Isotope fingerprints: origin of tequila with GC coupled with Isotope Ratio Mass Spectrometry

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ABSTRACT

Gas chromatography/isotope ratio mass spectrometry was used to measure carbon and oxygen isotope ratios from commercial tequila, sugar cane and the Agave tequilana plant to demonstrate how isotope fingerprints can identify Tequila adulteration. The results show that mixed tequila can be differentiated from pure tequila, which derives 100% from A. tequilana. Furthermore, isotope fingerprints differentiate sugar sources in mixed tequila.

INTRODUCTION

The blue agave (Agave tequilana Weber var. Azul) is a native plant of the Jalisco region in Mexico and is an important economic product that, by law, is the only one allowed to be used in the production of tequila (Figure 1). Tequila is produced from the fermented and distilled juices of the A. tequilana (Figure 2), which is cultivated for 6 – 12 years before being harvested. The raw material used for ethanol production comes from the central part of A. tequilana plant (piña). Piñas are cut, slowly baked and milled to extract the sweet juice. The subsequent fermentation determines the purity of the final product.

Globally, tequila is a popular alcoholic beverage, which has resulted in increased demand and thus production, with a subsequent increase in export value to the Mexican economy. This provides for 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[3,4]. 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).

Figure 1. Varieties of tequila.

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. These stable isotope data can subsequently be interpreted to determine the origin, correct-labeling and trace adulteration of food and beverage products, as summarized in Table 1.

With respect to Tequila, photosynthetically, A. tequilana is part of the CAM plant group, meaning it has a well-defined carbon isotope fingerprint of -12‰ to -14‰1. During plant growth, the biosynthesis of organic molecules in plants requires water that comes principally from rainfall (evaporation, sublimation, condensation and precipitation in the water cycle). Tequila is produced exclusively in 5 areas of Mexico: Jalisco, Nayarit, Michoacan, Guanajuato and Tamaulipas, meaning that the oxygen isotope fingerprint of the A. tequilana plant, and local sugars used in mixed tequilas, is primarily given by the rainfall water in those regions[2] and therefore can provide a geographical tool for origin[3,4].

Table 1. Stable isotopes and their interpretation in food and beverage origin and authenticity.

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 hence geographical area	Watering of beverages, place of origin of product	Coffee, wine, liquor, water, sugar, animal meat
Hydrogen	Related to local-regional rainfall and hence geographical area	Watering of beverages, origin of product	Coffee, wine, liquor, water, sugar, animal meat

Figure 2. Agave tequilana cultivation.

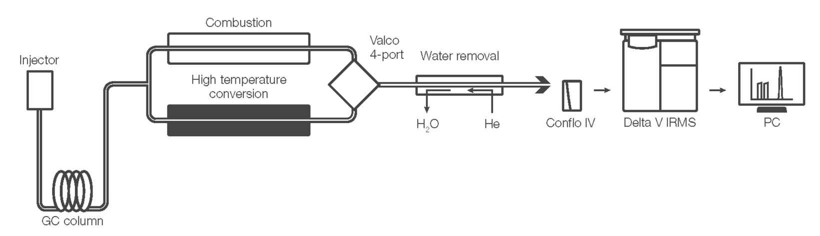
MATERIAL AND METHODS

All measurements can be performed using a Thermo Scientific™ TRACE™ 1310 GC coupled with a GC-IRMS system consisting of a Thermo Scientific™ GC IsoLink II™ IRMS System, Thermo Scientific™ ConFlo IV™ Universal Interface and a Thermo Scientific™ DELTA V™ Isotope Ratio Mass Spectrometer (Figure 3).

Figure 3. GC IsoLink IRMS System.

The GC IsoLink II IRMS System Conversion Unit incorporates a capillary design system built on high temperature combustion and high temperature conversion technology (Figure 4). This ensures complete conversion of compounds to simple gases. CO_2 provided by combustion imprints ^{13}C signature, where as CO provided by conversion imprints ^{18}O signature of compounds. Effluent from the GC passes through a micro channel device coupling GC with reactors and a temperature-controlled backflush system. True capillary design approach attains sharper peak shape and higher sensitivity. The GC IsoLink II IRMS System in combination with the TRACE 1300 Series GC, an extremely fast and easy to use GC, provides fully automated solution to meet the analytical challenges of isotope analysis.

Figure 4. GC-IRMS system schematics.



For measurement, 100 µL of the sample liquid was transferred into a 2 mL sample vial. Sampling was accomplished by transferring an aliquot of the headspace into the split/splitless injector of the GC by gastight syringe. The Thermo Scientific™ TriPlus™ RSH Autosampler utilizes robotic sample handling to advance sample handling cycles. Ethanol is the major compound dissolved and equilibrated in the headspace of the sample vial and was purified by gas chromatography using a thin layer GC column. System setup was adjusted as described in Table 1 and 2.

Table 1. GC conditions.

