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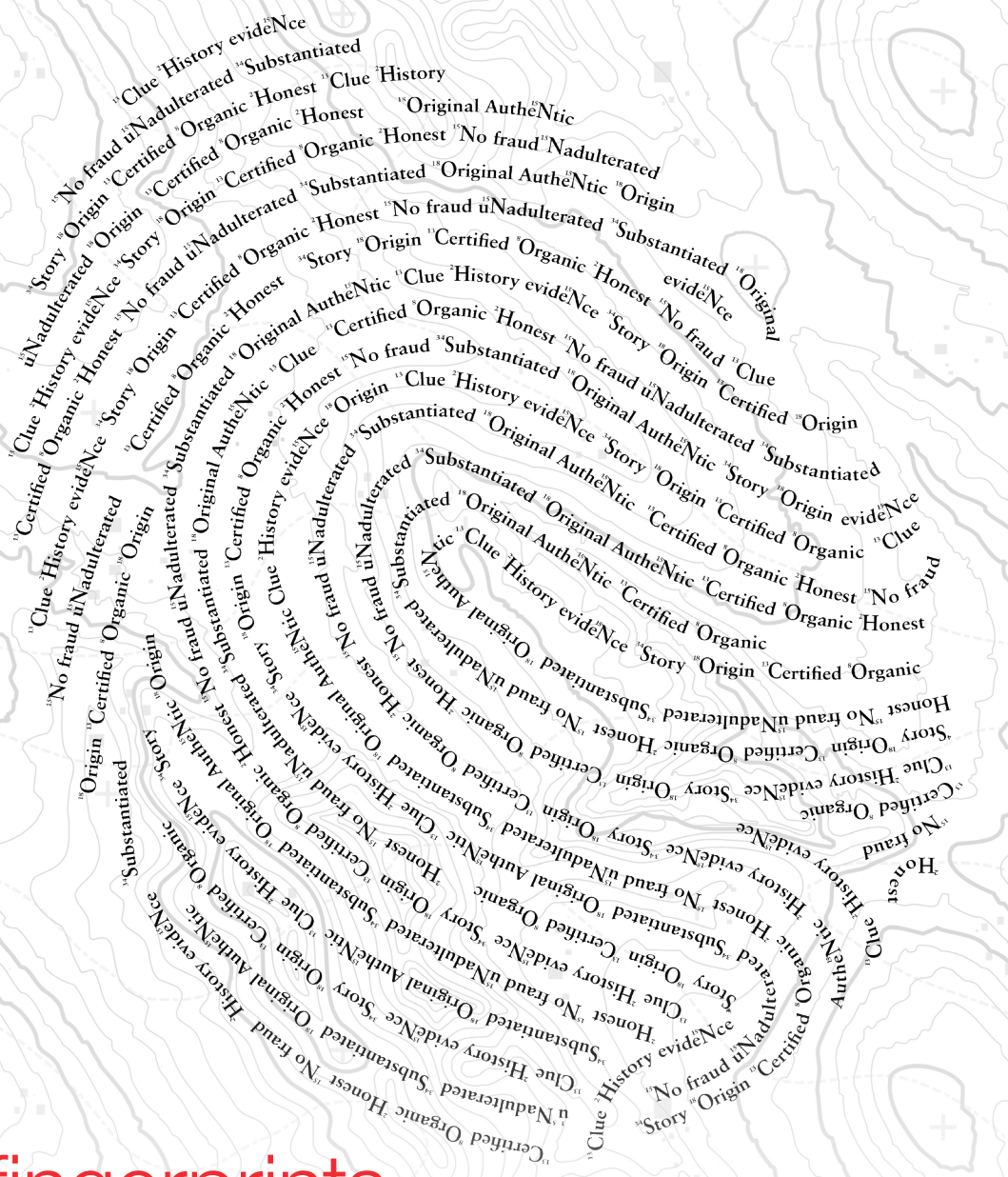

Isotope fingerprints in food integrity applications

Detecting ^{13}Clues, tracking ^{18}Origin, unraveling ^{2}History with isotope fingerprints

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Isotope fingerprints for food integrity applications

Food and beverage products have a fingerprint, a unique chemical signature that allows the product to be identified. To visualize this fingerprint, Isotope Ratio Mass Spectrometry (IRMS) can be used. The isotope fingerprint in food and beverage products is region or process specific (Table 1), which means that products can be differentiated based on geographical region (cheese, coffee, sugar, fish and animal feeding areas), botanical processes (beans, seeds, olive oil, vanilla), soil and fertilization processes (fruits and vegetables) and fraudulent practices (sugar addition to honey, watering of wines and spirits). These processes can be traced using carbon, nitrogen, sulfur, oxygen and hydrogen isotopes, with their variations indicating the origin and history of food and beverage products.

