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AN032

Induction Module-CRDS analysis of water isotopes in cheese II: Rapid method to discriminate cheese sources

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Keywords:

Material: Cheese, water cycle, Chevre, Camembert, matrix-bound water, food authentication, traceability
Process: Stable isotopes, δD, $\delta^{18}O$, IM-CRDS

Summary and Relevance:

Natural abundance stable isotope ratios have been identified as a potential metric for food source [1]. Understanding the source of food is relevant for brand fidelity of geographically protected foods [2], supply chain quality control [3] and tracing pathogen-infected foods to their origin. However, to date the approach has been hampered from widespread use because the analytical methodology (based on isotope ratio mass spectrometry, IRMS and/or inductively-coupled plasma mass spectrometry, ICP-MS) is expensive, and analyses are long and tedious. Another complication is the interpretation itself; often researchers will want to pinpoint exact origin with isotope measurements. This results in ever more complex analyses requiring ever more complex statistical tools. However, we posit that the appropriate question for commercial food entities to ask is not "where did this food come from?" (origination) but "did this food come from here?" (authentication).

In this, and a companion application note (AN031) we explore cheese. Previous studies measuring natural abundance stable isotopes to detect the origin of cheese are extremely complex. The most common approach is a highly involved chemical endeavor that combines the measurement of "light" isotope ratios (δD, $\delta^{13}C$, $\delta^{15}N$, and $\delta^{16}O$)[1] in a protein fraction, as well as concentrations of a number trace metals including strontium, titanium and beryllium [4-5] in the remaining fraction. This analysis requires the work of at least two expert analysts, takes multiple steps, and needs analysis time on two highly specialized, very expensive analyzers (IRMS and ICP-MS). Following the measurement, complex statistical tools such as principle components analysis, or partial least squares are needed to deconvolute the system.

[1]The delta notation is defined as: $\delta^n X=1000\times[(R_S - R_{Ref})/R_{Ref}]$; where *X* is some element, *n* is the number of its heavier stable isotope (e.g., 18 for oxygen), R_S is the ratio of the heavy to the light isotope of the sample (e.g., $^{18}O/^{16}O$) , and R_{ref} is the same ratio, but of a reference material. In this notation, rather than δ^2H, the convention is δD (for deuterium).

To amend the science to real-world applicability, we seek to define a "Yes/No" decision to the question of authenticity ("did this cheese come from here?"). This information will be much simpler to digest and interpret, while still providing useful information as to the origin of a given product. In a previous Application Note, we demonstrated that water in cheese retains its original isotopic signature (Picarro AN031). Thus, we restrict the analysis to isotopes of the water of the cheese (δD and δ^{18}O) [4]. This "matrix-bound" water is abundant in the cheese matrix (~40% by weight), will reflect regionality better than organic components, and requires only two isotopes (δD and δ^{18}O) so simple confidence intervals should suffice to make a Yes/No decision. The last simplifying step is in the technology itself. In place of the expensive, tedious and difficult to use IRMS, we exploit Picarro's Induction Module coupled to a Cavity Ring-Down Spectrometer (IM-CRDS). This is a radically simplified, more rapid approach for the analysis of water isotopes in food matrices. In this technique, the sample (cheese) is placed in a metal holder, and a localized electric field rapidly heats the sample for the complete extraction of matrix-bound water. During extraction, the water is simultaneously swept to the CRDS analyzer for measurement of δD and δ^{18}O allowing a single-step protocol. The whole process (extraction, detection and analysis) from cheese can be accomplished within 10 minutes. Contrast this to days-long preparation under hazardous conditions to isolate water, followed by additional purification and chemical conversion prior to a complicated, expensive and difficult analysis with IRMS. The IM-CRDS is a significant advance, both in terms of sample throughput efficiency and materials and training costs, over any other stable isotope analytical technique. It also carries the additional benefit of portability, so testing can happen on-site.

Process:

Soft cheeses (Chèvre, Camembert, and a ripened soft cheese), three originating from coastal California, and one each from Central California, Wisconsin, and France were purchased at a local grocery store (Figure 1). All surfaces and cutters were washed with isopropanol and dried in air to prevent cross-contamination between samples. A 3-cm piece was sliced from the edge and a small portion taken from the center and spread across the metal sample holder which was immediately crimped and placed in the sample vial, ready for analysis. Each sampling was taken from a fresh surface to avoid the effects of evaporation. Five (5) replicates of each cheese were

Figure 1 Cheeses purchased for this work. From left to right: Tradition Jacquin Chèvre, France; Cypress Grove Purple Haze Goat Milk Cheese, Coastal California; Laura Chenel's Chèvre, Central California; Montchvré Chèvre, Wisconsin; Rouge et Noir Camembert, Coastal California; and not pictured, Rouge et Noir Ripened Breakfast Cheese.

prepared, and data analysis based on the last four (4). Isotopes are reported in the delta notation (see note 1 above). Calibration was done once using three in-house water standards spanning δD of -106.10 to 4.56 ‰ and $\delta^{18}O$ = -14.11 to 0.54 ‰. A 6 mm diameter filter paper hole-punch was wet with 3 µL of the standard, placed in a stainless steel sample holder, crimped and analyzed immediately. Five replicates of each standard were analyzed, with a mean precision and accuracy of ±0.12/1.28 ‰ and 0.74/5.49 ‰ for $\delta^{18}O$/δD, respectively. Data generated by the IM-CRDS was exported to Microsoft Excel as an automatically generated *.csv file. Analysis was done using the AVERAGE (to calculate means) and STDEV (to calculate the 1-sigma standard deviation) functions.

