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Tracking sugar addition in food and beverage using isotope fingerprints

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ABSTRACT

The food and beverage industry suffers from fraudulent activities that include incorrect labeling of products and adulteration, which has a significant impact on food and beverage integrity, brand names and reputation and the market economy. Preventing food and beverage fraud is a key challenge that requires a reliable, cost-effective analytical process that can detect fraudulent changes in food and beverage products.

Stable isotope measurements can differentiate between food and beverage samples which otherwise share identical chemical composition: this is called the **isotope fingerprint**. Using **the isotope fingerprint of food and beverage products** is a reliable technique for fraud detection.

INTRODUCTION

The food and beverage industry suffers from fraudulent activities that include incorrect labeling of products and adulteration, which has a significant impact on food and beverage integrity, brand names and reputation and the market economy. According to the FDA, the most common forms of juice adulteration are addition of some form of sugar and water, addition of pulpwash solids, substitution of a less expensive juice, addition of unapproved preservatives, and labeling of reconstituted juice as fresh squeezed[1].

Detecting the added sugar can be achieved using stable isotope measurements because stable isotopes can differentiate between the sugar already present in the sample from the sugar which is added artificially. Carbohydrates carry an isotope fingerprint, a unique chemical signature which identifies their origin. To visualize this fingerprint, Isotope Ratio Mass Spectrometry (IRMS) can be used, identifying the isotope fingerprint of the product.

In this presentation, we provide examples of the use of isotope fingerprints in food and beverage for the determination of addition of sugar and provide an overview of the interpretation.

ISOTOPES IN FOOD AND BEVERAGE ORIGIN AND AUTHENTICITY

Stable isotopes of **carbon, nitrogen, sulfur, oxygen and hydrogen** can be measured from food and beverage products, such as honey, cheese, olive oil, animal meat, milk powder, vegetables, wine, liquor, water and so forth, using isotope ratio mass spectrometry techniques.[1-12] These stable data can be interpreted to verify the origin, correct-labeling and trace adulteration of food and beverage products (Table 1). Standardized methods (or official international methods) exist for stable isotope analysis, some specifically address the addition of sugars:

- OIV-MAAS312-06 for wine
- EU – CEN 1995, USA – AOAC 1981, EU – CEN 1998 for juices
- AOAC method 991.41, AOAC method 998.12 for honey.

Table 1. Isotope fingerprints in food and beverage samples.

Stable Isotope	What is the biogeochemical interpretation?	What is an example of food fraud interpretation?	What products can be affected?
Carbon	Photosynthesis (C3, C4 and CAM pathways)	Adulteration (e.g. sweetening with cheap sugar)	Honey, liquor, wine, olive oil, butter
Nitrogen	Fertilizer assimilation by plants	Mislabeling (differentiate organic and non-organic)	Vegetables, animal meat
Sulfur	Local soil conditions, proximity to shoreline	Origin of product	Vegetables, animal meat, honey
Oxygen	Principally related to local-regional rainfall and geographical area	Watering of beverages, place of origin of product	Coffee, wine, liquor, water, sugar, animal meat
Hydrogen	Related to local-regional rainfall and geographical area	Watering of beverages, origin of product	Coffee, wine, liquor, water, sugar, animal meat

DETECTION OF HONEY ADULTERATION USING ISOTOPE FINGERPRINTS

In the late 1990s, two brothers who ran a honey and syrup-making business in Mississippi were sentenced to prison after selling honey, maple syrup, and other syrups adulterated partially or wholly with corn syrup for more than 20 years[2]. In 1995, a large honey processing firm in the United States was indicted for adulterating the "pure" honey they sold to food producers with high-fructose corn syrup to increase profits[2].

Adulteration and dilution of honey has also been a widespread problem in China, where tests conducted in 1999 indicated that almost one-third of the brands were adulterated with other types of sugar[2].

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 13C 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 13C 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.

Table 2. Carbon isotope fingerprints of three honeys and their extracted proteins.