Production through to consumer: the spectre of economically motivated fraud

Complexities in the food and beverage supply chain from the production site through to the consumer have presented significant, and at times relatively easy, opportunity for economically motivated fraudulent activities to occur and be undetected. This means product adulteration, replacing a higher quality, original material with one of lesser quality, or extending a product by adding an adulterant and product mislabeling, including misrepresenting product origin and ingredients. Collectively, this affects consumer confidence, product brand reputation and may have a positive, or negative, impact on manufacturer revenue, depending on the source of adulteration. Consequently, there is an increase in retailer and consumer demand to see proof that food and beverage products are what the label claims them to be, including origin, authenticity and ingredient verification.

Legislation has been enacted globally to protect food and beverage products with respect to production processes and product labelling, either at the country level or cross-country level, such as in the European Union. For example, certifications on production practice and geographical origin add value to products, such as Protected Designation of Origin (PDO), Protected Geographical Indication (PGI) and Traditional Specialty Designation (TSG). The combination of legislation and food fraud practices demands a reliable analytical technique that can verify the origin, authenticity and label claims of food and beverage products.

Table 1. Isotope fingerprints in food and beverage products.

Isotope fingerprint	What is the biogeochemical interpretation?	What is an example of food fraud interpretation?	What products can be affected?
Carbon	Botanical origin (C_3, C_4 and CAM photosynthesis)	Adulteration (e.g. sweetening with cheap sugar)	Honey, liquor, wine, olive oil, butter, flavors
Nitrogen	Soil processes, plant fertilizer processes	Mislabeling (Differentiate organic and non-organic)	Fruits and vegetables, animal meat
Sulfur	Local soil conditions, proximity to shoreline	Origin of product	Fruits and vegetables, animal meat, honey
Oxygen	Principally related to local-regional rainfall and hence geographical area	Watering of beverages, place of origin of product	Coffee, wine, liquor, water, sugar, animal meat, flavors
Hydrogen	Related to local-regional rainfall and hence geographical area	Watering of beverages, origin of product	Coffee, wine, liquor, water, sugar, animal meat, flavors

Analytical solution: detecting isotope fingerprints

Using isotope fingerprints, food and beverage origin, authenticity and product label claims can be verified in a unique way. Isotope Ratio Mass Spectrometry (IRMS) works by detecting the "isotope fingerprint" of a sample, a unique chemical signature that changes from sample to sample. There are a number of approaches to preparing food samples for isotope analysis, however, the fundamental process for IRMS is the conversion of a solid or liquid sample to a gas under high temperature. In the case of EA-IRMS and GC-IRMS the conversion of the sample to a gas is performed by two processes: combustion and pyrolysis. Combustion, burning the sample at around 1000 ˚C with oxygen, is used to evolve carbon, nitrogen and sulfur from the sample in the form of N_2, CO_2 and SO_2. Pyrolysis, breaking down the sample at 1400 ˚C in a reductive environment, is used to evolve hydrogen and oxygen from the sample, in the form of H_2 and CO. After the gases are produced, they are transferred in helium carrier gas to a detector that measures the isotope fingerprint of the sample.

Food and beverage samples can be introduced into the Isotope Ratio Mass Spectrometer and analyzed for their isotope fingerprint via various analytical peripherals, for example an elemental analyzer or using a gas or a liquid chromatography interface.