Results:

Data for the six brands of cheese are presented in Table 1. Three cheeses from Coastal California (Chèvre, Camembert and a cow's milk-based ripened soft cheese) showed indistinguishable stable water isotope values (δD = -27.89 ± 1.09 ‰, $\delta^{18}O$ = -2.27 ± 0.21 ‰) that were significantly (p < 0.01) different from the other three regions. It is reiterated that the Coastal California cheeses are three varieties (Chèvre, Camembert, and a non-descript soft ripened cheese) made by two distinct brands (Rouge et Noir and Cypress Grove), made with milk from two species (cow and goat). Despite these differences, the water from these three cheeses are isotopically identical. This strongly supports the idea that regionality is preserved in the water isotopes despite milk type, curdling time and other processing.

Table 1 Stable isotope values of water extracted from six cheeses. Values are in ‰ according to the delta notation for stable isotopes. The data is based on four replicates of each cheese.

Region	Type	$\delta^{18}O$ (Stdev)	dD (Stdev)
Coastal California	Chèvre	-2.33 (0.25)	-28.22 (0.91)
Coastal California	Camembert	-2.18 (0.21)	-27.26 (1.07)
Coastal California	Soft	-2.32 (0.21)	-28.20 (1.27)
Central California	Chèvre	-5.97 (0.27)	-72.52 (1.02)
France	Chèvre	-4.34 (0.38)	-54.94 (2.37)
Wisconsin	Chèvre	-4.38 (0.35)	-37.62 (1.28)

To further simplify the interpretation, we focus on a single isotope, δD. The 1-sigma confidence interval of δD for the Coastal California Cheeses is ± 1.09 ‰, and the cheese with the nearest isotope value was from Wisconsin, with a difference of 9.73 ‰. The difference is large and distinct enough that complex statistical tools are not necessary to distinguish the two; simply calculate the averages and standard deviations and visualize any overlap (Figure 2). Interestingly, the cheese from inland California was the furthest in δD, 44.63 ‰ lower than the coast.

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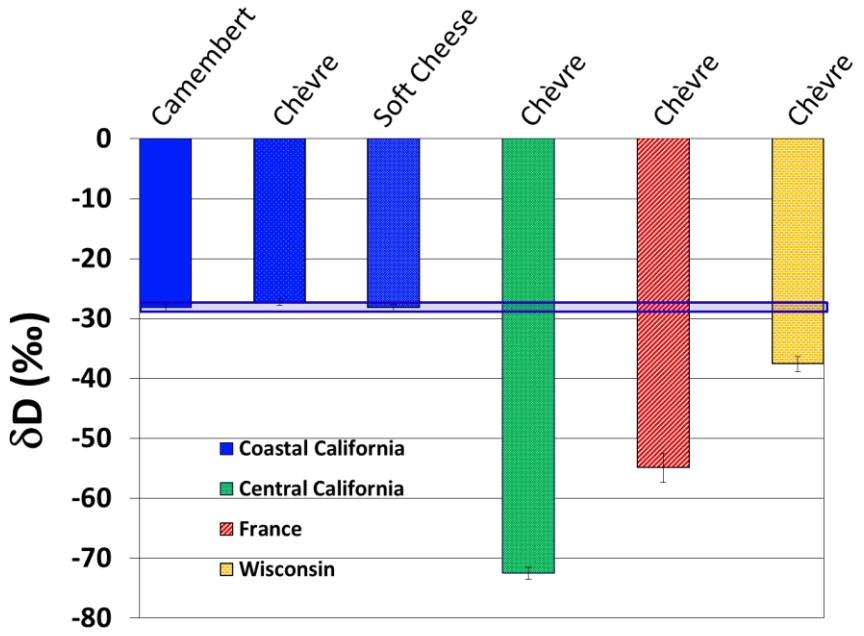

Figure 2 Graphical representation of the strong distinction between three Coastal California cheeses and three cheeses from other regions in the World. The shaded blue area across the plot represents the 1-sigma confidence interval defining whether a cheese is from Coastal California.

Comments:

In this application note, the ease and simplicity of ingredient authentication is demonstrated. We reduce the answer from near impossible certainty when asking origination, to a simple yes or no by shifting the question to authenticity. By understanding the values of a single isotope (δD), we are able to define what values constitute a particular region within a given confidence interval (in this case Coastal California) and test all other samples against this value. For geographically protected foods, or supply chains that demand ingredients from a specific location, this approach is much more desirable and just as, if not more effective than the complex analyses required to define origination. The regionality of the signal is strong enough that species or variety had no impact on the isotope values. The distinction between regions is clear enough that cheese produced ~100 miles apart in California were clearly distinguishable. The simplicity is even further enhanced through the use of the IM-CRDS, which allowed collection of the data in a single step at ~10 minutes and a cost of $0.50 USD per sample.

References:

[1] Kelly *et al.* (2005) Trends in Food Science and Technology 16:555 - 567
[2] Gonzavalez *et al.* (2009) Trends in Analytical Chemistry 28:1295-1311
[3] Picarro, Inc. (2010) AN24
[4] Renou *et al.* (2004) Food Chemistry 85:63-66

A companion Application Note (AN031) detailing the theory and relation of stable isotopes in cheese to environment can be downloaded from:
http://www.picarro.com/resources/application_notes

Induction Module product details can be found at:
http://www.picarro.com/isotope_analyzers/im_crds