clearly measurable using this instrument.

Process:

Six honey samples and the protein extracted from each sample were sourced from a honey importer for analysis. The CO_2 resulting from combustion of samples was collected via Picarro's Liaison high throughput interface. After an appropriate mixing time to ensure isotopic equilibration, the collected CO_2 was automatically passed into the CRDS sampling chamber for $^{13}C/^{12}C$ analysis.

Results:

Photosynthetic carbon isotope fractionation is related to carbon dioxide uptake and enzymatic processes[3]. The so-called C3 plants, named due to the number of carbons in an intermediate molecule in the relevant biochemical pathway, discriminate more heavily against ^{13}C than the so-called C4 plants and therefore have more negative $\delta^{13}C$ values. Corn and cane sugar, having been derived from C4 plants, show clearly distinguished $\delta^{13}C$ values, typically in the proximity of -9 to -14 ‰. C3 plants are typically in the range of -25 to -29 ‰. Thus, the high-precision of the CM-CRDS system allows detection of honey adulterated with High Fructose Corn Syrup (HFCS) to concentrations as low as 5% (AN022).

In addition, the protein found in honey comes from the bees themselves. The bees feed on C3 plants. As such, bee protein will show a C3 appropriate $\delta^{13}C$ value. The protein $\delta^{13}C$ value may be subtly different from that of the honey. This difference likely reflects a time lag due to food metabolism by the bees versus their honey production.

Table 1 (below) shows the $\delta^{13}C$ values obtained from the six samples. The precision for the honey samples (column 3) was in the range of 0.2 ‰, while that of the protein (column 2) was a little higher (0.3 ‰), probably reflecting the smaller sample sizes.

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Sample	Protein (‰)	Honey (‰)	C4 Sugars (%)
Sample 1	-26.57	-27.35	-4.6
Sample 2	-26.79	-27.57	-4.6
Sample 3	-26.27	-25.45	5.0
Sample 4	-26.21	-27.84	-9.8
Sample 5	-26.55	-26.19	2.1
Sample 6	-27.80	-27.45	1.9

Comments:

The ISCIRA method, AOAC 998.12, shows how to calculate the percentage of C4 sugars in a sample using a sample's honey and protein $\delta^{13}C$ values. It indicates that negative values should be reported as 0% and only values at, or above 7% are indicative of significant amounts of C4 sugars. The data in Table 1 shows that all of the samples tested here are unadulterated using these criteria.

This study confirms that an AOAC method, 998.12 can be run on a Picarro CM-CRDS system. $\delta^{13}C$ values derived from these instruments can be used as a fast screening tool for various food adulteration and origin problems.

References:

1. AOAC Official Method 998.12, C4 Plant Sugars in Honey, AOAC International, Gaithersburg, MD
2. See for example, Stable Carbon Isotope Ratio Analysis of Honey: Validation of Internal Standard Procedure for Worldwide Application, J.W. White, K. Winters, P. Martin, A. Rossmann, Journal of AOAC International, Vol 81, No. 3, 1998, 610.
3. Carbon Isotope Discrimination and Photosynthesis, G D Farquhar et al, Annual Review of Plant Physiology and Plant Molecular Biolog, Vol. 40: 503-537 (1989)