The dedicated solutions of the Thermo Fisher Scientific™ isotope fingerprinting portfolio are designed to offer different capabilities and performances, with dedicated features for the coupling to the Thermo Scientific™ IRMS Systems, according to the varying analytical needs of modern laboratories working for routine and research applications:

- the Thermo Scientific™ EA IsoLink™ IRMS System, for analysis of bulk samples
- the Thermo Scientific™ GC IsoLink II™ IRMS System, for analyzing volatile compounds within a sample
- the Thermo Scientific™ LC IsoLink™ IRMS System, for analyzing polar compounds within a sample
- the Thermo Scientific™ GasBench II System, for the analysis of gas samples from beverages

For more information read SN30410

The Isotope Fingerprints and What They Tell Us for Food & Beverage

For more information read the infographic IN30475

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

^{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

^{15}Nitrogen
Interprets: Soil processes, plant fertilizer processes
Identifies: Mislabeling (organic vs. non-organic)
Foods Affected: Fruits, vegetables and animal meat

^{34}Sulfur
Interprets: Local soil conditions, proximity to shoreline
Identifies: Product origin
Foods Affected: Fruits, vegetables, animal meat and honey

^{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

Detecting ^{13}Clues, tracking ^{18}Origin, unraveling ^{2}History with isotope fingerprints

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Official methods for food and beverage product origin, authenticity and label claims

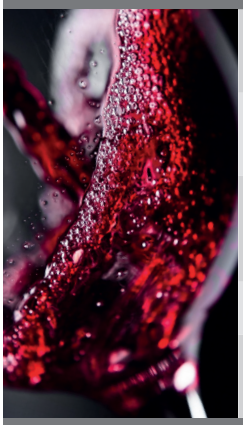
Standardized methods (or official international methods) exist for stable isotope analysis of food and beverage samples and are aimed at protecting product origin, authenticity and label claims. These official methods, alongside the analytical solutions, are listed in Table 2.

These methods have been formalized to create standardized approaches to sample analysis between laboratories, allowing laboratories to obtain conclusive answers for consumers, manufactures and governmental bodies, pursuing food and beverage adulteration and fraud.

Food and beverage products have a fingerprint, a unique chemical signature that allows the product to be identified. To visualize this fingerprint, Isotope Ratio Mass Spectrometry (IRMS) can be used, which identifies the isotope fingerprint of the product. The isotope fingerprint in food and beverage products is region or process specific (Table 2), which means that products can be differentiated based on geographical region (cheese, coffee, sugar, fish and animal feeding areas, coffee, sugar), botanical processes (beans, seeds, olive oil, vanilla), soil and fertilization processes (fruits and vegetables) and fraudulent practices (sugar addition to honey, watering of wines and spirits). These processes can be traced using carbon, nitrogen, sulfur, oxygen and hydrogen isotopes, with their variations indicating the origin and history of food and beverage products.

For more information read SN30414

Table 2. Official methods for isotope analysis using isotope ratio mass spectrometry.

Product	Official method	Isotope fingerprint	Sample	What does it address?	Analytical solution
Wine					
	OIV-MA-AS2-12	$\delta^{18}O$	Water	Adulteration, Geographical origin, Year of vintage	Thermo Scientific™ GasBench II System, Thermo Scientific™ Dual Inlet
	OIV-MA-AS312-06	$\delta^{13}C$	Ethanol, Wine must, Grape sugar	Adulteration, origin	Thermo Scientific™ EA IsoLink™ IRMS System, Thermo Scientific™ GC IsoLink II™ Interface for GC-IRMS
	OIV-AS312-07	$\delta^{13}C$	Glycerol in wines	Adulteration by addition of glycerol from C_4 maize or Fossil sources	GC IsoLink II Interface for GC-IRMS, Thermo Scientific™ LC IsoLink™ Interface for IRM-LC/MS
	OIV-OENO 510-2013	$\delta^{13}C$	Acetic acid in wine, vinegar	Adulteration	GC IsoLink II Interface for GC-IRMS, EA IsoLink IRMS System
	OIV-OENO 510-2013	$\delta^{18}O$	Water in wine, vinegar	Adulteration, Geographical Origin, Year of Vintage	Thermo Scientific™ GasBench II System, Dual Inlet
Sparkling wine					
	OIV-MA-AS314-03	$\delta^{13}C$	CO_2 in sparkling wine	Origin and authenticity of sparkling wine	GasBench II System, EA IsoLink IRMS System, GC IsoLink, Dual Inlet
Spirits					
	OIV-AS312-07	$\delta^{13}C$	Glycerol in spirits	Adulteration by addition of glycerol from C_4 maize or Fossil sources	GC IsoLink II Interface for GC-IRMS, LC IsoLink Interface for IRM-LC/MS
Fruit Juice					
	EU – CEN 1995	$\delta^{13}C$	Sugars	Adulteration	GasBench II System, LC IsoLink Interface for IRM-LC/MS, GC IsoLink II Interface
	USA – AOAC 1981	$\delta^{13}C$	Sugars	Adulteration	GasBench II System, LC IsoLink Interface for IRM-LC/MS, GC IsoLink II Interface
	EU – CEN 1998	$\delta^{13}C$	Sugars and pulp	Adulteration	GasBench II System, LC IsoLink Interface for IRM-LC/MS, GC IsoLink II Interface
	EU – CEN 1995	$\delta^{2}H$ and $\delta^{18}O$	Water	Adulteration	GasBench II System, LC IsoLink Interface for IRM-LC/MS, GC IsoLink II Interface
	AOAC method 2004.01	$\delta^{13}C$	Ethanol (From Fermentation)	Adulteration	GasBench II System, LC IsoLink Interface for IRM-LC/MS, GC IsoLink II Interface
Fruit Juice (Concentrate)					
	AOAC 1992	$\delta^{18}O$	Water	Adulteration	GasBench II System, LC IsoLink Interface for IRM-LC/MS, EA IsoLink IRMS System
Honey					
	AOAC method 991.41	$\delta^{13}C$	C-4 plant sugars at concentration >7%	Adulteration of honey	EA IsoLink IRMS System
	AOAC method 998.12	$\delta^{13}C$	C-4 plant sugars at concentration >7%	Adulteration of honey	EA IsoLink IRMS System
Cheese					
	EU Reg 548/2011	$\delta^{13}C$	PDO	PDO Grana Padano	EA IsoLink IRMS System

