*AN029*

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# Geographical Analysis of Olive Oil Samples Originating from Eight Countries Using Picarro's Novel Simultaneous $^{13}C$ + D CM-CRDS Isotope Analyzer

A clear distinction in dual-isotopic ratio measurements of individual samples indicates the applicability of the new combustion analyzer to geographical origin verification and product authentication.

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**Keywords:**
Material: Olive oil
Process: Stable isotopes, $\delta^{13}C$ + $\delta D$
Instrument: $^{13}C$+D CM-CRDS Isotope Analyzer

**Abstract:**
Olive oil samples were analyzed using the world's first simultaneous $\delta^{13}C$ + $\delta D$ instrument, the Picarro $^{13}C$+D Combustion Module-Cavity Ring-Down Spectroscopy (CM-CRDS) Isotope Analyzer. Simultaneous measurements of $\delta^{13}C$ and $\delta D$ of the whole oil have been performed on commercially available olive oils produced in Greece, Spain, Italy, California, Lebanon, Israel, Australia and Turkey. Together, the isotopic ratios produce statistically significant differentiation between olive oils from different locations around the globe.

## Summary and Relevance:

The discerning power of the novel Picarro $^{13}C$+D CM-CRDS Isotope Analyzer vis-a-vis the separation of various olive oil samples based on their geographical origin was put to task. By using a dual-isotope analysis approach, simultaneous measurements of $\delta^{13}C$ and $\delta D$ of the whole oil have been performed on commercially available olive oils produced in Greece, Spain, Italy, California (USA), Lebanon, Israel, Australia and Turkey. Together, the isotopic ratios produce statistically significant differentiation between olive oils from different locations around the globe.

Stable isotope D/H ratio analysis of precipitation reveals, on average, a global geographic pattern that can be mapped to produce global precipitation isotope landscapes, or *isoscapes*[1]. The same is true of the $^{18}O/^{16}O$ isotope ratio. Because the hydrogen atoms incorporated into organic molecules during biosynthesis comes from the water available to the plant, plant tissues from different geographic locations should record isotopic variations in source water. Geographic variations in the hydrogen isotopes of water used during cultivation should therefore be reflected in the hydrogen isotopes of olive oil.

Similarly, carbon isotope ratios also record aspects of a plant's growth environment. Carbon in plant material reflects the isotopes of $CO_2$ incorporated during photosynthesis and the stomatal responsiveness to humidity in the growth environment. Thus carbon isotope ratios have the potential to indicate whether olive trees were grown in shady or sunny areas. In general, enzymatic fixation of $CO_2$ during

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photosynthesis discriminates against the heavier isotope of carbon ($^{13}C$), resulting in plant tissues with lower carbon isotope ratios than atmospheric $CO_2$.

In this application, olive oil samples were analyzed using the world's first simultaneous $\delta^{13}C$ + $\delta D$ instrument, the Picarro $^{13}C$+D Combustion Module-Cavity Ring-Down Spectroscopy (CM-CRDS) Isotope Analyzer, and the dual-isotope correlation have given a solid evidence that isotopic ratios from the olive oil samples tend to cluster according to the different climatic areas of growing environment of the olives.

**Process:**

3µl of olive samples were placed in tin capsules with the top carefully sealed to avoid squeezing the capsule too tight and squirting the oil to the outside. The samples were then placed on the carousel of the CM autosampler. The CM has a single combustion/reduction reactor packed with $Cr_2O_3$ as a combustion catalyst and elemental copper wires as the reduction agent. The column was held at 980$^{o}$C at its center which is optimal for the combustion while a temperature gradient keeps the copper at its optimal reduction temperature of 650$^{o}$C. The flow rate on the CM is set to 200ml/min and coupled to the heated interface on the CRDS with an open-split at a 7:1 split ratio. The CRDS draws a constant flow at a rate of 25 ml/min. The CM-CRDS operation software was then triggered with the appropriate number of samples populated in the Picarro coordinator with their respective description typed in. Samples were run in 6 replicates. For $\delta D$, the first 3 replicates were tossed out to avoid memory effect and the last three replicates were used in the average calculation of $\delta D$. However, for $\delta^{13}C$, we used all 6 replicates to calculate the $\delta^{13}C$ values. Each replicate runtime was approximately 15min. The $H_2O$ and $CO_2$ peaks were integrated in real-time and both $\delta^{13}C$ and $\delta D$ values were tabulated within the analyzer coordinator. The average reported standard deviation for the $\delta^{13}C$ analysis was 0.09‰ on average (n=3) while the $\delta D$ analysis yielded an average standard deviation of 0.83‰ (n=3) for the various olive oil samples analyzed.

**Results:**

The $\delta^{13}C$ and $\delta D$ measurements of the various olive oil samples clearly resolve the country of origin of each oil (Table 1, Fig. 1). However, the $\delta^{13}C$ isotopic ratio alone could not have distinguished olive oils of California from Turkey and of Lebanon from Spain. Similarly, $\delta D$ alone could not have distinguished olive oils from Australia from Turkey or of California from Italy. Together, these isotopic ratios produce statistically significant differentiation between olive oils from various locations around the globe. It is important to keep in mind that with the exception of the authentic olive oils sourced from Lebanon and Israel, we have depended on the product label for the country of origin information. In order to authenticate olive oil products, there is an absolute need to acquire authentic samples with verified geographical origin in order to build a repository of authenticated samples and generate a database with their corresponding $\delta^{13}C$ and $\delta D$ values for comparative purposes of commercially acquired olive oil products.

