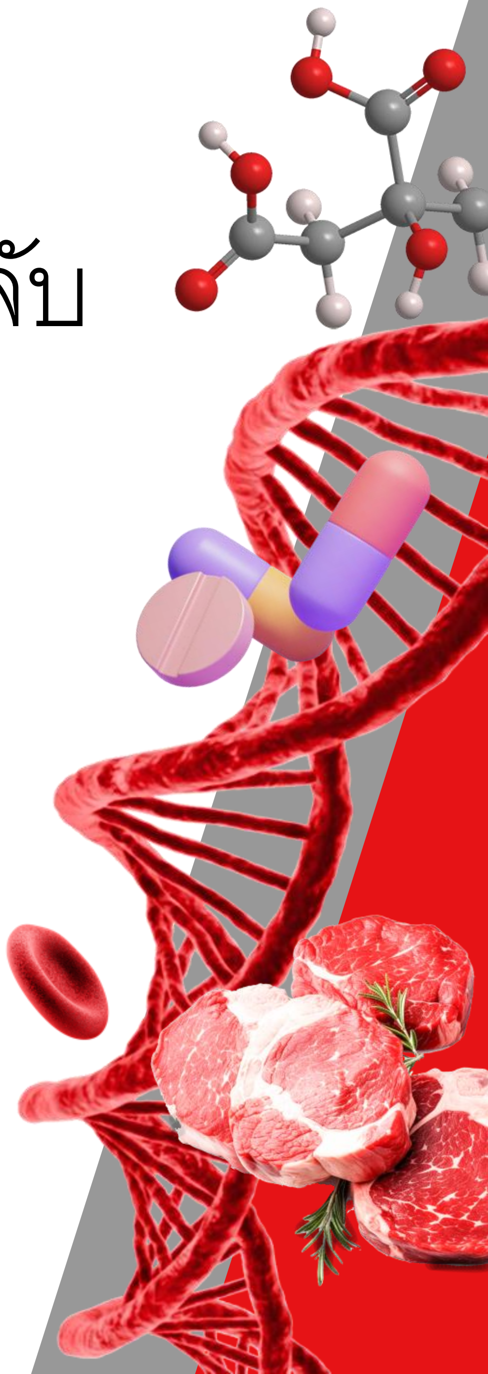
การใช้ Isotope Ratio ในการตรวจสอบย้อนกลับการตรวจสอบการปลอมปนของสินค้าเกษตร

PRESENTED BY

มหิทธิ เพื่อนงาม



Isotope Fingerprints

Chemically similar but physically different

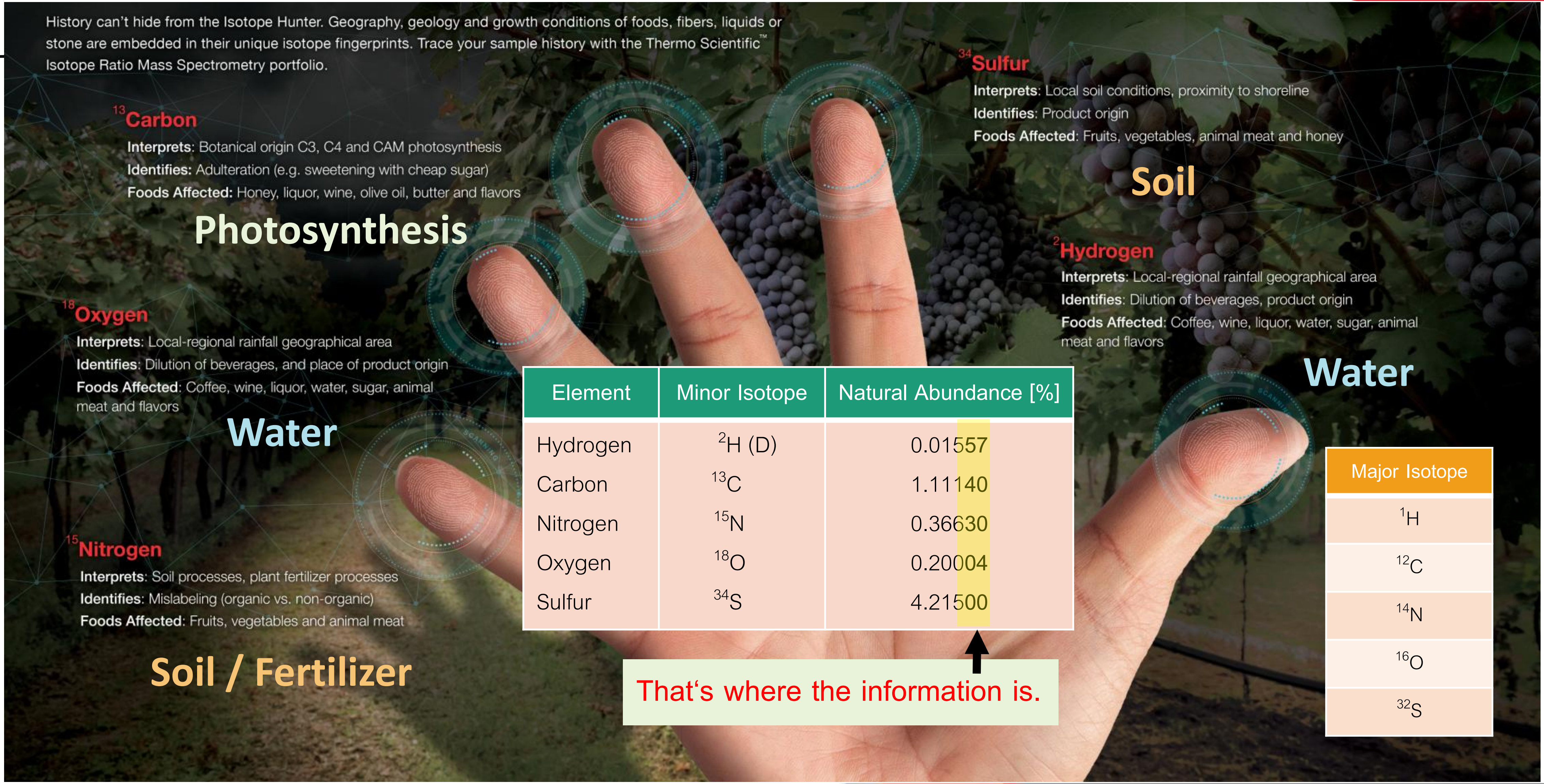
- Materials have a fingerprint, a unique chemical signature that allows the sample to be identified.
- To visualize this fingerprint, Isotope Ratio Mass Spectrometry (IRMS) is used to measure stable isotopes and identify the isotope fingerprint of a material or product.
- IRMS traces carbon, nitrogen, sulfur, oxygen, and hydrogen isotopes by detecting their natural variations, which can reveal to the origin and history of samples.