Application contents

For more information read AB30461

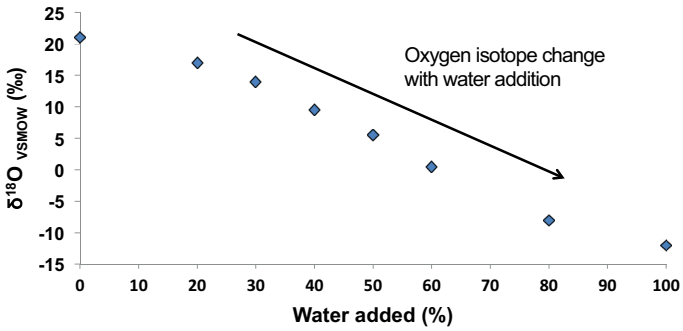
Tracking wine adulteration using isotope fingerprints

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. One example is the addition of exogenous sugar to wines during the fermentation process to artificially increase the alcohol grade, a process known as “chaptalisation”. Adulterated wine is then labeled as the original product, generally an expensive brand, and sold on the market as if the original product. It also relates to the re-labeling of wines, by adding the label of a more expensive wine to a bottle of a different, cheaper version and selling it on the market as an original product. In the European Union, for example, European Commission Regulation (EC) No 607/2009 regulates the origin and labelling of wine, with bilateral agreements in place with Australian, Mexico, Chile, USA, Croatia, Switzerland, amongst others.

The isotope fingerprint of wine

Oxygen and hydrogen isotope fingerprints can be used to identify the geographical origin of wine. The grapes, from which wine is produced, carry a fingerprint derived from local-regional rainfall, but that can also be influenced by cultivation practices, soil processes and geological characteristics of the local area, altitude and proximity to the shoreline. Oxygen and hydrogen isotope fingerprints change in rainfall as you move further inland from the shoreline and with increasing altitude because heavier isotopes are released from the clouds first, meaning heavier isotopes are closer to the coast line compared to further inland.

The carbon isotope fingerprint ($\delta^{13}C$) of plants are different because of photosynthetic processes and broadly grouped as C_3, C_4 and CAM plant types. C_3 plants utilize the Calvin photosynthetic pathway to fix CO_2. C_4 plants utilize the Hatch-Slack photosynthetic pathway and CAM by Crassulacean Acid Metabolism. Therefore, C_3 plants have a carbon isotope fingerprint between -33‰ to -22‰, C_4 plants a carbon isotope fingerprint between-16‰ to -8‰. And CAM plants between -20‰ to -10‰.



Oxygen isotope fingerprints detect watering of wine.

The correct labeling of wine 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, oxygen and hydrogen isotope fingerprint of wine allows the identification of water addition in commercial wine, i.e. adulteration. This helps protect producer reputation and consumer confidence by detecting fraudulent activity and supports (EC) No 606/2009.