	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

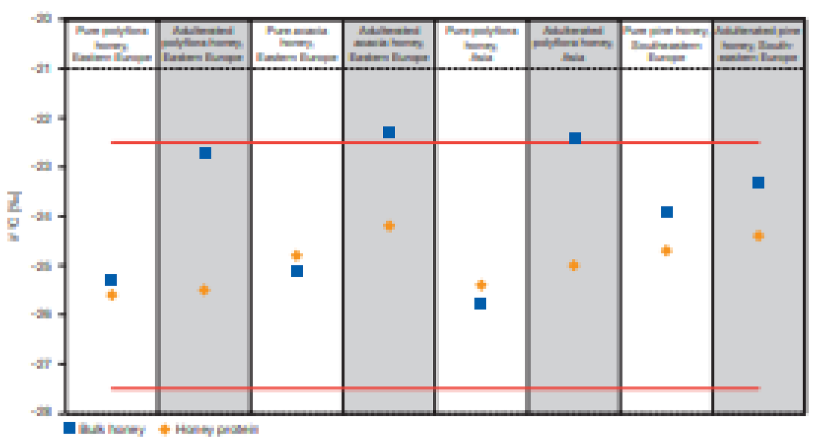

Figure 1. Carbon isotope fingerprints of honey and related proteins. Limit of detection due to natural variation: 7% C4 sugar (agreed value). The red lines show the natural variation of δ^{13}C in honey.

COMPOUND SPECIFIC ISOTOPE ANALYSIS OF HONEY

Carefully selected mixtures of sugars can mimic both, the bulk 13C composition and the sugar profile of the natural product. Floral honey is composed mainly of glucose and fructose with sucrose, the disaccharide of glucose and fructose, as a minor compound. Such mixtures or compounds can be added from other sources, like from high fructose corn syrup (C4 based sugars), to adulterate honey. However, compound specific isotope analysis by LC-IRMS can refine the authenticity fingerprints of honey. LC-IRMS methodology is based on the chromatographic separation of the carbohydrates and carbohydrate fractions and the subsequent determination of 13C isotopic value of every individual sugar in honey. The comparison of the δ13C of fructose and glucose, the detection of other unusual sugars as well as the determination of the sugar pattern can be determined within a single HPLC run.

The table shows eight honey samples which have been analyzed by LC-IRMS and by EA-IRMS. This multi parametric methodology approach demonstrates how different cases of adulterated honey can be detected by combining compound specific and bulk analysis.

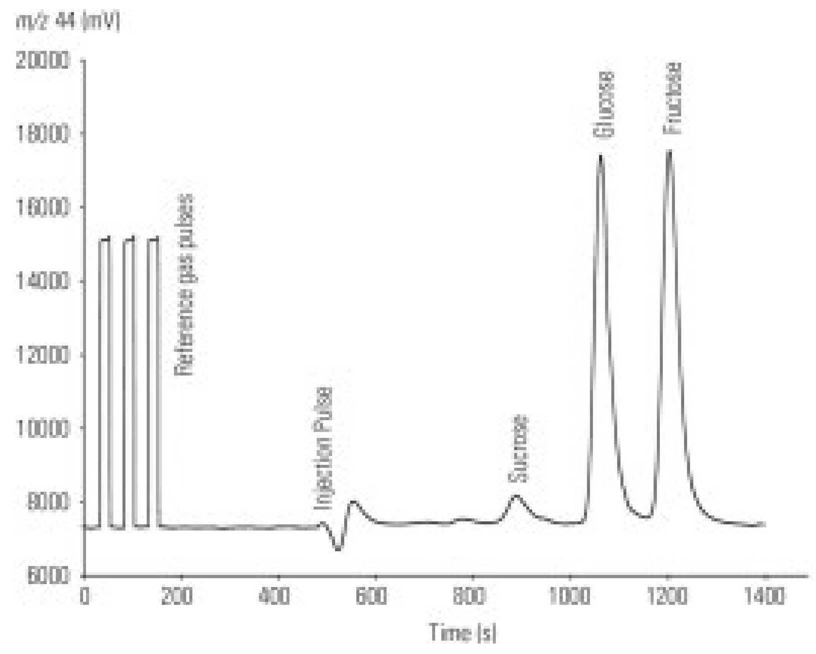

Figure 2. Chromatographic separation of honey carbohydrates by LC-IRMS.

Table 3. LC-IRMS and EA_IRMS analysis of eight honey samples.

