Verifying the origin of olive oil using Stable Isotope Ratio Analysis (SIRA)

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Known authenticity challenges in olive oils and other edible oils

There are many known challenges in the authenticity of olive oils, some are general to all oils and some are specific to olive oils. Isotope testing can directly help with some of these challenges and may be able to indirectly spot other issues. Here are a few examples:

- Dilution of raw input olives with olives from other origins
- Dilution of olive oils with olive oils from other origins/qualities
- Dilution of olive oil with other vegetable oils
- Dilution of olive oil with prohibited oils (e.g. waste oil, animal feed oil, mineral oil)

Dilution of olive oil with inputs from undeclared origins

It is well known that when poor harvests occur in certain countries, the demand for olive oil doesn’t drop. A poor harvest in early 2016 skyrocketed the price of olive oil. Regulators commonly report suspicions that producers are finding ways to ‘stretch’ what they currently have to meet demand or take advantage of the high price when the rest of the market is hampered by a poor harvest [European Commission and International Olive Council 2013].

One of the ways producers may be stretching out their olive supply is by simply buying more olives from outside of their normal appellation. Whereas a producer may be producing ‘Greek’, ‘Spanish’ or ‘Italian’ labelled oils, they may be using olives bought from outside the declared country. This has been detected in tests in conjunction with the German testing magazine Stiftung Warentest. Fig 1 shows three oils from the same producer but with different batch codes (best before dates, “MHD”). Isotope analysis of the three samples revealed that the input materials were not from consistent origins throughout the season. This evidence supports the speculation many have that at the start of a season when olives are plenty, there are unlikely to be issues with the authenticity of the oils. However, later in the season, fraudulent producers may be using olives from other regions to make their oils despite maintaining consistent geographic origin labelling.

As it is possible for some producers to blend oils together from different origins to control for flavour and quality, it is also suggested unscrupulous producers may be using this opportunity to dilute premium oils with lower grade olive oils (e.g. pomace oil) [Angerosa et al 1997]. As these lower grade oils are more likely than not to be of different origin, isotope testing can play a helpful role in identifying this issue. However, producers may also incorporate their own lower grade oils into their higher grade oils. This dilution can be more challenging to spot, if it is suspected then it can be in part controlled for by comparison across batches both at the product level and at the producer level. Chemical-specific isotope techniques may help more accurately resolve this issue even at low levels of pomace oil dilution (5%) [Angerosa et al 1997].
Figure 1. Graph showing the hydrogen isotope ratios (δD) and oxygen isotope ratios (δ¹⁸O) of three tested olive oils (Proben) from the same producer from different ‘Best before dates’ (MHD) in comparison to reference olive oils of known provenance.

Dilution of olive oils with non-allowed oils

Testing magazines such as Stiftung Warentest and regulators also report that cooking oils may be diluted with non-allowed oils or non-olive oils [European Commission and International Olive Council 2013]. The Taiwanese ‘gutter oil’ scandal of 2014 showed that oil producers were blending cooking oil with recycled waste oil [China post, 2014]. Though this scandal only included cooking oils from Taiwan it showed some of the types of fraud that can occur within the edible oils sector. Examples of non-allowed oils include:

- Non-olive vegetable oils
- Waste oil
- Mineral oil
- Animal feed oil
- Petroleum hydrocarbons

(Ref. [China Post 2014, Stiftung Warentest 2016])

Isotope testing focused on geographic origin is not able to directly identify these issues, however as these ingredients almost certainly have different isotope ratios from the legitimate products it should be possible to identify whether there are authenticity issues in olive oils. Further analysis by specialists may be able to identify each of these issues using other forensic methods (e.g. petroleum hydrocarbons can be identified in olive oil using ¹³C/¹²C and radiocarbon (¹⁴C) analysis).

Olive oil based cosmetics containing non-allowed products from sharks

Squalene and squalane are used extensively in cosmetics such as moisturisers. Squalene and squalane can be produced from both olive oil or, at lower cost, from deep-sea shark livers. Camin et al 2010 (2) identified this risk as an environmental concern and a method based on hydrogen and carbon isotope ratio analysis to distinguish olive oil from shark squalene/squalane has been developed. It should be noted that Agroisolab does not currently offer this test.
Solutions to authenticity issues

Random testing of retail products

Random testing programs allow for a different verification method to conventional traceability systems which are based on paper trails. By checking whether a product is or isn’t consistent with its declared origin can give insight into current traceability practices to improve them, can highlight other authenticity issues, and can give valuable evidence that supply chains are functioning as they should.