For more information read AB30399

Detecting organic grown vegetables

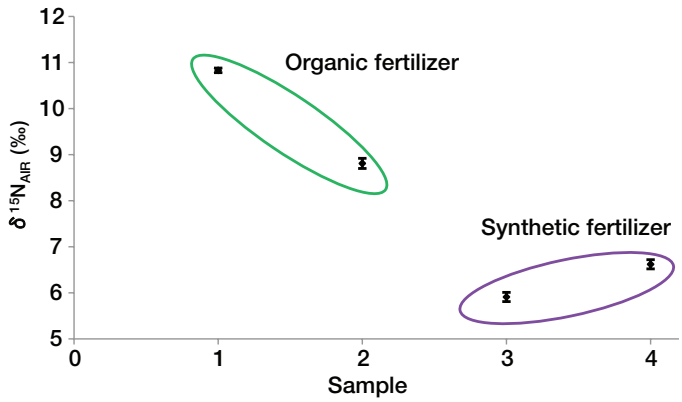
Organic grown products, such as fruits and vegetables, attract higher prices on the market because they are healthier and safer for consumers and the farming practices are cleaner and more environmentally friendly. The higher consumer cost is directly related to greater costs in growth and production of organic fruits and vegetables, and in the certification process that allows produce to be labeled and certified as organic. The certification process follows a set of standards (EC Council Regulation No 834/2007) and excludes the use of synthetic fertilizer during plant growth.

As organic fruit and vegetables attract a higher price on the market, this can lead to economically motivated fraud through mislabeling produce as “organic” when they have been grown using synthetic fertilizer. The identification of mislabeled fruit and vegetables represents a challenge as laboratories need a technique that identifies fruits and vegetables grown using organic fertilizers and synthetic fertilizers with full confidence in results. The identification of mislabeled products subsequently protects consumer confidence, brand market reputation related revenue-generating capabilities.

The nitrogen isotope fingerprint of vegetables

The nitrogen isotope fingerprints of vegetables are used to differentiate whether the fertilizer used for plant growth was organic or synthetic. Vegetables grown using organic fertilizers, such as peat, sewage sludge and animal manure, tend to have nitrogen isotope values between +8‰ to +20‰.Vegetables grown using synthetic fertilizers, such as potash and ammonia, tend to have nitrogen isotope values of +3‰ to +6‰.This differentiation provides a framework to detect vegetables grown using organic or synthetic fertilizers thanks to a strong ^{15}N isotope resulting from ammonia volatilization, denitrification, nitrification and other N transformation processes prior to plant uptake

In the graph, it can be seen that the tomatoes grown using organic fertilizer can be differentiated from tomatoes grown using synthetic fertilizer using nitrogen isotope fingerprints. This illustrates the potential of a simple tool for verifying label claims associated with organic fruit and vegetables.



Nitrogen isotope fingerprints detect organic grown tomatoes.

For more information read AB30477

Detecting purity and adulteration of tequila with isotope fingerprints

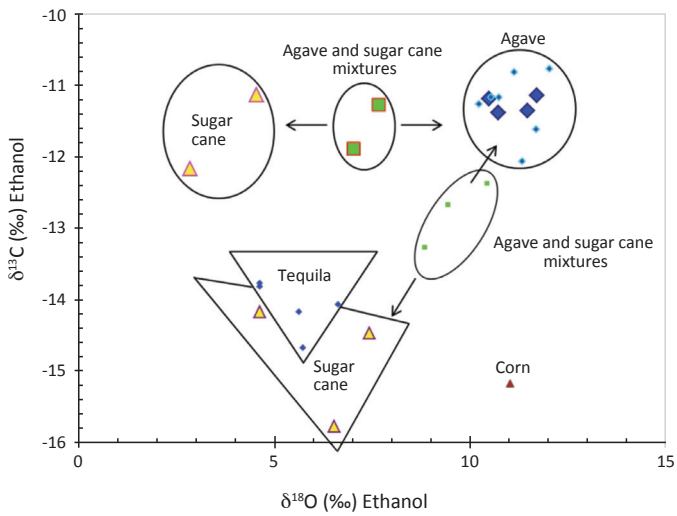
The blue agave (*Agave tequilana* Weber var. Azul) is a native plant of the Jalisco region, Mexico and is an important economic product that, by law, is the only one allowed to be used in the production of tequila. Globally, tequila is a popular alcoholic beverage, which has led to increasing demand and thus production, with a subsequent increase in export value to the Mexican economy. This provides an opportunity of economically motivated fraud either by adulteration and mislabeling of original tequila or production of fake tequila.