Topics

Things that we will cover on today

- What an Isotope Fingerprint is
- How Isotope Fingerprints from
- How Isotope Fingerprints are measured
- Where Isotope Fingerprints are useful
    - Origin
    - Authenticity
    - Adulteration
    - Mislabeling

Isotope Fingerprints



History can't hide from the Isotope Hunter. Geography, geology and growth conditions of foods, fibers, liquids or stone are embedded in their unique isotope fingerprints. Trace your sample history with the Thermo Scientific™ Isotope Ratio Mass Spectrometry portfolio.

^{13}Carbon

Interprets: Botanical origin C3, C4 and CAM photosynthesis
Identifies: Adulteration (e.g. sweetening with cheap sugar)
Foods Affected: Honey, liquor, wine, olive oil, butter and flavors

Photosynthesis

^{18}Oxygen

Interprets: Local-regional rainfall geographical area
Identifies: Dilution of beverages, and place of product origin
Foods Affected: Coffee, wine, liquor, water, sugar, animal meat and flavors

Water

^{15}Nitrogen

Interprets: Soil processes, plant fertilizer processes
Identifies: Mislabeling (organic vs. non-organic)
Foods Affected: Fruits, vegetables and animal meat

Soil / Fertilizer

^{34}Sulfur

Interprets: Local soil conditions, proximity to shoreline
Identifies: Product origin
Foods Affected: Fruits, vegetables, animal meat and honey

Soil

^{2}Hydrogen

Interprets: Local-regional rainfall geographical area
Identifies: Dilution of beverages, product origin
Foods Affected: Coffee, wine, liquor, water, sugar, animal meat and flavors

Water

Element	Minor Isotope	Natural Abundance [%]
Hydrogen	^{2}H (D)	0.01557
Carbon	^{13}C	1.11140
Nitrogen	^{15}N	0.36630
Oxygen	^{18}O	0.20004
Sulfur	^{34}S	4.21500

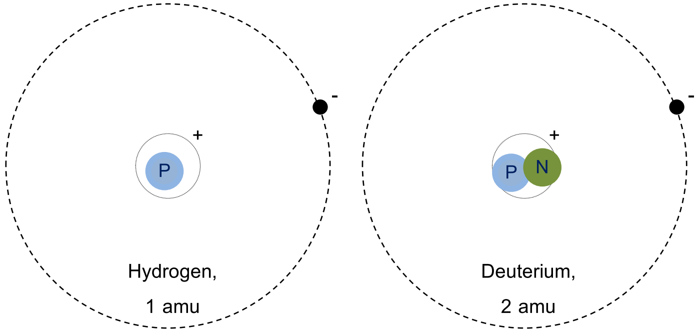
That's where the information is.

Major Isotope
^{1}H
^{12}C
^{14}N
^{16}O
^{32}S

Measuring the Isotope fingerprints

Isotope Ratio Mass Spectrometry (IRMS)

- Isotope ratio mass spectrometry (IRMS) is a specialization of mass spectrometry, in which mass spectrometric methods are used to measure the relative abundance of isotopes in each sample.[1]
- The isotope ratio mass spectrometer (IRMS) allows the precise measurement of mixtures of stable isotopes.[2] The analysis of 'stable isotopes' is normally concerned with measuring isotopic variations arising from mass-dependent isotopic fractionation in natural systems.



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Sulfur	^{34}S	4.21500

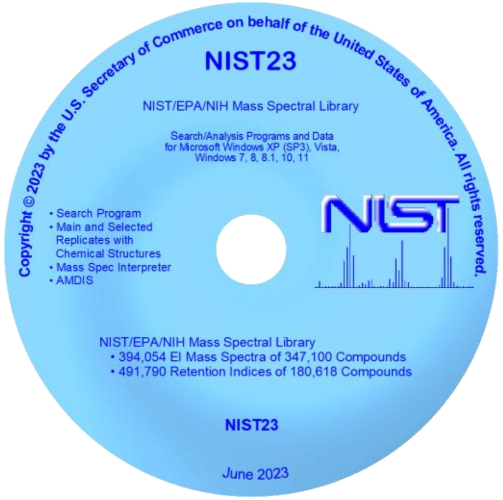
Isotropic Fractionation: any process that changes the relative abundances of stable isotopes of an element.

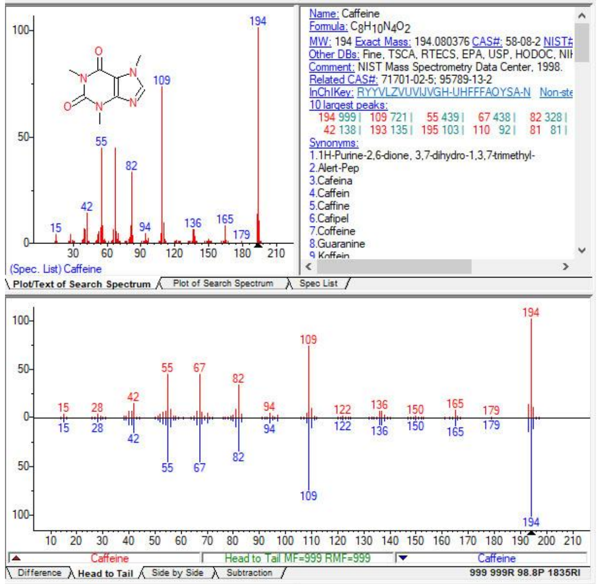
[1] Paul D, Skrzypek G, Fórizs I (2007). "Normalization of measured stable isotopic compositions to isotope reference scales - a review". Rapid Commun. Mass Spectrom. 21 (18): 3006–14.
[2] Townsend, A. (ed) (1995). Encyclopaedia of Analytical Science Encyclopaedia of Analytical Science. London: Academic Press Limited.