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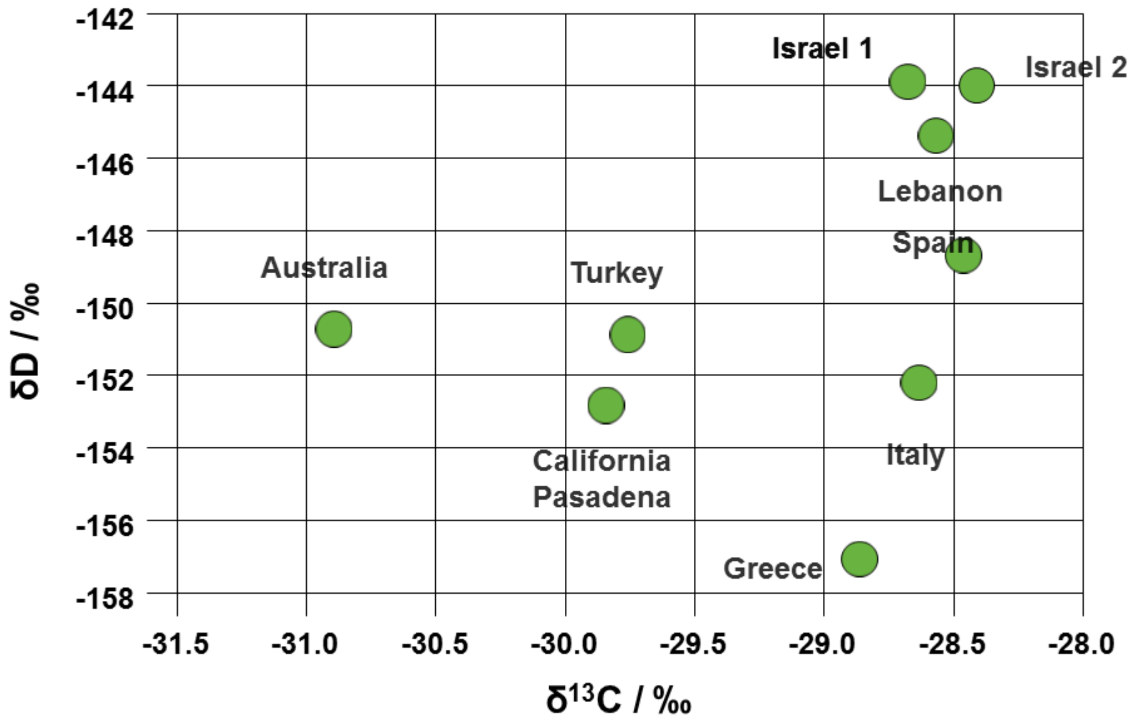


**Figure 1:** Graph of the $\delta^{13}C$ and $\delta D$ isotopic analysis of nine olive oil samples originating from eight countries using Picarro's $^{13}C$+D CM-CRDS.

| Olive Oil Country of Origin | Average $\delta^{13}C$ (permil) | SD (n=6) of $\delta^{13}C$ (permil) | Average $\delta D$ (permil) | SD (n=3) of $\delta D$ (permil) |
|---|---|---|---|---|
| **Spain** | -28.50 | 0.07 | -148.47 | 0.78 |
| **Turkey** | -29.87 | 0.16 | -150.70 | 0.80 |
| **Greece** | -28.84 | 0.05 | -156.77 | 0.89 |
| **Lebanon** | -28.57 | 0.08 | -145.21 | 2.00 |
| **California** | -29.88 | 0.08 | -152.48 | 0.67 |
| **Israel sample 1** | -28.68 | 0.11 | -143.59 | 1.00 |
| **Israel sample 2** | -28.46 | 0.10 | -143.90 | 0.58 |
| **Italy** | -28.66 | 0.07 | -151.92 | 0.28 |
| **Australia** | -30.87 | 0.11 | -150.27 | 0.49 |

**Table 1:** $\delta^{13}C$ and $\delta D$ isotopic analysis of nine olive oil samples originating from eight countries using Picarro's $^{13}C$+D CM-CRDS Isotope Analyzer. Samples were analyzed in 6 replicates. For $\delta^{13}C$, all 6 replicates were used in the average and standard deviation calculation. for $\delta D$, only the last 3 replicates were used for those calculations.

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**Additional resources:**

For an animated view of the above graph, please click on the link below:
**http://www.picarro.com/isotope_analyzers/13cd_cm_crds**

Original data: The Picarro coordinator output file for this data is attached to this pdf. If the attachment does not appear in Acrobat Reader in a sidebar on the left side, go to:

**View > Show/Hide > Navigation Pane > Attachment**

Clicking on this will pop up the sidebar with the attachment in a window.

**References:**

1. www.waterisotopes.org