Tequila can come in two broad varieties: pure tequila, derived 100% from *A. tequilana*, or mixed tequila, deriving from *A. tequilana* with up to 49% sugar cane addition. Tequila is protected under the North American Free Trade Agreement (NAFTA) and local bilateral trade agreements, alongside regulations to combat fraudulent activities, such as the European Union Regulation (EC) 110/2008 (including a 2016 application pursuant to tequila).

The isotope fingerprints of Agave tequilana and Mexican rainfall

Photosynthetically, *A. tequilana* is part of the CAM plant group, meaning it has a well-defined carbon isotope fingerprint of -12‰ to -14‰. During plant growth, the water that comes principally from rainfall (evaporation, sublimation, condensation and precipitation in the water cycle) is used.

Tequila is produced exclusively in 5 areas of Mexico: Jalisco, Nayarit, Michoacán, Guanajuato and Tamaulipas, meaning that the oxygen isotope fingerprint of the *A. tequilana* plant, and of the local sugars used in mixed tequilas is primarily given by the rainfall water in those regions and therefore can provide a geographical tool for origin. The carbon and oxygen isotope fingerprint plot allows differentiating the original branded mixed tequila from *A. tequilana* and sources of sugar (corn and cane). This indicates that mixed tequila can be clearly differentiated from pure tequila.



Carbon and oxygen isotope fingerprints of tequila.

For more information read AN30427

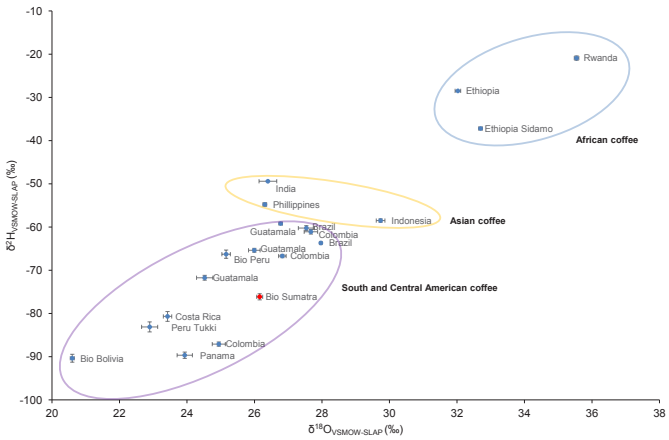
Tracing the geographical origin of coffee using isotope fingerprints

Coffee is one the most popular beverages worldwide. Coffee from different geographical regions are imported through a commercial chain that usually involves several intermediates, presenting significant, and at times relatively easy, opportunity for economically motivated fraudulent activity. To ensure that coffee beans come from their labelled locations, laboratories need an analytical tool for geographical origin discrimination with a special emphasis on the country of origin. 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. Investigating food origin and authenticity in laboratories is one of the key ways of monitoring and enforcing legislation for food integrity and labelling (EC Council Regulation No 1169/2011) and protecting consumers and brands.

Hydrogen and oxygen isotopic fingerprints of coffee

The hydrogen and oxygen isotope fingerprints in coffee beans can be used to differentiate their geographical origin. The Coffea plants, from which coffee beans are cultivated, carry a local-regional fingerprint primarily associated with local and regional rainfall, but can also be influenced by cultivation practices, soil processes and geological characteristics of the local area, altitude and proximity to the shoreline.

This effect can be tracked in the oxygen and hydrogen isotopic fingerprints of plants and their fruits (e.g. green coffee beans). The graph shows hydrogen and oxygen isotope fingerprints of coffee beans indicating they can be clearly differentiated at the continent scale. Additionally, the Bio Sumatra coffee measured is grouped with coffee from South and Central America rather than from Asia (red marker), illustrating that mislabeling can be identified.