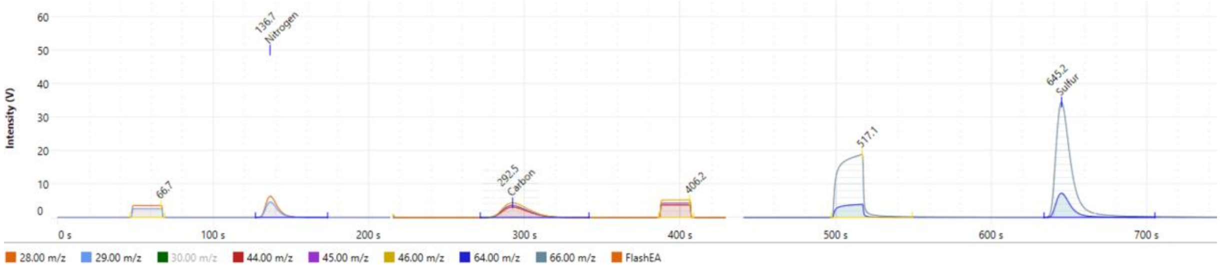
Isotope ratio mass spectrometer (IRMS)

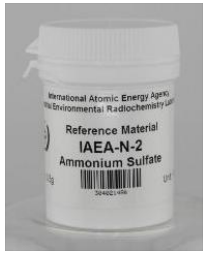
designed to measure the relative abundances of isotopes with very high precision

Conventional MS	IRMS
• Provide structural information	• Provide an isotope ratio of elements









IAEA

International Atomic Energy Agency

USGS science for a changing world

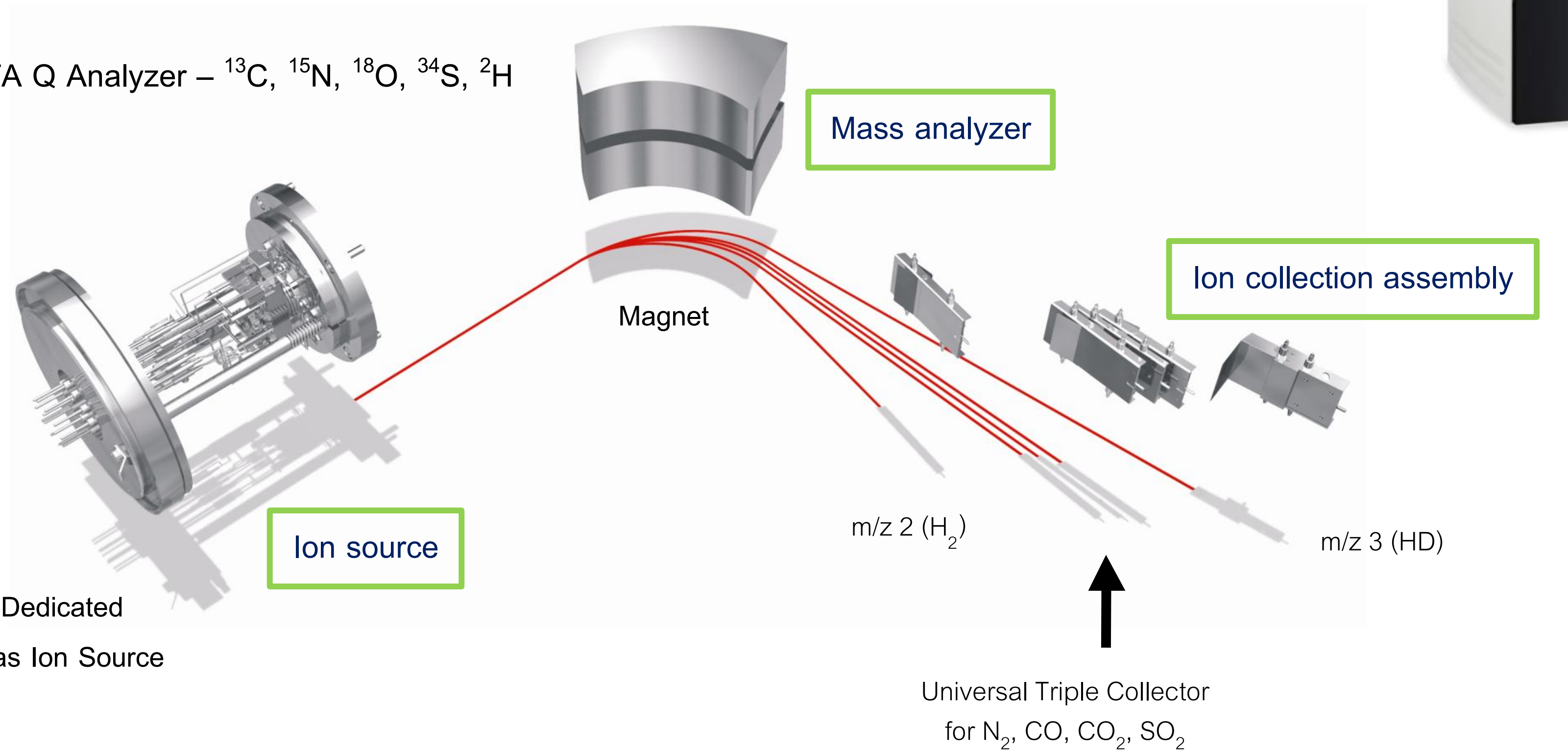
U.S. Geological Survey

Isotope ratio mass spectrometer (IRMS)

designed to measure the relative abundances of isotopes with very high precision

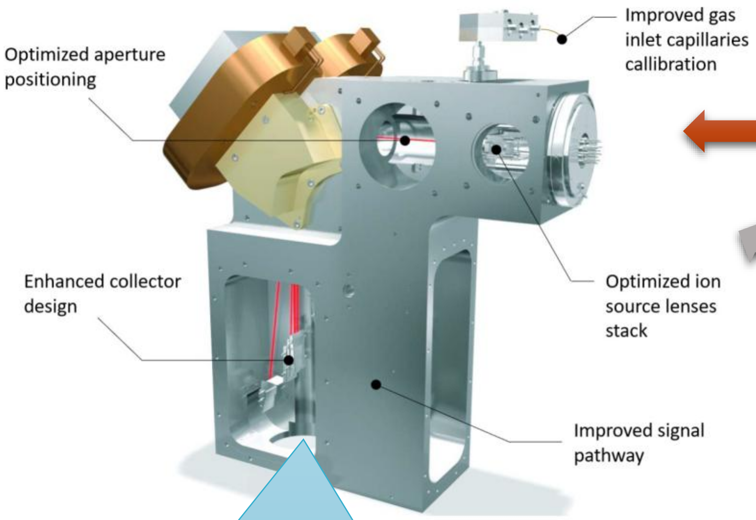
The mass spectrometers used for isotopic analysis generally comprise three basic sections; an ion source, a mass analyzer and an ion collection assembly.