Trace 1310 GC Inlet Parameters	
Injection Volume	100 µL
Liner	Split Straight Liner, Deactivated
Injector	180 °C
Injector Module and Mode	SSL, split
Carrier Gas	Helium
Oven Temperature Program	
Initial Temperature	110 °C
Hold Time	2 min
Rate 1	5.0 °C/min
Temperature 1	140 °C
Hold Time 1	0 min
Initial Temperature	110 °C

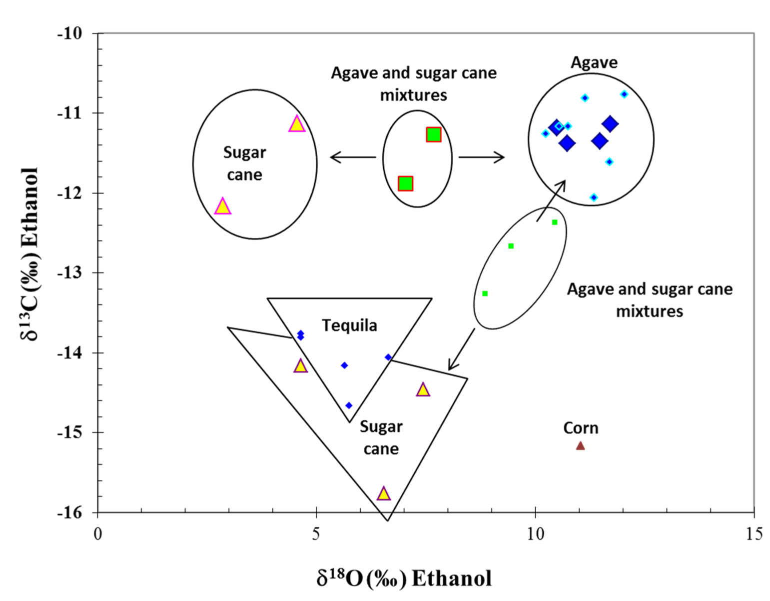
Table 2. Isotope Ratio MS Conditions.

GC-IRMS Parameters for $^{13}C/^{12}C$ Combustion Analysis	
Combustion Reactor	Ceramic tube, ID 0.5 mm with NiO, CuO and Pt wiring
Reactor Temperature	950 °C
GC Flow	1.8 mL/min
Split Ratio	30:1
Injection Volume	40 µL
GC-IRMS Parameters for $^{18}O/^{16}O$ HTC Analysis	
HTC Reactor	Ceramic tube, ID 0.5 mm with Pt shielding, Ni wiring
Reactor Temperature	1280 °C
GC Flow	1.8 mL/min
Split Ratio	15:1
Injection Volume	100 µL

RESULTS

All measurements can be performed using a Thermo Scientific™ TRACE™ 1310 GC coupled with a GC-IRMS system consisting of a Thermo Scientific™ GC IsoLink II™, Thermo Scientific™ ConFlo IV™ Universal Interface and a Thermo Scientific™ DELTA V™ Isotope Ratio Mass Spectrometer (Figure 3).

Figure 6. Carbon and oxygen isotope fingerprints of tequila.



The data in Figure 6 show measurements for mixed tequila, the A. tequilana plant and pure sugar cane. The carbon and oxygen isotope fingerprint plot allows 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, which derives 100% from A. tequilana. In addition, it also shows the difference between A. tequilana, original mixed tequila and sugar sources, meaning that adulterated and mislabeled tequila can be differentiated from original tequila and original source ingredients.

CONCLUSIONS

Isotope fingerprints provided by GC-IRMS differentiate tequila purity and can inform on adulteration and product mislabeling, upholding the regulations cited in the introduction. With the GC IsoLink IRMS System, laboratories gain an effective analytical solution based on the identification of the isotope fingerprint in beverage samples, which provides fast and reliable analysis with full automation.

REFERENCES

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- **AB30477**: GC-IRMS Detecting purity and adulteration of tequila with isotope fingerprints
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- **AB30418**: EA-IRMS Tracing the geographical origin of coffee using isotope fingerprints
- **AB30427**: EA-IRMS Tracing the geographical origin of green coffee beans using isotope fingerprints
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- **AB30424**: EA-IRMS Testing sugar package label claims using carbon isotope fingerprints
- **AN30048**: GB-IRMS Isotope analysis in Water, Fruit Juice and Wine
- **AN30052**: GC-IRMS Food labeling and FAME analysis
- **AN30024**: LC-IRMS $\delta^{13}C$ of Carbohydrates in Honey
- **AN30276**: EA-IRMS Detection of squalene from animal and vegetable sources

TRADEMARKS/LICENSING

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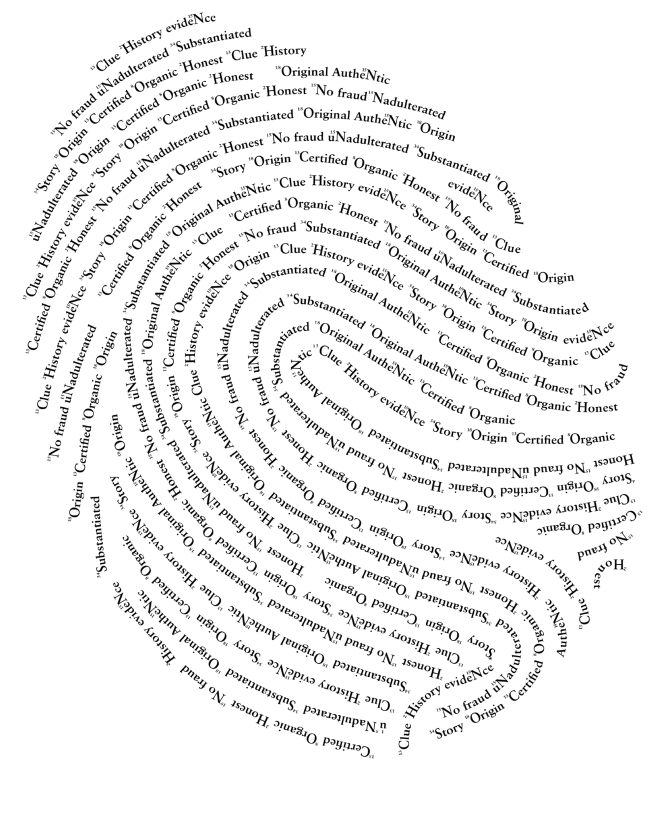

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