Isotope ratio MS

GC-MS-IRMS: Addressing authenticity of fish oils by carbon and hydrogen isotope fingerprints

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Abstract

As fish oils become a popular and precious source of omega-3 fatty acids, the risk of mislabelling and adulteration has risen significantly. The fatty acid profiles of different fish oils do not often allow the discrimination between different sources and geographical origins. In this study, the compound specific multi-isotope analysis of fatty acids in 30 salmon oils and 43 cod liver oils were analyzed allowing the discrimination of fish oils from different provenances, following risk-based comparisons from market experience. In the light of emerging cases of food fraud, we present how GC-MS-IRMS advanced technology can tackle these problems for addressing the authenticity of fish oils. By coupling GC-IRMS with an organic mass spectrometer (MS), isotopic compositions and comprehensive qualitative and quantitative sample information with high levels of selectivity, sensitivity, and confidence are accessible simultaneously from a single injection.

Introduction

Growing consumer awareness of the benefits of omega-3 fatty acids supplements for their health has caused significant growth in market demand for fish oils. This opens opportunities for economically motivated fraud and highlights the need for an analytical solution allowing robust assessment of fish oil authenticity. Fish oil label claims are focusing on differentiating species from which the oil originates, the geographical area of origin, sustainability practices and traceability. The ability to distinguish different regions of origin is especially challenging for fish oils from same species as there is no significant compositional difference that can be determined with traditional methods used in food integrity investigations. But these claims and processes can be traced using stable isotope fingerprints of carbon and hydrogen, with their variations indicating the origin and history of food and beverage products and ingredients.

Isotope fingerprints

Stable isotopes of carbon, nitrogen, sulfur, oxygen and hydrogen can be measured from food and beverage products such as oil, honey, cheese, animal meat, milk powder, vegetables, wine, liquor, water and so forth, using isotope ratio mass spectrometry techniques. This stable isotope data can be interpreted to verify the origin, correct labelling and adulteration of food and beverage products (Table 1).

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, oil, butter
Nitrogen	Fertilizer assimilation by plants	Mislabelling (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

Analytical configuration

All measurements were performed using a Thermo Scientific™ GC IsoLink II™ IRMS System (Figure 1), consisting of the Thermo Scientific™ TRACE™ 1310 GC, the Thermo Scientific™ GC IsoLink II™ Interface, the Thermo Scientific™ ConFlo IV™ Universal Interface and the Thermo Scientific™ DELTA Series Isotope Ratio Mass Spectrometer. The GC-IRMS system setup allows for automated software controlled switching between carbon and hydrogen isotope analysis of the sample.

Figure 1. Thermo Scientific GC IsoLink II IRMS System.

Sample preparation of the oils included a derivatization procedure using CH_3COCl in MeOH to gain Fatty Acid Methyl Esters (FAMEs). Sample volume of 1 µl was injected via liquid injection using the Thermo Scientific™ TriPlus™ RSH™ Autosampler. The separation of C14 to C22 FAMEs was achieved using a non-polar and a high polar column at the conditions described in Table 1. The GC-IRMS system has been coupled with the Thermo Scientific™ ISQ™ single quadrupole system for concomitant compounds identification. Combination of IRMS and quadrupole MS with a single TRACE 1310 GC provides vital complementary information. From just a single injection, the structure and isotope ratio of each compound can be determined (Figure 2).

Figure 2. GC-MS-IRMS System workflow

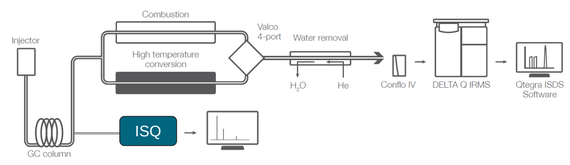


Table 1. GC conditions.

GC Inlet parameters		
GC column	Non-polar TG-5MS (30 m x 0.25 mm x 0.25µm)	High polar column (60 m x 0.32mm x 0.25µm)
Injection Volume (µL)	1	1
Injector ˚C	250	250
Carrier gas (mL/min)	Helium, 1.8	Helium, 1.8
Oven temperature program		
Initial temperature (˚C)	100	100
Hold time (min)	1	1
Rate 1 (˚C/min)	70	20
Temperature 1 (˚C)	180	180
Hold time 1 (min)	0	0
Rate 2 (˚C/min)	5	5
Temperature 2 (˚C)	330	215
Hold time 2 (min)	6	0
Rate 3 (˚C/min)	-	30
Temperature 3 (˚C)	-	250
Hold time 3 (min)	-	10

Results

In this study, 30 salmon oils and 43 cod liver oils were analyzed. Carbon isotope fingerprints ranged from -19,0‰ to -33,9‰ with STDEV of individual measurements <1,1‰ (n=3), and hydrogen isotope fingerprints ranged from -182‰ to – 282‰ with STDEV of individual measurements <3,9‰ (n=3).

The FAMEs of the respective fatty acids shown in Table 2 were analyzed and their carbon and hydrogen isotope ratios were determined. Statistical analysis of the isotope data allowed the selection of certain parameters for the subsequent statistical model (Table 3). These contributed to the discrimination of the given clusters for each model (salmon and cod liver oils).