DELTA Q Analyzer – ^{13}C, ^{15}N, ^{18}O, ^{34}S, ^{2}H



Isotope ratio mass spectrometry

sample need to be converted to simple gases before enter the ion source

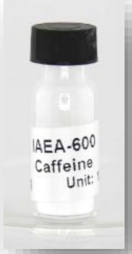


$^{14}N^{14}N$, $^{15}N^{14}N$
$^{12}C^{16}O^{16}O$, $^{13}C^{16}O^{16}O$,
$^{12}C^{18}O^{16}O$, $^{32}S^{16}O^{16}O$,
$^{34}S^{16}O^{16}O$

combustion

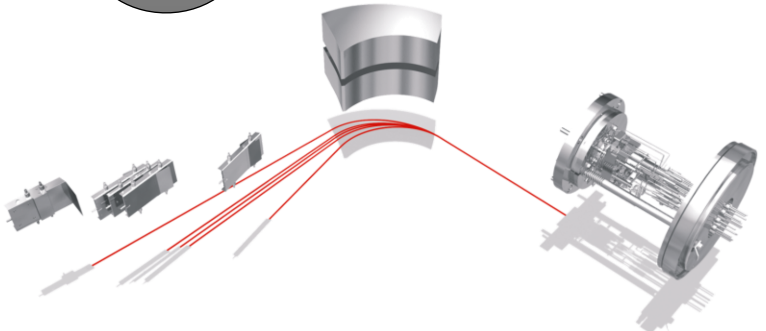
$^{1}H^{1}H$, $^{2}H^{1}H$
$^{12}C^{16}O$, $^{13}C^{16}O$,
$^{12}C^{18}O$

pyrolysis



m/z

2, 3, 28, 29, 30, 44, 45, 46, 64, 66



Continuous flow IRMS (CF-IRMS)

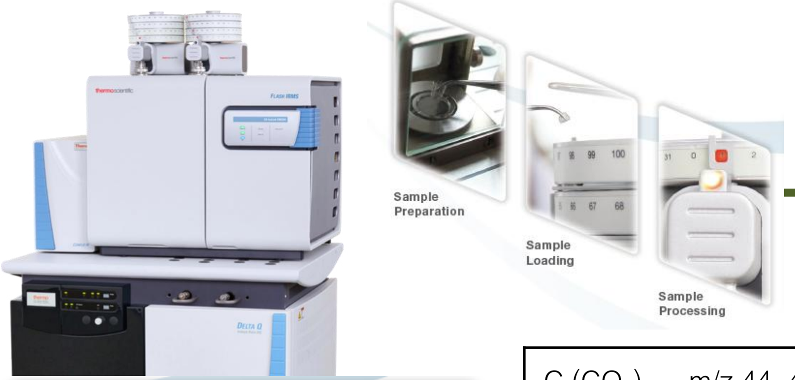
automated sample introduction and conversion

The CF-IRMS sample introduction technique consists of a helium carrier gas that carries the analyte gases into the ion source of the IRMS. This technique is used to connect an IRMS to a range of automated sample preparation devices.

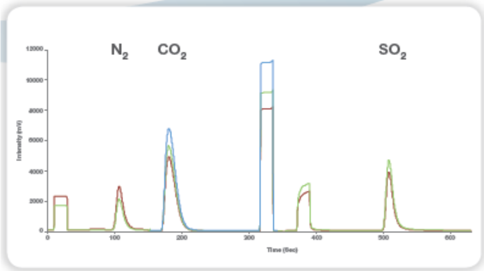
- Bulk Stable Isotope Analysis
  - Whole sample (all components) provide isotopic values
- Compound Specific Isotope Analysis
  - Each components provide individual isotopic values

Bulk Stable Isotope Analysis

based on the use of an elemental analyzer (EA-IRMS)



He
O_2
Combustion tube
oxidation catalyst,
~1000 - 1700 °C
N_2, NO_x, CO_2, SO_2, O_2 and H_2O
Reduction tube
Reduce NO_x to N_2
and remove excess O_2
~600 °C
H_2O trap
N_2, CO_2 and SO_2
Packed GC column
IRMS



C (CO_2),	m/z 44, 45, 46
N (N_2),	m/z 28, 29
S (SO_2),	m/z 64, 66

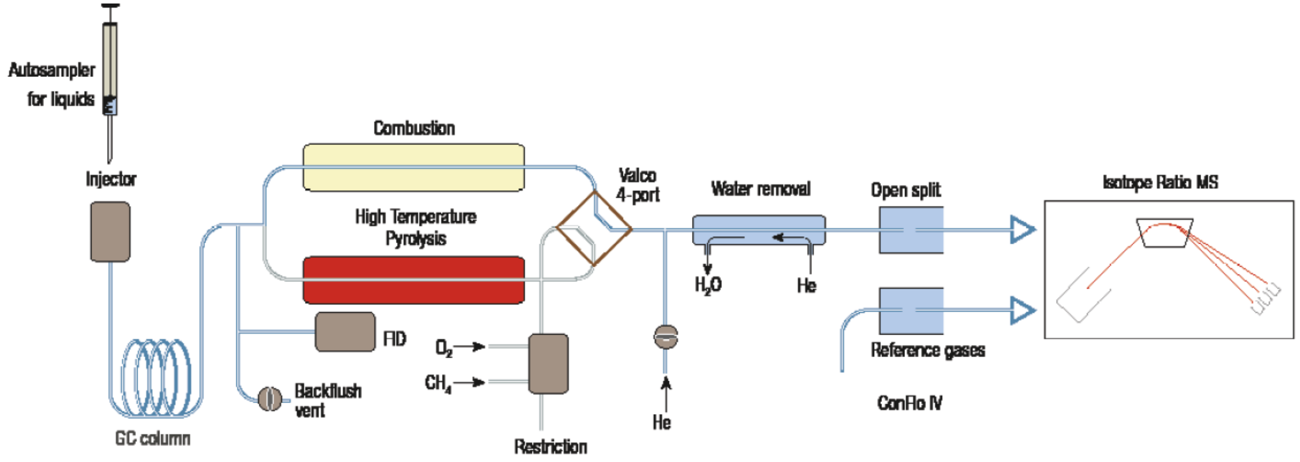
Sample
He
Pyrolysis tube
oxidation catalyst,
>1400 °C
H_2 and CO
Packed GC column
IRMS