Agroisolab believes many of the risks in olive oil can be first detected using tests focusing on geographic origin. If there are clear issues with the geographic provenance of an oil, this normally calls for further investigation. However, if there are also concerns related to specific issues such as entry of petroleum hydrocarbons in olive oils Agroisolab can focus on these areas more specifically.

Archiving samples from producers

In order to ensure consistency of products from suppliers, particularly in high-risk products such as olive oil, Agroisolab recommends archiving samples from producers from each batch supplied. This significantly enhances origin testing work as it enables a 1:1 analysis in addition to verifying the general geographic origin claims made on the oil. In this situation, the product should match the reference sample with very high confidence.

Archiving helps overcome the following challenges:

- Authentication against broad origin claims (e.g. Italian) as the authentication can be made against the wider claim and the direct reference sample(s).
- Ensuring that consistent input materials are used to make the retail product
- Ensuring that the sample tested by quality managers/testers is the same product that reaches retail shelves

Labelling the product with the isotope signature

This interesting concept was brought forward by mass spectrometer manufacturer Isoprime as a promotional item. Nevertheless, there are producers interested in labelling their products with the isotope ratios to give their customers assurance of the provenance of their products.

If samples of oils were regularly tested from producers it should be possible to incorporate isotope testing results with other ‘typical value’ data included in the product packaging.
What are stable isotopes?

The stable isotope method exploits the fact bioelements (hydrogen, carbon, nitrogen, oxygen and sulfur) exist in two stable variants in natures/isotopes. For example, in addition to the “normal” nitrogen nature also has a heavy version of nitrogen (1 additional neutron). Due to various global processes, the ratios of light to heavy isotopes varies geographically. This subtle, but profound difference offers the opportunity to track produce.

Fig.2: ‘Light’ nitrogen and ‘heavy’ nitrogen. Heavy nitrogen is chemically identical to light nitrogen but has an extra mass-containing sub-atomic particle called a neutron.

Stable isotopes, by their very nomenclature, are ‘stable’. Stable isotopes do not decay. Bioelemental stable isotopes (hydrogen, carbon, nitrogen, oxygen and sulfur) are all products of fusion. On Earth the stable isotopes originated from a supernova event predating the current sun, their abundance has not changed significantly for the 4.5 billion years earth has existed for.

How is stable isotope analysis used in olive oil?

Multi-element Stable Isotope Ratio Analysis (SIRA) is a commonly used analytical tool to determine/verify the provenance of once-living materials such as timber, agricultural products and foodstuffs. Chiefly SIRA is used in ecology but is also used in monitoring climate change, biogeochemistry, forensics, anthropology, and trade regulation.

Today Agroisolab performs routine analysis determining the origin for: wine (European wine database EU 2729/2000.), Ivory (CITES, WWF [Ziegler et al 2016]), pork (BMPA, AHDB), Caviar (German customs), Timber (EUTR, Regulation (EU) No 995/2010), Eggs (KAT), as well as supporting labelling claims for various European retailer and producer groups.
Table 1. Bio-elemental isotope ratios analysed + expected major origin of the bioelement in olive oil

<table>
<thead>
<tr>
<th>Bioelement</th>
<th>Fraction analysed</th>
<th>Isotope ratios analysed</th>
<th>Main origin of bioelement in olive oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrogen</td>
<td>Lipids (Bulk analysis of the oil)</td>
<td>D/H</td>
<td>Water the olive tree takes from various water sources. Known to be influenced by the mean annual temperature of the location, distance the olive orchard is from the sea, and elevation [Camin et al 2010, Bontempo et al 2009]. Hydrogen/Deuterium from water is initially ‘fixed’ into sugars by photosynthesis, this is later converted into lipids for storage in olives.</td>
</tr>
<tr>
<td>Oxygen</td>
<td>Lipids (Bulk analysis of the oil)</td>
<td>$^{18}$O/$^{16}$O</td>
<td>Water the olive tree takes from various water sources. Known to be influenced by the mean annual temperature of the location, the distance the olive orchard is from the sea, and elevation [Camin et al 2010, Portarena et al 2015].</td>
</tr>
<tr>
<td>Carbon</td>
<td>Lipids (Bulk analysis of the oil)</td>
<td>$^{13}$C/$^{12}$C</td>
<td>Local atmospheric CO$_2$. This parameter reflects the local climate conditions the olive trees live in. Carbon ratios in olive oil are influenced by annual temperature and climatic conditions such as humidity [Angerosa et al 1997, Portarena et al 2014].</td>
</tr>
<tr>
<td>Sulfur</td>
<td>Organosulfur compounds (Bulk analysis of the oil)</td>
<td>$^{34}$S/$^{32}$S</td>
<td>Sulfur in olive oil mainly originates from sulfur containing compounds taken up by the olive trees that produce the olives. Sulfur isotope ratios are regarded as a ‘geological parameter’ as they reflect the local chemical cycling within the soil a plant is grown in [McCutchan et al 2003].</td>
</tr>
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Why do stable isotope ratios vary geographically?