Honey	Sucrose ‰	Glucose ‰	Fructose ‰	Fru/Glu ratio of areas	EA Honey (4) ‰	EA Prot. (4) ‰	Adult. (4) ‰	
1	-23.3	**-23.2**	**-22.9**	1.07	**-21.8**	-24.2	**16.7**	**adulterated**
2	-11.3	**-11.2**	**-13.9**	**0.65**	**-11.9**	n.a.	n.a.	**adulterated**
3	-25.3	-24.9	-24.9	1.42	-24.8	-24.8	0.0	
4	-26.4	-26.5	-26.4	0.97	-25.4	-21.6	0.0	
5	n.d.	-26.1	-26.0	**4.53**	-25.8	-26.1	1.9	**adulterated**
6	-26.1	-25.0	-25.3	1.62	-24.3	-24.3	0.0	
7	-25.0	-25.2	-25.1	1.16	-24.2	-24.7	3.4	
8	n.d.	**-25.1**	**-26.4**	2.17	-24.8	-25.1	1.5	**adulterated**

HPLC/EA-IRMS: IDENTIFYING ADULTERATED COCONUT JUICE USING ISOTOPE FINGERPRINTS

The authenticity of commercially available coconut water is of increasing importance because of its designation as a juice by the European Fruit Juice Association (AIJN) and the increasing consumer perspective that it is a healthy, low-carbohydrate beverage. It has been noted that recent trends in addition of sugar to enhance taste and attractiveness of the coconut juice have resulted in an increased sale, however, opening the possibility to fraudulently mis-label coconut juice packaging with respect to the addition of sugar, meaning declarations such as "100% natural" would no longer be valid.

The carbon isotope fingerprint (δ^{13}C values) of plants are different because photosynthetic processes and broadly grouped as C3, C4 and CAM plant types. Consequently, the δ^{13}C values of coconut juice is unique and distinguishable from sugar derived from sugar cane, for example. Coconut juice is extracted from the center of a coconut, which grow on coconut trees (Cocos nucifera) and are part of the C3 plant family. Sugar derived from sugar cane (Saccharum spp.) are part of the C4 plant family. It is known that C3 plants have a carbon isotope fingerprint between -33‰ to -22‰2,3 and C4 plants have a carbon isotope fingerprint between -16‰ to -8‰2,3, providing a framework to differentiate.