H (H_2),	m/z 2, 3
O (CO),	m/z 28, 29, 30

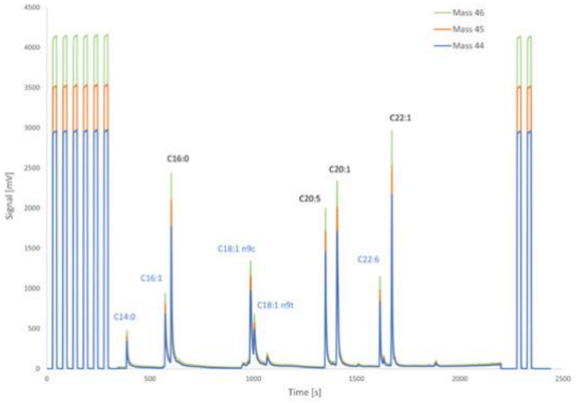
Compound Specific Isotope Analysis

based on the use of gas chromatography system (GC-IRMS)

Organic compounds eluting from a GC column are converted into simple gases when traversing a capillary micro-reactor. Accordingly, all compound specific isotope ratios can be analyzed in the IRMS.



Schematic of the GC-IRMS system with the Thermo Scientific GC IsoLink II conversion unit



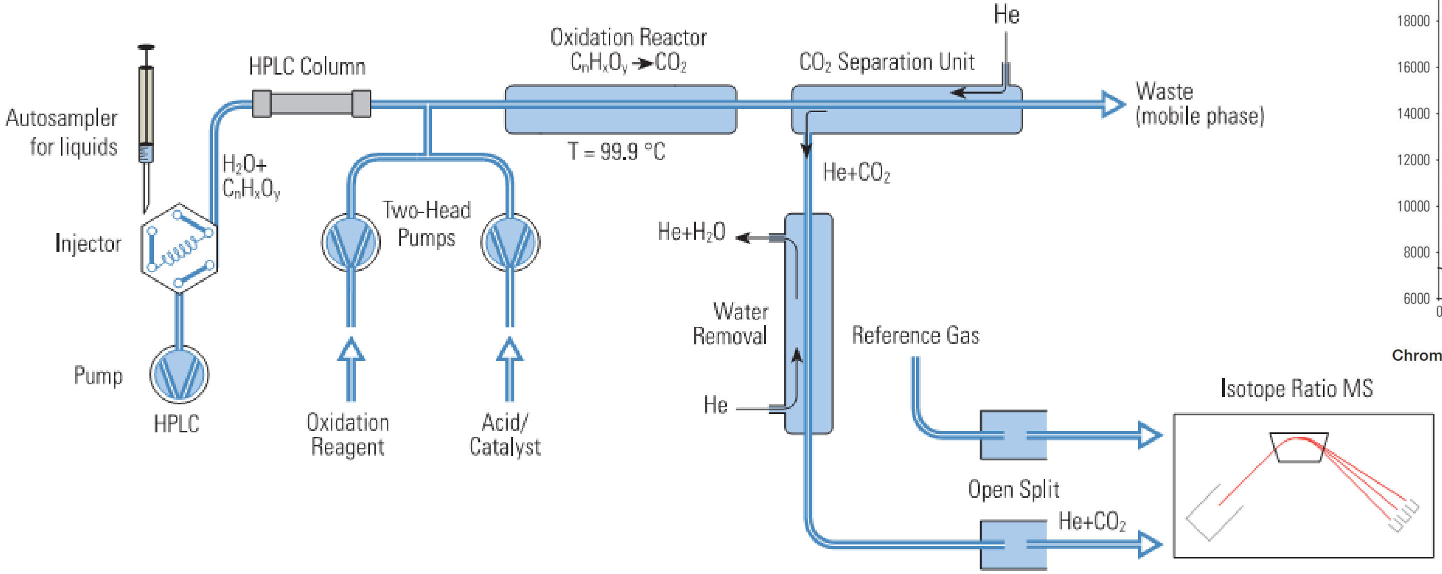
Complex organic mixtures are separated on the capillary GC column. Baseline separated peaks are the basis for high precision CSIA.

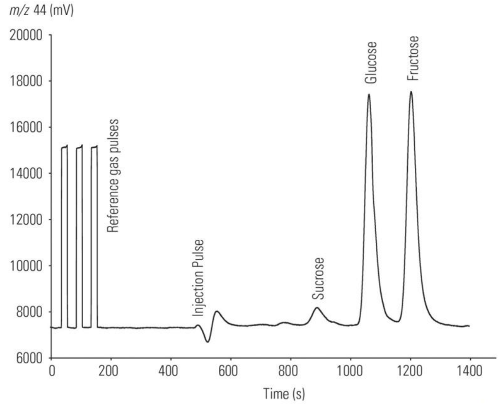
A splitter at the end of the GC column sends >95% of the sample to a combustion or pyrolysis tube. The remainder is sent to an optional FID, MS, or is vented to the atmosphere.

Compound Specific Isotope Analysis

based on the use of liquid chromatography system (LC-IRMS)

Quantitative oxidation of all individual organic compounds eluting from the HPLC column, followed by fractionation-free separation of the resulting CO_2 gas from the liquid phase.

