Application Note

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AN031

Induction Module-CRDS analysis of water isotopes in cheese I:
Water in cheese retains its environmental isotopic signature

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Keywords:

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

Summary and Relevance:

Natural abundance stable isotope ratios in food are defined by environmental conditions at the time of growth and are a focal point in the effort to define a 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 stable isotope 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) can be expensive, long and tedious.

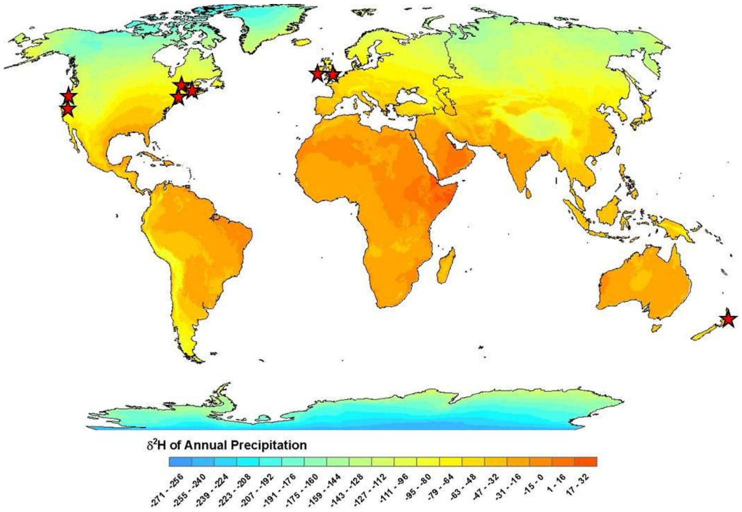

Figure 1 IsoMAP™ of global mean annual meteoric δD in precipitation. Red stars indicate approximate locations listed on labels of cheddar cheese used in this study. Map from http:\\www.waterisotopes.org

In this and the following application note (see Picarro AN032), we explore cheese. Previous studies measuring natural abundance stable isotopes to detect the origin of cheese are extremely complex and are analogous to many other stable isotope-based methods for food study. A common approach is a highly involved chemical endeavor that combines the measurement of “light” isotope ratios[1] (δD, $\delta^{13}C$,

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[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).

$\delta^{15}N$, and $\delta^{16}O$) in a specific protein fraction, as well as concentrations of multiple trace metals including strontium, molybdenum and uraniumin the remaining fraction [4-5]. This analysis requires the work of at least two expert analysts, takes multiple steps, and analysis time on two highly specialized, very expensive mass spectrometers (IRMS and ICP-MS). Following the measurement, complex statistical tools such as principle components analysis, or partial least squares are needed to grasp all the data generated. We believe the analysis should, and can, be simplified on all levels, starting with the target analyte

Any organic molecules in food (carbohydrates, proteins, fats) carry signatures of environmental conditions at the time of growth. However the biosynthetic pathways that define the stable isotope values of these individual components can vary greatly in ways that are very complex and difficult to predict. Detailed studies have even found that carbon isotopes from north and south-facing sides of a single tree can vary significantly [6]. In addition, the isotopes of these organic components can also be significantly altered by processing, such as bacterial growth, and aging. By selecting a food component that is as little affected by these biosynthetic and processing alterations as possible, the original environmental (i.e., regional) isotopic signature should be better preserved. An ideal candidate for cheese is water contained in the structure of the cheese, referred to as "matrix-bound water". Water constitutes a significant portion of cheese (~40 % by weight), there is little evidence collected to date to support significant biosynthetic alteration of water isotopes during milk production [8-9], and water is potentially the best single indicator of regionality due to well understood global meteoric trends in precipitation.[2] In addition, water carries information on only two isotopes (δD and $\delta^{18}O$), which will greatly simplify the analysis and interpretation.

The next simplification 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 $\delta^{18}O$ 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

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[2] A relationship termed the Global Meteoric Water Line has long been exploited by hydrologists to understanding shifting precipitation patterns and primary water sources [7, and http://wateriso.eas.purdue.edu/]. It describes the constant relationship between δD and $\delta^{18}O$ in precipitation, which is given as $\delta D = 8\times\delta^{18}O + 10$. Water follows this relationship has not been altered and reflects precipitation (e.g., many tap waters). Water that falls off this relationship underwent some sort of additional process, such as evaporation of irrigation water or sun-drying of vegetables.

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. In this application note, IM-CRDS is used to assess the feasibility of cheese water to retain its regional isotopic signature.

Process:

Nine cheddars whose labels claimed production from around the world 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 0.5 – 1.0 mm thin slice taken from the fresh surface. A manual hole-puncher (o.d. 6 mm) was used to immediately subsample from the center of the slice, and the subsample placed in a metal sample holder which was crimped with pliers and immediately placed in the sample vial for analysis. Each sampling was taken from a fresh surface to avoid the effects of evaporation. Five (5) replicates of each cheese were prepared, and data analysis based on the last four (4). Isotopes are reported in the delta notation. Calibration was done once using three in-house water standards spanning δD of -106.10 to 4.56 ‰ and δ^{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 strip 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 δ^{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. The automatic graphing and linear regression function was also used for analysis and visualization.