In order for a geographic origin test to work, it is necessary for there to be a demonstrable link between a tested value (e.g. an isotope ratio) and a geographical location. The stronger the link between the tested attribute and geography, the more robust the test.

Water isotope ratios – hydrogen and oxygen

In the 1950’s research in the stable isotope field was particularly well funded. Harmon Craig in the 50s and 60s was one of the earliest scientists to discover the Global Meteoric Water Line (GMWL). He found that the isotope ratios of oxygen and hydrogen in ground water were correlated irrespective of geography, though had different values in different geographical locations. The Global Meteoric Water Line is an equation that describes the correlation of any particular hydrogen isotope ratio with an oxygen isotope ratio in ground water. The varying geographical patterns in water isotopes are the product of the global water cycle and other influencing factors. Hydrogen and oxygen isotope ratios in precipitation (rain) are primarily dependent on the annual temperature of the location [Dansgaard 1964] and, to a lesser degree, altitude, latitude, and the ‘continental effect’. This results in broad global variability in hydrogen and oxygen stable isotope ratios in groundwater.

Climate isotope ratios - carbon

Carbon ratios in biological matter are principally derived from plant matter. The carbon in organisms originates from the plants they consume. In plants carbon isotope ratios are primarily dictated by the photosynthetic pathway the plant uses (CAMS, C3 or C4) [O’Leary 1988], this aspect isn’t geographically distinct but can be exploited in other ways (e.g. calculating the % maize in the diet of an animal or determining whether vanilla is natural or synthetic). However, there is strong fractionation of carbon stable isotope ratios dependent on stomatal conductance and photosynthetic assimilation; both are influenced by environmental factors such as humidity, light...
and temperature which are ultimately tied to geography [Farquhar 1982]. It can be summarised that carbon isotope ratios reflect the local climate in plant products.

**Anthropogenic isotope ratios - nitrogen**

Nitrogen in plants is derived from the nitrogen immediately available to them. This can either be the nitrogen that exists within the ground in various compounds, nitrogen fixed from the air into bioavailable compounds by bacteria, nitrogen from animal waste naturally deposited, or nitrogen placed anthropogenically in the form of fertilisers. Since 1974, it is well known that organic fertilisers such as composts, manures and green manures show greater enrichment of heavy nitrogen (\(^{15}\text{N}\)) than conventional mineral fertilisers. Organic fertilisers typically have nitrogen ratios of +10%/-4 in comparison to mineral fertiliser which has nitrogen ratios of roughly 0% [Shearer 1974] (see Figure 3).

![Figure 3: \(^{15}\text{N}/^{14}\text{N}\) isotope ratios of various fertilisers, Bateman 2007](image)

The enrichment of heavy nitrogen (\(^{15}\text{N}\)) in biomass derived fertilisers (compost, manure, bonemeal) is the product of metabolism. Transaminase, an enzyme that exists in all organisms and is involved in protein/urea metabolism, prefers heavy nitrogen (\(^{15}\text{N}\)) as opposed to normal nitrogen (\(^{14}\text{N}\)). Further heavy nitrogen enrichment in manure-based fertilisers can be attributed to the preferential volatilisation of \(^{14}\text{N}\) ammonia [Sturm et al. 2011]. As a result, biomass-derived fertilisers are enriched in heavy nitrogen and can be easily differentiated from mineral fertilisers (figure 3).