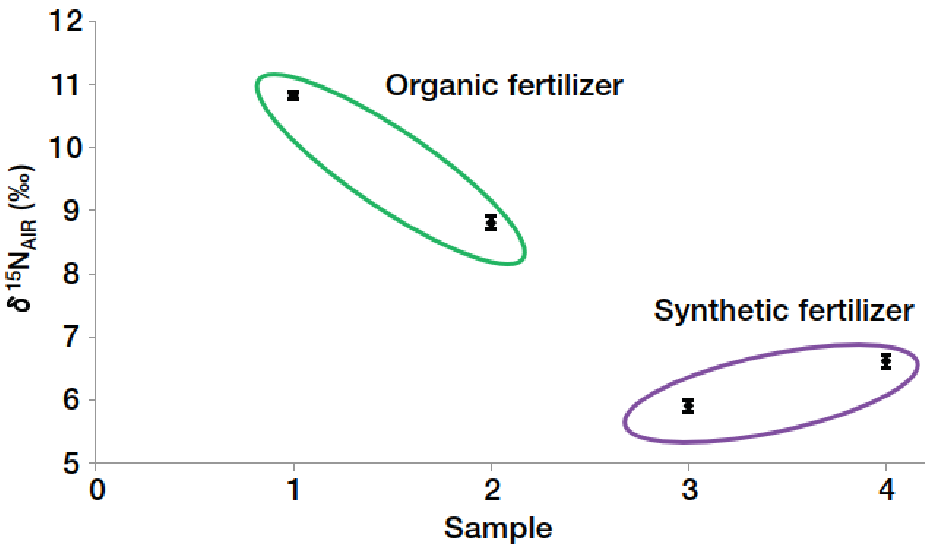




Chromatographic separation of honey carbohydrates by LC-IRMS.

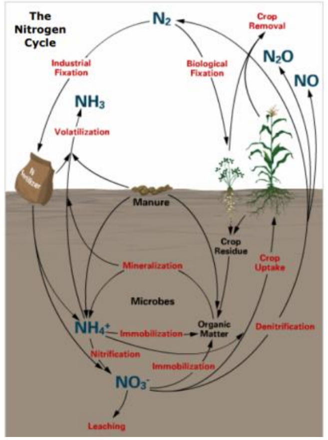
Detecting organic grown vegetables

As organic fruit and vegetables attract a higher price on the market, this can lead to economically motivated fraud through mislabelling produce as "organic" when they have been grown using synthetic fertilizer.



Nitrogen isotope fingerprints detect organic grown tomatoes.

- Nitrogen Isotope Fingerprint
- Organic vs Synthetic fertilization
- Differentiation of nitrogen isotopes in plants and soils due to ammonia volatilization, denitrification, nitrification, etc.
- Organic fertilization fingerprint: +8‰ to +20‰
- Synthetic fertilization fingerprint: +3‰ to +6‰



Tracing the geographical origin of coffee

Green coffee beans have a fingerprint, a unique chemical signature that allows them to be identified: isotope fingerprints have been reliably used for origin, authenticity and product label claim verification.

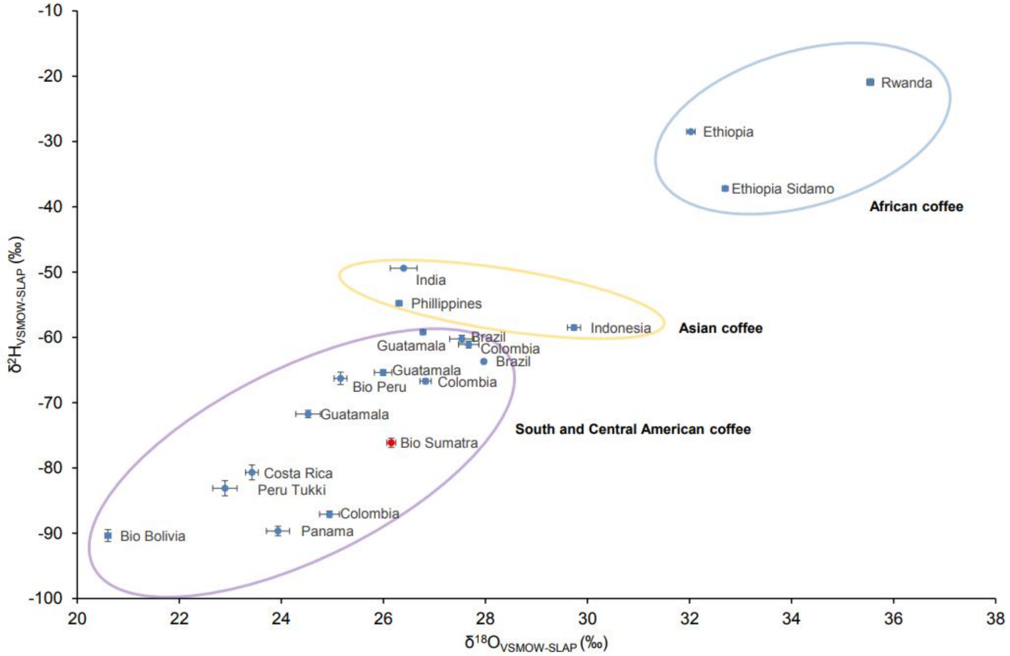
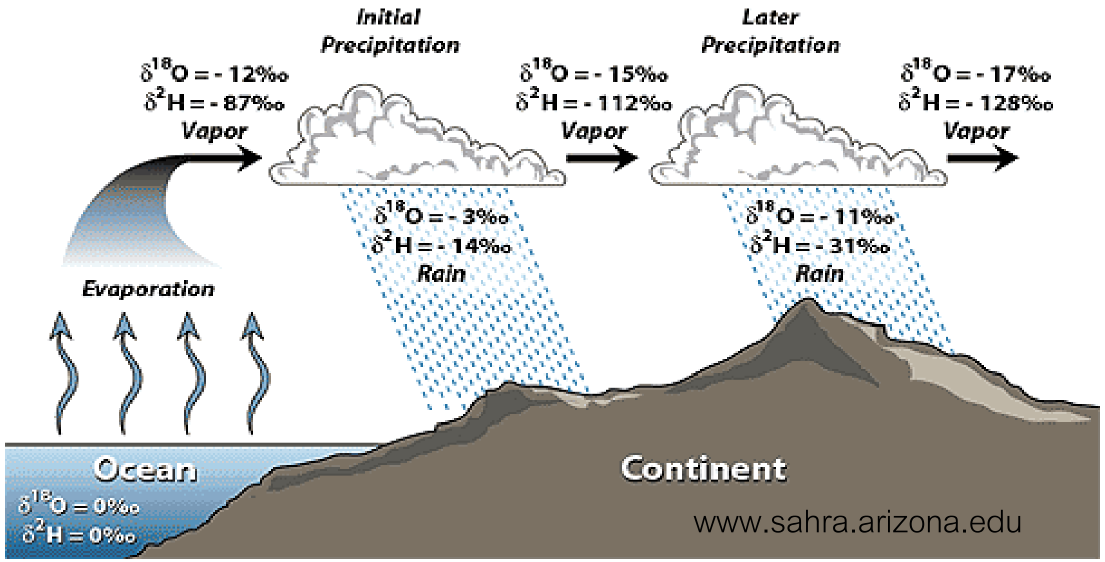


Figure 1. Hydrogen and oxygen isotope fingerprints of roasted coffee beans from Africa (blue), Asia (brown) and central and South America (purple).