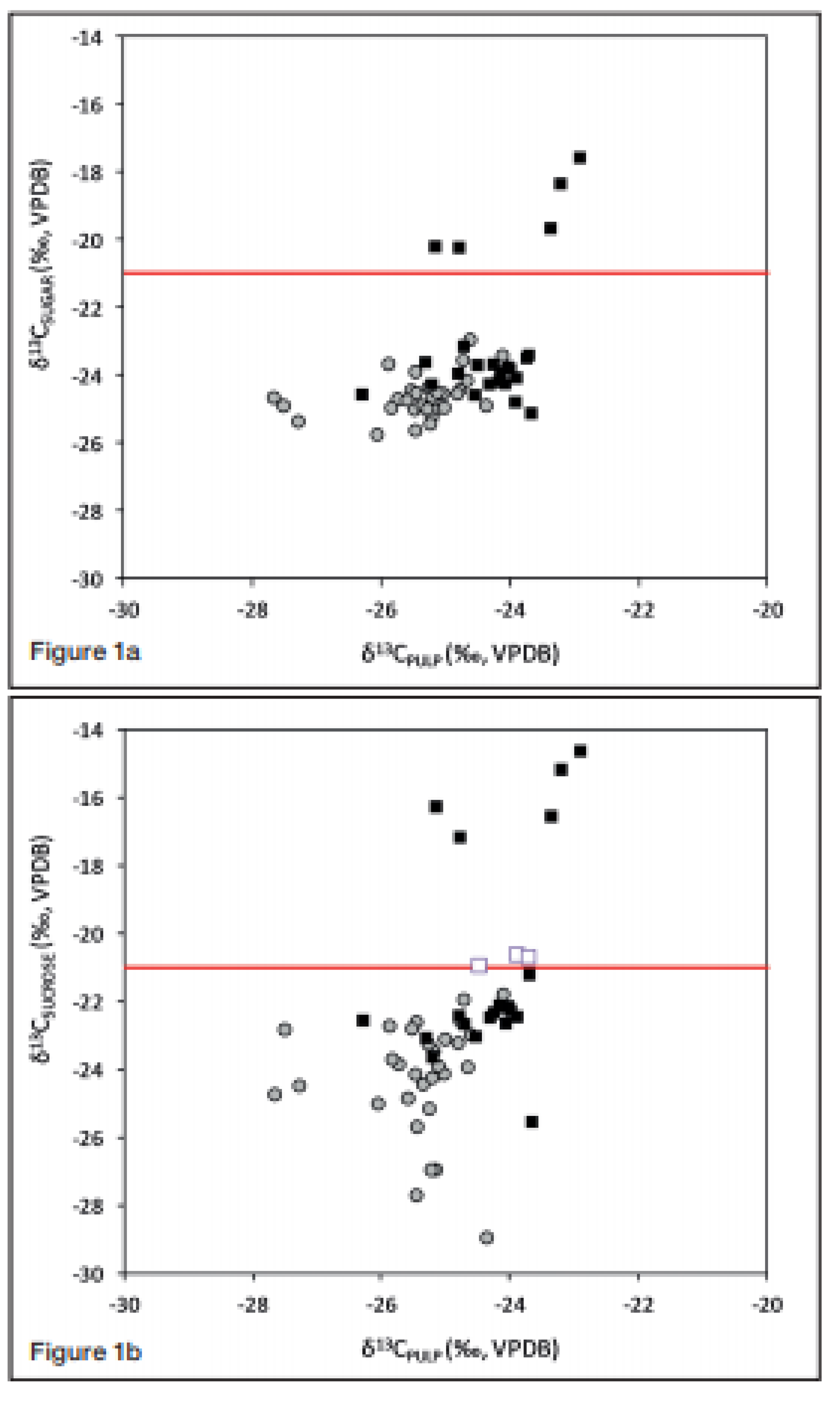

Figure 3. Carbon isotope composition of (a) pulp and total sugars and (b) pulp and sucrose in authentic coconut waters (grey circles) and in commercial bottled coconut waters (squares). The horizontal solid red line indicates the upper limit for C3-plants (- 21% VPDB). The blank (white) squares represent the samples identified as adulterated only by the sucrose carbon isotope analysis (and not by the total sugars).

The addition of C4-plant sugar to coconut juice was better detected using the carbon isotope fingerprints of individual sugars (sucrose, glucose and fructose) by comparison with the isotope fingerprint of total sugar (Figure 3, 1b). For the authentic coconut juices, the carbon isotope fingerprints of sucrose, glucose and fructose fell within the expected range. However, when applied to the commercially purchased coconut juices, the approach resulted in enhanced detection of C4-sugar addition by identifying 38% of adulterated samples. Moreover, the limit of detection was improved such that sugar additions of less than 10% can be detected.

ISOTOPE FINGERPRINTS FOR SUGAR ADDITION

Food and beverage products have a unique chemical signature from the biogeochemical processes that occurred during the formation of their base ingredients. Isotope Ratio Mass Spectrometry (IRMS) works by detecting the "isotope fingerprint" of a sample, a unique chemical signature that changes from sample to sample across the environment. These isotope fingerprints can provide conclusive information on the origin and the authenticity of a product.
By using isotope fingerprints, laboratories can:
- Trace unique answers on origin and authenticity.
- Extend their analytical capabilities.
- Work with an integrated analytical solution, driven by a single software for automated high sample throughput.

Thermo Fisher Scientific provides dedicated Isotope Fingerprinting solutions with a portfolio designed to offer different capabilities and performances, with dedicated features for the coupling to the Thermo Scientific™ IRMS Systems, according to the varying laboratory analytical needs.

REFERENCES

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3. Psomiadis, D., Zisi, N., Koger, C., Horvath, B., Bodiselitsch, B. (2018). J Food Sci Technol. 55: 2994

INVESTIGATE MORE

Visit thermofisher.com/IsotopeFingerprints and learn more about food and beverage investigations by isotope fingerprints by reading more application reports:

- **SN30414** - Official methods for food and beverage
- **AN300024** - LC-IRMS δ^{13}C of Carbohydrates in Honey
- **AB30583 -** HPLC/EA-IRMS: Identifying adulterated coconut juice using isotope fingerprints
- **AN30147 -** EA-IRMS Analysis of Ethanol in Wine.

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