Nitrogen ratios from the fertilisers are reflected in crops grown in the fertilised soil; if the soil was fertilised with biomass derived fertilisers (organic fertilisers) which show the enriched heavy nitrogen signature, this will be evident in the crop [Yoneyama 1990]. The reverse also applies, if a crop is fertilised with mineral fertiliser (\(^{15}\text{N}/^{14}\text{N} = 0\%\)) the nitrogen ratio of the crop will reflect in this signature too.

It should be noted that while nitrogen isotope ratios in olives can be tested, it is not possible to test them in olive oil as there are insufficient nitrogen-containing compounds to work with current extraction and analytical methods.
Geological isotope ratios – strontium, sulfur and nitrogen

Geologically dependent isotopes can vary within a country as they reflect the local geology of the soil and do not reflect the wide-scale patterns like water isotopes do. This is due to the fact that they primarily reflect local geological and chemical cycling.

As mentioned in the above section, nitrogen isotope ratios can function as a geological parameter, though this is usually overwritten in agriculture where the anthropogenic use of nitrogen has a greater significance than the nitrogen from the soil.

Sulfur and strontium isotope ratios can both reflect chemical cycling in the soil.

Trace elements

Trace elements reflect soil mineralogy [Kreitals 2013], due to this trace element concentration can vary significantly even over short distances. Consequently, when using trace elements to track the origin of a particular product, it is necessary to have a lot more samples mapped in order to adequately describe a geography. As it can often be challenging to obtain enough samples to make appropriate use of trace elements for origin tracking, more data-efficient methods such as stable isotope analysis are often preferred. Nevertheless, there are highly successful trace-element based tracking systems such as the Pig-Pass/Physi-Trace system in Australia [Watling et al 2010], the high sampling involved allows for farm-level traceability.

Chemical fingerprinting techniques

The analysis of the chemical fingerprint of olive oil with NIR (Near infrared), NMR, or Metabolomics are new applications which are still under development. Historically NIR measurement is mainly used to verify the quality of the oil [Mailer 2004], however, some publications have already demonstrated that the NIR analysis could be used to predict the origin as well [Galtier et al 2007].

One of the current known risks with chemical fingerprinting is that the variability of the measured parameters isn’t necessarily related to geographic origin. This is because the origin is calculated from an enormous dataset for each sample (>500 parameters). A good description of a chemical fingerprint based test is that it is a test of similarity between known reference samples. In that regard, it necessary to have a huge dataset of geographical samples in order to achieve a good discrimination. It is also a risk that chemical fingerprinting analyses may assign the wrong origin especially when a test sample has a new ‘unknown’ spectrum.

Sampling efficiency – how many reference samples are needed to describe an area?

It should be established that in order to develop a robust geographic origin test that is suited to verifying the declared origin of products, it is necessary to get a view of what the attributes of the product are like in the same origin as it. In order to do this, it is necessary to obtain samples from the same geographic origin as the product that is to be tested.

It is often questioned about how many samples are required to do this; unfortunately, there are no hard and fast rules with how many samples are needed from an area to describe it and re-trace products to it using analytical tools. The majority of the experience of how many samples are required to describe an area comes from research into weather/climate mapping [Holland, EPA 2004].
Principally the number of samples required to describe the vast majority of an area depends on:

- How variable the attribute being measured is
  - The more variable the attribute being measured, the more samples are required over a given area. For example trace element concentrations in most products are generally more variable than stable isotope ratios. More samples are required to adequately describe trace element variation but in return greater differentiation of origin can be achieved.

- The distance between the sample points (the aim should be to obtain samples from the evenest spread possible)

The geographic variance in the stable isotope ratios of olive oil

Agroisolab’s core database of olive oils consists of over 200 samples from Portugal, Spain, Italy and Greece. Samples were obtained by both Agroisolab and Dr Gertz of MaxFry GmbH.

![Hydrogen stable isotope ratios in European olive oils](image)

**Fig. 4** Hydrogen stable isotope ratios in European olive oils. Map created using Universal Kriging (Global) (sample n=226).

Hydrogen isotope ratios in olive oil originate from the rainwater the olive trees use to create sugar via photosynthesis. Sugars are subsequently metabolised into fatty acids and other lipid compounds and placed into the fruits of the olive tree. The above map shows the mean values of hydrogen isotope ratios in olive oils across Europe. ‘Red’ denotes that there is relatively more of the heavy isotope (hydrogen, 2H or ‘D’), ‘Blue’ denotes that there is relatively less heavy hydrogen. As hydrogen isotope ratios are dependent on temperature, latitude, altitude and the ‘continental effect’ it is no surprise that there is a similarity between some areas.