Table 2. List of the fatty acids (as FAMEs) screened and analyzed by GC-C/P-IRMS.

Analyzed fatty acids	Parameters (20)
C14:0	$\delta^{13}C$, $\delta^{2}H$
C16:0	$\delta^{13}C$, $\delta^{2}H$
C16:1	$\delta^{13}C$, $\delta^{2}H$
C18:0	$\delta^{13}C$, $\delta^{2}H$
C18:1	$\delta^{13}C$, $\delta^{2}H$
C18:2	$\delta^{13}C$, $\delta^{2}H$
C20:1	$\delta^{13}C$, $\delta^{2}H$
C20:5	$\delta^{13}C$, $\delta^{2}H$
C22:1	$\delta^{13}C$, $\delta^{2}H$
C22:6	$\delta^{13}C$, $\delta^{2}H$

The parameters shown in Table 3 were statistically processed using Discriminant Analysis, in order to achieve the discrimination of the given clusters (different geographical origin). For better assessment of the discrimination potential, a third cluster was used in each case. In Discriminant Analysis, a number of principal components or functions are generated (Fx), integrating the information from the analytical parameters (carbon and hydrogen stable isotope ratios of the selected FAMEs, as shown in Table 3).

By using different Fxs, bivariate or multivariate illustrations are possible. Commonly, F1 and F2 represent the largest amount of the information (up to 100%), as depicted in Figures. 3 and 4. The statistical models generate also a cross-validation stage, where a prediction potential is estimated (as % of correct prediction).

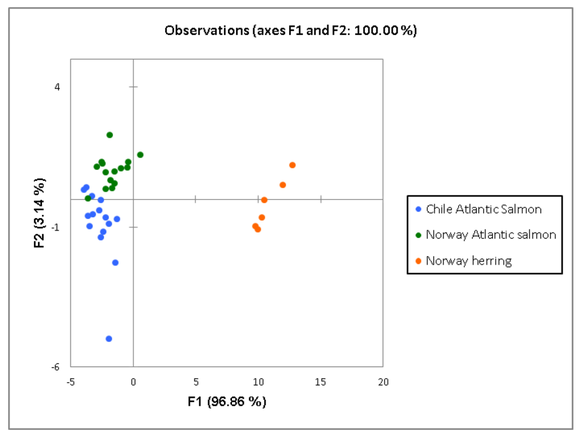
Table 3. List of the fatty acids (as FAMEs) used in the statistical model.

Fatty acids used in the model	Parameters used in the model (9)
C14:0	$\delta^{13}C$, $\delta^{2}H$
C16:0	$\delta^{13}C$, $\delta^{2}H$
C16:1	$\delta^{2}H$
C18:1	$\delta^{13}C$
C20:5	$\delta^{13}C$
C22:6	$\delta^{13}C$, $\delta^{2}H$

Salmon

Most salmon products come from either Norway or Chile, and the two have significant price differences and values. It is therefore crucial that the label claims of any fish oil supplement can be verified. Discriminant analysis gave a correct prediction of 94.29% (Figure 3), showing that the two regional products could be clearly determined.

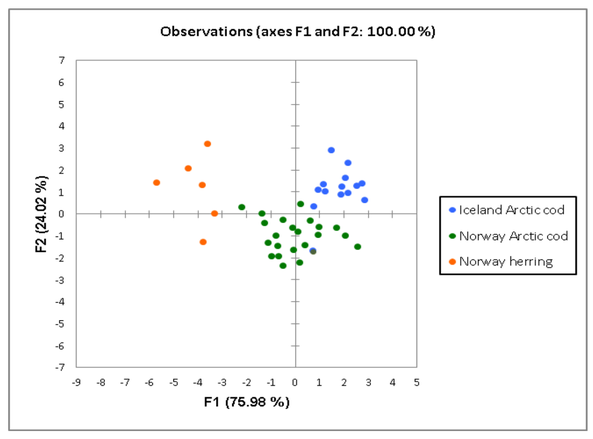
Figure 3. Discriminant analysis: Atlantic salmon (Norway) vs. Atlantic Salmon (Chile). Correct prediction: 94,29%



Cod

Similar to the salmon situation, Iceland and Norway have price discrepancies between products derived from each region's cod. However, the two countries are physically very close, meaning there may be less extreme differences between the two diets and habitats, and therefore more similarity between isotopic fingerprints. Despite the close proximity of the cod species, the multi-isotope method was able to discriminate the fish oil origin with a correct prediction of 97.22% (Figure 4). Based on this score, we can see the technique is highly accurate and reliable, making it a strong choice for fish oil determination.

Figure 4. Discriminant analysis: Arctic cod (Iceland) vs. Arctic cod (Norway). Correct prediction: 97,22%



Conclusions

GC-MS-IRMS is a powerful technique that can determine the origin of fish oil by elucidating structure and isotope ratio. The study here shows the potential of GC-MS-IRMS in verifying the geographical origin of matrices with emerging commercial value and high adulteration risks — and validates the method, demonstrating that the resulting data provides conclusive answers about fish oil origins. Crucially, the technique is suitable even for products deriving from geographic regions close to one another.

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