Table 1 Stable isotopic values of water extracted from nine cheddars produced in eight locations. Values are the reported mean and standard deviation from four samples collected in different parts of a single. Units are in ‰.

Location of Production	δ^{18}O (± StDev)	δD (± StDev)
California, USA 1	-6.05 (0.31)	-40.26 (1.88)
California, USA 2	-5.47 (0.35)	-39.87 (1.86)
Eastern Canada	-9.79 (0.93)	-69.34 (1.87)
England	-3.84 (0.62)	-28.24 (2.39)
Ireland	-3.04 (0.05)	-21.00 (1.84)
New York, USA	-9.04 (0.77)	-65.14 (2.46)
New Zealand	-1.52 (0.45)	-15.36 (1.14)
Oregon, USA	-7.44 (0.19)	-53.05 (0.31)
Vermont, USA	-10.43 (1.02)	-67.98 (1.89)

Results:

Data is summarized in Table 1. The precise locations of sampling are not available for all products so a direct comparison to estimated meteoric water isotopes is not possible. Still, the isotopic values do fall within the expected range for meteoric water (e.g. precipitation in the Northeastern USA would be in the range of δD -60 to -70, which is precisely where the cheddar water falls, Table 1). In addition, the expected trends hold true, such as samples presumed to be produced in coastal areas (New Zealand, Ireland and England) show enriched δD and δ^{18}O, while samples from colder regions (Canada and Northeast USA) are more depleted in δD and δ^{18}O (Table 1). In addition, the two samples from California are indistinguishable in their δD values.

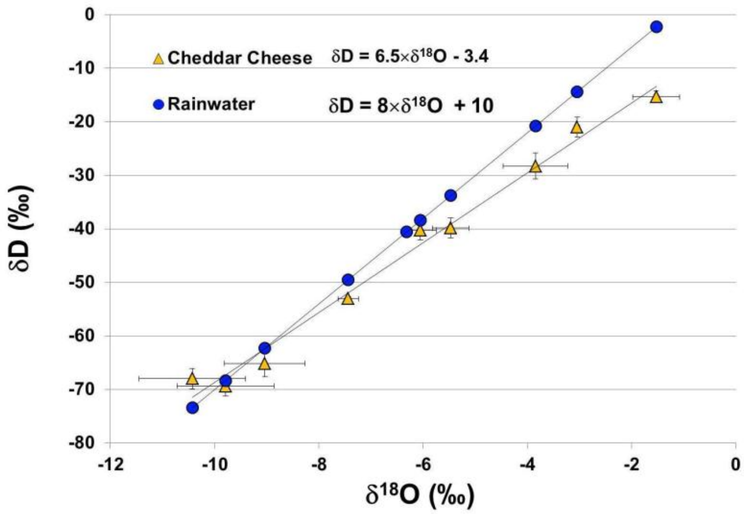

Figure 2 Isotopic cross plot of water isotopes calculated from the GMWL for rainwater (●) and matrix-bound water extracted from cheddar (▲).The lower slope and y-intercept value are indicative of evaporative processes, likely during production.

To further solidify the interpretation as to whether matrix-bound water in cheese retains the meteoric water signal, we can look at the data through the lens of hydrology. Plotting Cheddar water δD as a function of δ^{18}O and comparing to the calculated GMWL shows striking similarities between environmental and cheese water isotopes (Figure 2). The slightly lower slope and y-intercept of the Cheddar line are indicative of evaporative processes, likely during the curdling phase of cheese production. Despite this shift, the work reveals that cheeses produced in different locales with different water isotope regimes carry that signal through production and can be clearly distinguished. The δD isotopes provide better distinction than the δ^{18}O isotopes due to improved relative precision.

Comments:

In this application note, the relationship between environmental water isotopes, and water isotopes in cheese is presented. The flow of water from rain to irrigation through ruminant to milk and finally cheese goes through with very little alteration. This means cheeses made in a particular region should share particular water isotope values. Finally, the use of the IM-CRDS for this task allowed collection of the data in a single step at ~10 minutes and a cost of $0.50 USD per sample. In the companion Application Note

(AN032), the question is shifted from origination to authenticity, in which the task is not to clearly demonstrate where a cheese came from, but to show whether the cheese belongs to a particular region.

References:

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[8] Renou *et al.* (2004) Food Chemistry 85:63-66
[9] Chesson *et al.* (2010) Journal of Agricultural and Food Chemistry 58:2358-2363

A companion Application Note (AN032) detailing real-life applicability of matrix-bound water to distinguish cheese authenticity 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