Nevertheless, the above map only describes the general trend in hydrogen isotope ratios across European olive oils, there is also local variance which can’t easily be displayed on the same map but can be exploited for provenancing using SIRA (see Fig. 7). Additionally, the colour scaling of the map only reflects 95% of the possible values and does not show extreme values, the same is also true for Fig. 5 and 6.
As hydrogen and oxygen are related to each other through groundwater as described by the GMWL, it can be expected that there will be relatively similar geographical patterns in oxygen stable isotope ratios and hydrogen stable isotope ratios. However, as oxygen ends up in lipid compounds (e.g. fatty acids, TAGs) in slightly different metabolic pathways to hydrogen there are also some differences. Whereas the previous map (Fig. 4) showed similarity between hydrogen isotope ratios in Portuguese olive oils and Cretan olive oils, the oxygen isotope ratios show clear differences between the two origins.

Carbon ratios reflect the local climate. As Southern European countries all share a similar ‘hot’ climate it is easy to see similarity on the above map. The opposite is also true, colder Northern regions have olive oils with more depleted carbon isotope ratios. This map shows a striking resemblance to maps of daylight hours per year in Europe (see appendix), recent research supports that factors relating to daylight hours cause the difference in carbon ratios in Mediterranean olive oils [Portarena et al 2014].
Regional level declarations provide greater granularity in determining origin

![Centroids](image)

**Fig. 7** Discriminant analysis (Hydrogen, Oxygen and Carbon stable isotope ratios) showing the centroids of European olive oils.

When evaluating the origin of olive oils labelled with just a country declaration, it is necessary to use broader evaluation criteria in order to encompass the variance of the isotope ratios within the country. However, if more detailed regional information is available, this allows for a narrowing of the evaluation criteria increasing the discrimination possibilities. Further discrimination between different regions may be achieved with the use of further parameters (e.g. Sulfur isotope ratio analysis, trace elements, chemical profiling).

**Sulfur isotope ratios – a key regional parameter**

Agroisolab is currently developing a method to extract sulfur from olive oil in order to perform sulfur isotope ratios analysis ($^{34}$S/$^{32}$S), as this parameter reflects local conditions the olives were grown in, it is anticipated that regional differentiation will improve with its inclusion.

Nevertheless, it should be noted that sulfur isotope analysis is, and likely will, remain challenging as the amount of sulfur in olive oil is tiny. Agroisolab is working on concentration tools to gain a reliable amount of sulfur from oil for routine analysis.
Further possibilities for analysis – strontium isotope ratios/strontium concentration

Fig. 8 Strontium isotope ratios in natural mineral waters across Europe. [Vorkelius S. et al 2010]

The basis for using strontium isotope ratio analysis to determine or verify the origin of olive oils is not strong. The map above (Fig. 8) shows that Strontium isotope ratios in natural mineral water across the olive oil producing regions of Europe are largely homogenous. On this basis, it is unlikely that strontium isotope ratios deliver much help in terms of geographical differentiation despite the high cost of analysis. Similar results have been found in other commodities such as Iroko timber across Africa, however, strontium concentration was found to be a useful parameter [Boner et al 2015].
Analytical challenges in olive oil analysis

Determination of the origin of olive oil is, and always will be, a challenge due to the nature of the product. Olive oil is often sold as a blended product. It is not uncommon to see olive oils labelled as ‘blend of EU olive oils’ or simply with only one country declaration e.g. ‘Italian’. Where products are blended it can sometimes be difficult to detect the entry of olive oils from undeclared origins.

Nevertheless, it is possible (Fig. 1) and is currently a routine analysis for Agroisolab. Other challenges include:

- Annual variation
- Harvest/processing influences
- Variation due to olive varieties (minor)
- Variation due to ageing effects (minor)

Most of these challenges can be overcome by obtaining samples more regularly from producers and controlling for them appropriately.

References:

30 WWF Germany. Blind Test Evaluation Report. 2015. ITTO-Project PD 620/11 M; Rev. 1
Fig. 9. Sunshine hours in Europe. Credit: Wikipedia user XL3