- Hydrogen and oxygen Isotope Fingerprints
- The Coffea spp plants, cultivated as the source of the coffee beans, carry an isotopic fingerprint associated with local-regional rainfall.
- Differentiation of American, Asian and African Coffee beans (Green and Roasted)
- Identification of mislabeled coffee beans.

Isotopes in Nature

^{18}O and ^{2}H in the Water Cycle



The isotopic composition of water samples can be affected by several environmental parameters :

- Seasonality
- Amount of precipitation
- Altitude
- Continentally
- Temperature

All these parameters can be characterizing the source region of a water sample.

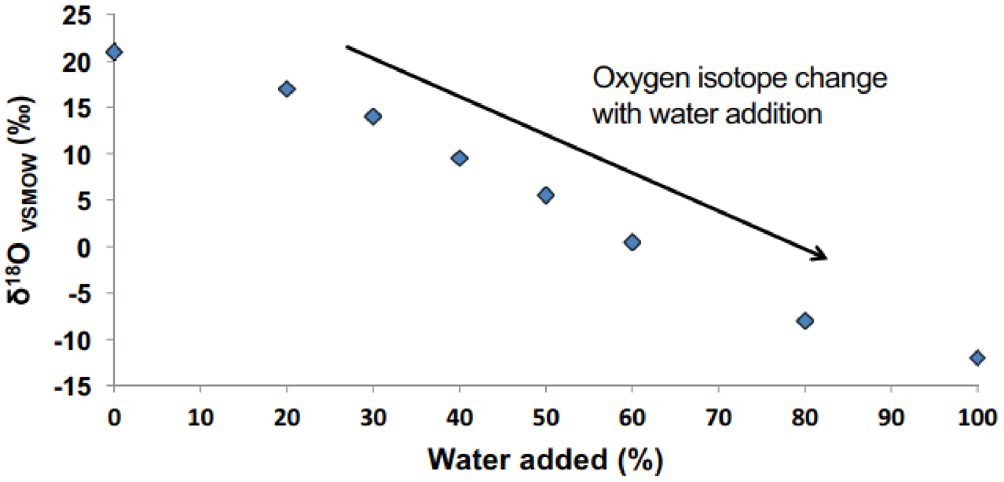
IAEA water standard	$\delta^{18}O$	$\delta^{2}H$
SLAP2 (Standard Light Antarctic Precipitation 2)	-55.50 ‰	-427.5 ‰
GISP (Greenland Ice Sheet Precipitation)	-24.76 ‰	-189.5 ‰
VSMOW2 (Vienna Standard Mean Ocean Water 2)	0 ‰	0 ‰

https://nucleus.iaea.org/rpst/ReferenceProducts/ReferenceMaterials/Stable_Isotopes/2H18O-water-samples/index.htm

Applications

Tracking wine adulteration using isotope fingerprints

The most common type of wine adulteration is the addition of cheaper products to the original wine, such as fruit juices, water and sweeteners, which are not related to the grapes or fermentation process that the wine was originally produced from.



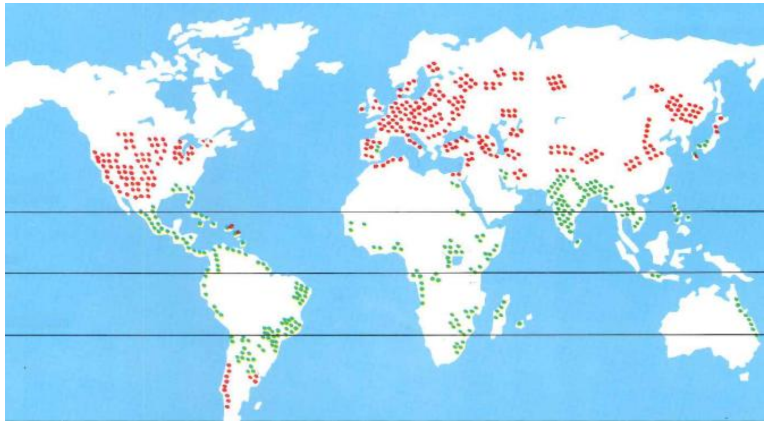
Oxygen isotope fingerprints detect watering of wine.

- Oxygen Isotope Fingerprint
- Geographical origin and adulteration
- Grapes have local-regional rainfall
- If adulterated by water or juices, oxygen isotope fingerprint changes
- Official method OIV-MS-AS2-12