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

Testing sugar package label claims using carbon isotope fingerprints

Sugar is primarily refined from Saccharum spp. (sugar cane), which grows above the ground under tropical climates, and Beta vulgaris (sugar beet), which grows underground under temperate climates. The refining process for beet is simpler and faster than for cane. Furthermore, beet can grow in a variety of climates beyond tropical regions and thus can be sourced locally. Consequently, beet sugar is cheaper to manufacture and source. This economic consideration may lead to fraud with the mislabeling of beet sugar. Testing the accuracy of product label claims is one of the key ways of monitoring and enforcing legislation on food product labelling (EC Reg. No. 1169/2011 and FDA-2012-N-1210). The identification of mislabeled products subsequently protects consumer confidence, brand market reputation and related revenue-generating capabilities.

Carbon Isotope fingerprints of sugar

Sugar has a fingerprint, a unique chemical signature that allows it to be identified. The carbon isotope fingerprint ($\delta^{13}C$) of plants are different because of photosynthetic processes and broadly grouped as C_3 and C_4 plant types. C_3 plants like Beta vulgaris, cultivated as the source of beet sugar, utilize the Calvin photosynthetic pathway to fix CO_2 and incorporate less ^{13}C than other plants. C_4 plants, like Saccharum spp., cultivated as the source of cane sugar, utilize the Hatch-Slack photosynthetic pathway which does not fractionate atmospheric carbon dioxide to the same extent as the Calvin pathway. Therefore, C_3 plants have a carbon isotope fingerprint between -33‰ to -22‰ and C_4 plants have a carbon isotope fingerprint between -16‰ to -8‰. In this study, 28 sugar packages from 25 countries were analyzed to verify the accuracy of package label claims using carbon isotope fingerprints. Although some of the sugars analyzed did not have a label claim, the differences in their carbon isotope fingerprints enabled us to distinguish between cane sugar and beet sugar (Table below).

Carbon isotope fingerprint of sugar.

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**

For more information read AB30572

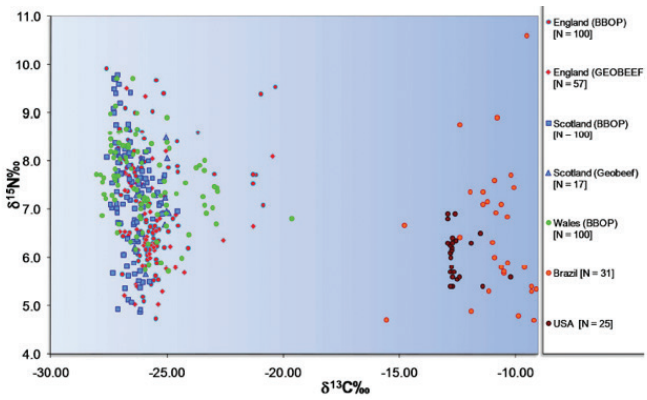
Tracing the origin of beef based on diet using carbon isotope fingerprints

The introduction of pan-European compulsory beef labelling rules, from the 1st September 2000 onwards was designed to provide consumers with correct, complete and transparent information to enable them to make an informed choice on the type and origin of beef they purchased (Council Regulation (EC) No. 2772/1999). As a consequence of this legislation it is reasonable to suggest that there should be analytical methods in place that can verify the information provided on origin labels describing where an animal has been reared.

Carbon isotope fingerprints in beef

The origin of beef can be tracked using the carbon isotope fingerprint which is related to the photosynthetic signature of the plants consumed by the animals during their grazing. To identify beef of UK origin relative to beef of Brazilian origin, this can be readily differentiated using carbon isotope fingerprints. The carbon isotope fingerprint ($\delta^{13}C$) of plants are different because of photosynthetic processes and broadly grouped as C_3, C_4 and CAM plant types. C_3 plants utilize the Calvin photosynthetic pathway to fix CO_2. C_4 plants utilize the Hatch-Slack photosynthetic pathway and CAM by Crassulacean Acid Metabolism. Therefore, C_3 plants have a carbon isotope fingerprint between -33‰ to -22‰, C_4 plants a carbon isotope fingerprint between-16‰ to -8‰, and CAM plants between -20‰ to -10‰.

Cattle in the UK and northern Europe are reared on pastures with C_3 plant types whilst in Brazil and USA they are reared on pastures with C_4 plant types. As a result, the animal meat carries the dietary carbon isotope fingerprint. The nitrogen isotope fingerprint can further differentiate by tracking differences in plant fertilization and also pastures with leguminous plants. The Figure clearly shows the differentiation, for example, between beef sourced in the UK and North and South America.