Applications

Testing sugar package label claims using carbon isotope fingerprints

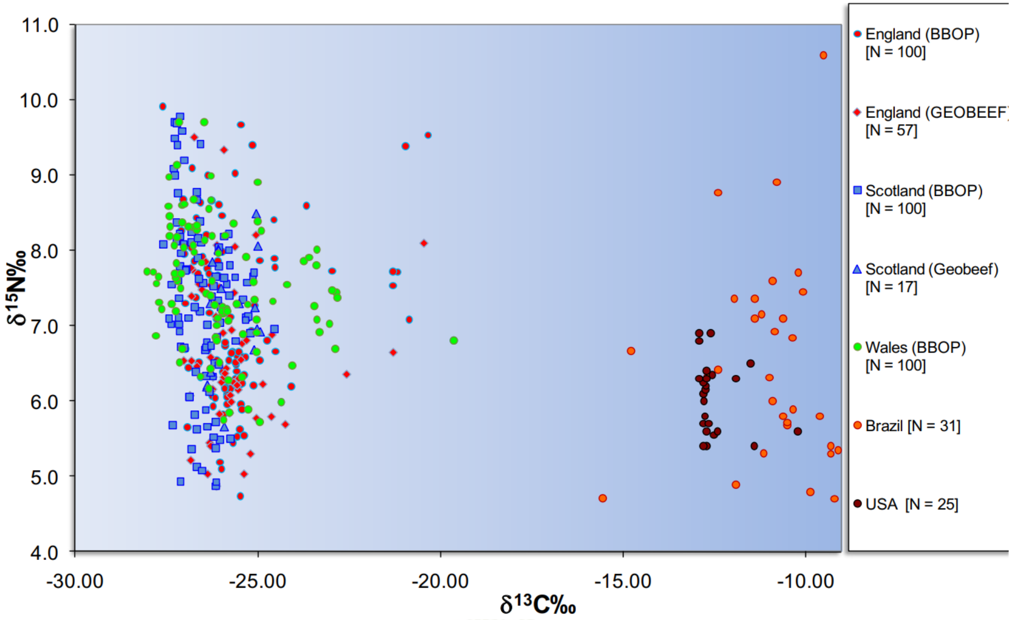
Sample name	$\delta^{13}C_{VPDB}$ ± 1SD [‰, n=3]	Label claim	Identified by $\delta^{13}C$ as
Australia	-12.59±0.15	Not Stated	Cane sugar
Brazil	-12.21±0.17	Not Stated	Cane sugar
China (Shanghai)	-12.49±0.17	Not Stated	Cane sugar
China (Nan Jing)	-12.63±0.11	Not Stated	Cane sugar
Cuba	-12.46±0.06	Not Stated	Cane sugar
Denmark	-26.69±0.05	**Beet sugar**	**Beet sugar**
Egypt	-13.11±0.02	Not Stated	Cane sugar
Estonia	-13.19±0.08	Not Stated	Cane sugar
France	-12.14±0.12	**Cane sugar**	**Cane sugar**
France	-12.02±0.35	**Cane sugar**	**Cane sugar**
Germany	-26.69±0.08	**Beet sugar**	**Beet sugar**
Italy	-12.22±0.05	**Cane sugar**	**Cane sugar**
Ivory Coast	-12.24±0.19	**Cane sugar**	**Cane sugar**
Lebanon	-27.08±0.02	Not Stated	Beet sugar
Malaysia	-12.21±0.12	Not Stated	Cane sugar
Morocco	-12.58±0.03	Not Stated	Cane sugar
New Zealand	-12.33±0.10	**Cane sugar**	**Cane sugar**
Philippines	-12.95±0.09	**Cane sugar**	**Cane sugar**
Portugal	-12.51±0.04	Not Stated	Cane sugar
Romania	-12.47±0.04	Not Stated	Cane sugar
Senegal	-12.42±0.25	**Cane sugar**	**Cane sugar**
Taiwan	-13.08±0.01	Not Stated	Cane sugar
Thailand	-12.24±0.02	Not Stated	Cane sugar
Turkey	-13.29±0.12	Not Stated	Cane sugar
UAE	-25.02±0.02	Not Stated	Beet sugar
United Kingdom	-12.75±0.04	**Cane sugar**	**Cane sugar**
USA (Hawaii)	-12.41±0.13	**Cane sugar**	**Cane sugar**
USA (San Francisco)	-12.89±0.04	**Cane sugar**	**Cane sugar**



- Carbon Isotope Fingerprint
- The carbon isotope fingerprint of plants differ because of photosynthetic processes
- C3 plants have a carbon isotope fingerprint between -33‰ to -22‰ and C4 plants between -16‰ to -8‰.
- Can identify beet and cane sugar and verify product label

Applications

Tracing the origin of beef based on diet using carbon isotope fingerprints



Carbon and nitrogen isotope fingerprints of beef muscle.

- Carbon Isotope Fingerprint
- Pasture determines the diet of cattle
- Pasture varies between C3 and C4 plant groups, which result in difference in animal (i.e. dietary differences)
- Takes 167 days for “meat” to change from C3 to C4 signature, and vice versa
- UK cattle reared on C3 diet, whilst Brazilian cattle

Table 1: Features of C3 and C4 grasses

NSW Department of Primary Industries

	C3	C4
Initial molecule formed during photosynthesis	3 carbon	4 carbon
Growth period	Cool season or yearlong	Warm season
Light requirements	Lower	Higher
Temperature requirements	Lower	Higher

Detection of honey adulteration

AOAC Official Method 998.12

C-4 Plant Sugars in Honey Internal Standard Stable Carbon Isotope Ratio

- Carbon Isotope Fingerprint
- Honey adulteration by addition of exogenous sugars
- If adulterate by sugar addition, carbon isotope fingerprint changes
- Official method AOAC 998.12

Carbon isotope fingerprints of three honeys and their extracted proteins.

	Honey-1	Protein-1	Honey-2	Protein-2	Honey-3	Protein-3
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

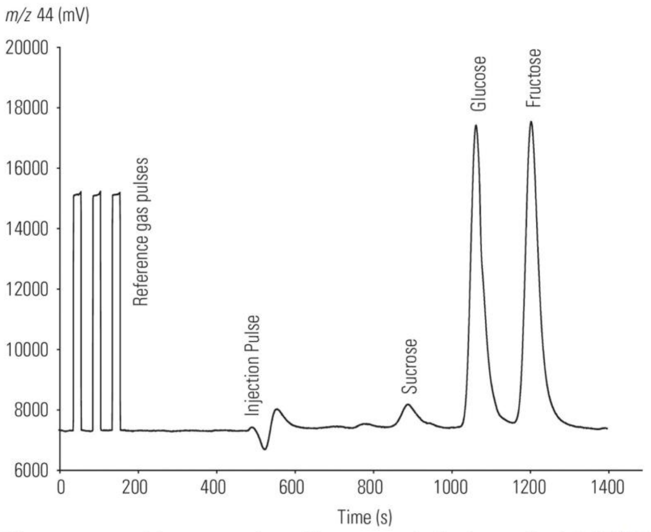
Calculate apparent C-4 sugar content as follows:

$$\text{C-4 sugars, \%} = \frac{\delta^{13}C_P - \delta^{13}C_H}{\delta^{13}C_P - (-9.7)} \times 100$$

where $\delta^{13}C_P$ and $\delta^{13}C_H$ are $\delta^{13}C$ values, ‰, for protein and honey, respectively, and −9.7 is the average $\delta^{13}C$ value for corn syrup, ‰. Report negative values from this calculation as 0%. Product is considered to contain significant C-4 sugars (primarily corn or cane) only at or above a value of 7%.

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**
2	-11.3	**-11.2**	**-13.9**	**0.65**	**-11.9**	n.a.	n.a.
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
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



Chromatographic separation of honey carbohydrates by LC-IRMS.

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