Carbon isotope fingerprints of beef from S. America and Europe.

For more information read AB30177

Detection of honey adulteration using isotope fingerprints

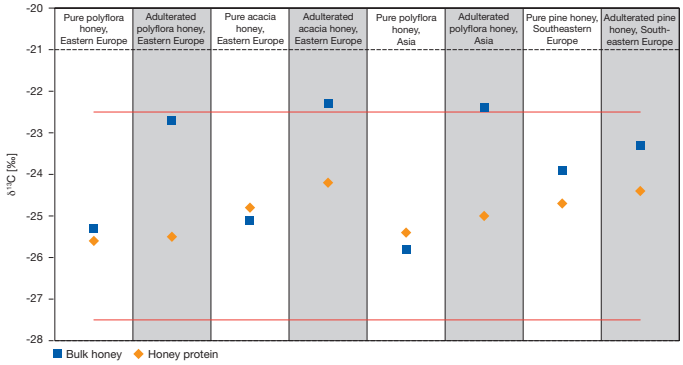
Honey is subject to fraud by adulteration with low price sugar syrups. Saccharides in syrups derived from cane, corn or beet sugar are difficult to distinguish from those in pure honeys. Sugar cane and corn syrups, the most widely used adulterants, have distinctive ^{13}C isotope fingerprints because both sugar cane and corn plants use the C_4 photosynthetic pathway in contrast to most honey which is derived from plants that use the C_3 photosynthetic pathway. These differences in ^{13}C isotopic composition allow detection of > 7% addition of such sugars.

Carbon Isotope fingerprint of honey

The isotope fingerprint of honey Carbon isotope fingerprints can be used to identify adulteration of honey that results from the addition of exogenous sugars. The carbon isotope fingerprint ($\delta^{13}C$) of plants are different because of photosynthetic processes and broadly grouped as C_3, C_4 and CAM plant types. C_3 plants utilize the Calvin photosynthetic pathway to fix CO_2. C_4 plants utilize the Hatch-Slack photosynthetic pathway and CAM by Crassulacean Acid Metabolism. Therefore, C_3 plants have a carbon isotope fingerprint between -33‰ to -22‰, C_4 plants a carbon isotope fingerprint between-16‰ to -8‰, and CAM plants between -20‰ to -10‰. The carbon isotope fingerprint of sugar and plants from which honey is derived, allows the identification of sugar addition in commercial honey, i.e. C_4-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 C_4-syrups according to the AOAC 998.12 guideline.

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



Carbon isotope fingerprints of honey and related proteins. Limit of detection due to natural variation: 7% C_4 sugar (agreed value). The red lines show the natural variation of $\delta^{13}C$ in honey.

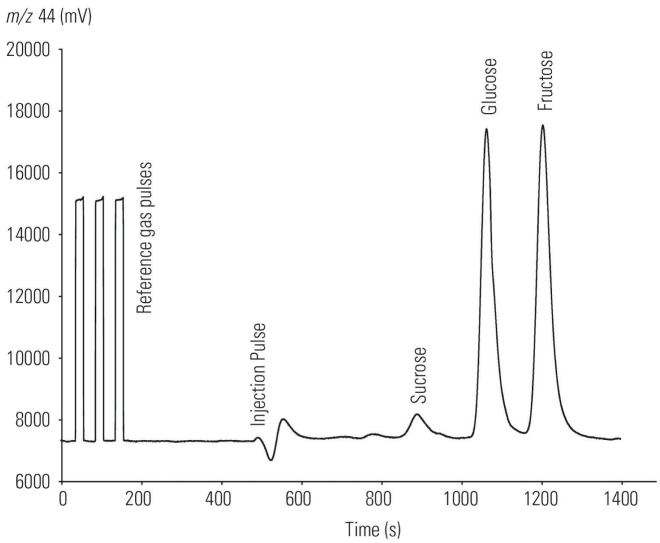
For more information read AN30024

Compound specific isotope analysis of honey

Carefully selected mixtures of sugars can mimic both, the bulk ^{13}C 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 (C_4 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 ^{13}C isotopic value of every individual sugar in honey. The comparison of the $\delta^{13}C$ 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.



Chromatographic separation of honey carbohydrates by LC-IRMS.

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**

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