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Analysis of the hydrogen and oxygen stable isotope ratios of beverage waters without prior water extraction using isotope ratio infrared spectroscopy

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Hydrogen (δ^2H) and oxygen (δ^{18}O) stable isotope analysis is useful when tracing the origin of water in beverages, but traditional analytical techniques are limited to pure or extracted waters. We measured the isotopic composition of extracted beverage water using both isotope ratio infrared spectroscopy (IRIS; specifically, wavelength-scanned cavity ring-down spectroscopy) and isotope ratio mass spectrometry (IRMS). We also analyzed beer, sodas, juices, and milk 'as is' using IRIS. For IRIS analysis, four sequential injections of each sample were measured and data were corrected for sample-to-sample memory using injections (a) 1-4, (b) 2-4, and (c) 3-4. The variation between δ^2H and δ^{18}O values calculated using the three correction methods was larger for unextracted (i.e., complex) beverages than for waters. The memory correction was smallest when using injections 3-4. Beverage water δ^2H and δ^{18}O values generally fit the Global Meteoric Water Line, with the exception of water from fruit juices. The beverage water stable isotope ratios measured using IRIS agreed well with the IRMS data and fit 1:1 lines, with the exception of sodas and juices (δ^2H values) and beers (δ^{18}O values). The δ^2H and δ^{18}O values of waters extracted from beer, soda, juice, and milk were correlated with complex beverage δ^2H and δ^{18}O values ($r = 0.998$ and 0.997, respectively) and generally fit 1:1 lines. We conclude that it is possible to analyze complex beverages, without water extraction, using IRIS although caution is needed when analyzing beverages containing sugars, which can clog the syringe and increase memory, or alcohol, a known spectral interference. Copyright © 2010 John Wiley & Sons, Ltd.

Recent studies have documented that the hydrogen and oxygen stable isotope ratios of some widely available bottled beverages co-vary and that the relationships between the δ^2H and δ^{18}O values closely resemble the Global Meteoric Water Line (GMWL, defined as $\delta^2H = 8^*\delta^{18}O + 10‰$[1]), suggesting that the water within a beverage (i.e., beverage water) records the isotopic composition of the water used to produce the beverage.[2–4] Because the stable isotope ratios of meteoric waters vary predictably spatially,[5,6] it may be possible to measure the δ^2H and δ^{18}O values of water within a beverage and infer the beverage's original water source or location of production. The rapid analysis of a suspect beverage such as bottled water, milk, or wine to independently verify its geographic origin would be a major boon to investigations of beverage authenticity.[7–11]

Until very recently the stable isotope ratios of hydrogen and oxygen in water had been exclusively measured using stable isotope ratio mass spectrometry (IRMS).[12] Water cannot be directly analyzed using mass spectrometry. Instead, it must first either be converted into H_2 and CO gases or equilibrated with H_2 and CO_2 gases so that it can be analyzed in a gaseous form. To analyze water contained within a material of interest (e.g., a beverage) by the classic IRMS analysis method, water must first be collected using time-consuming offline extraction methods.[13]

The introduction of isotope ratio infrared spectroscopy (IRIS) analyzers, based on wavelength-scanned cavity ring-down spectroscopy (WS-CRDS; Picarro Inc.) or off-axis integrated cavity output spectroscopy (OA-ICOS; Los Gatos Research), has provided a new technique to rapidly measure the stable isotope ratios in water.[14–17] Because of their smaller size and weight, it is possible to use WS-CRDS and OA-ICOS water analyzers in a field setting.[18,19] The analytical precision and accuracy of IRIS-based approaches are similar to that of IRMS.[12,17,8] However, similar to IRMS, the conventional applications for IRIS are the analyses of pure waters or waters extracted from samples of interest.

In this study we investigated the possibility of analyzing beverage water hydrogen and oxygen isotopic compositions without prior water extraction using IRIS. The beverages considered included several bottled drinks widely available to and commonly imbibed by the modern consumer: beer, carbonated soft drinks (sodas), citrus juices, and milk. We first compare the analysis of bottled waters, tap waters, and

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waters extracted from beverages using both the traditional IRMS analytical technique and a recently introduced IRIS analytical technique, wavelength-scanned cavity ring-down spectroscopy. We then demonstrate the feasibility of measuring the stable isotope ratios of beverages 'as is' (i.e., unextracted) using IRIS, and finally discuss some of the potential complications and analytical interferences that arise from the analysis of complex, unextracted beverages by IRIS.

EXPERIMENTAL

Beverage collection and preparation

Beverages were collected in four regions of the contiguous United States: in Santa Clara and Marin Counties, CA; the Orlando metropolitan area, FL; the Salt Lake City metropolitan area, UT; and the Spokane metropolitan area, WA. Collected beverages included Coors Light® beer, beers produced by breweries local to the collection regions, Coca-Cola® Classic soda, Diet Coke® soda, Dasani® bottled water, Fiji® bottled water, not-from-concentrate orange and grapefruit juices, whole milks from commercial dairies in the collection areas, and tap water. In addition, a single sample of local freshly squeezed apple juice was purchased in the Salt Lake City metropolitan area. The collected beverages were immediately sub-sampled into 2- or 4-oz plastic watertight bottles. The bottles were half filled, then frozen and stored frozen until processed.

In the laboratory, frozen beverages were thawed and a 1-mL sub-sample of each beer, soda, juice, and milk sample (hereafter, 'complex' beverages) was transferred onto clean glass wool, frozen, and cryogenically extracted.[8,13] The extracted waters were not filtered, processed, or treated further. The extracted beverage waters as well as the bottled and tap water samples were transferred (0.5 mL) into 1.8-mL crimp-top vials, sealed, and stored in a cool, dark location prior to analysis. Sub-samples (0.5 mL) of the complex beverages were also transferred into crimp-top vials for analysis 'as is', with no additional filtration, treatment, or processing.

Stable isotope analysis

The stable isotope abundances are reported in δ-notation in parts per thousands (‰), where

$$\delta = (R_A/R_S - 1) \times 1000$$

and R_A and R_S are the molar ratios of the rare to abundant isotope (e.g., $^2H/^1H$ or $^{18}O/^{16}O$) in the sample of interest and an international standard, respectively. The international standard for both hydrogen and oxygen stable isotope analysis is Vienna Standard Mean Ocean Water (VSMOW).

The stable isotope abundances of water samples were first analyzed at IsoForensics Inc. by IRIS (WS-CRDS) on a model L1102-*i* water analyzer (Picarro, Sunnyvale, CA, USA).[18] Water samples were introduced into the vaporization chamber using an attached PAL autosampler (Leap Technologies, Carrboro, NC, USA). Each sample was analyzed four times (four consecutive replicate injections) alongside a set of three laboratory reference materials, which had previously been calibrated to the VSMOW scale. After IRIS analysis, the crimp-top caps on sample vials were replaced and samples were analyzed a second time by IRMS at the Stable Isotope Ratio Facility for Environmental Research (SIRFER) on the University of Utah campus (Salt Lake City, UT, USA). The stable isotope compositions were measured on a ThermoFinnigan Delta+ XL isotope ratio mass spectrometer (Bremen, Germany) with a high-temperature conversion elemental analyzer (TC/EA; Costech Analytical, Valencia, CA, USA) attached. During IRMS analysis samples were injected in triplicate (three consecutive replicate injections) into the TC/EA using a PAL autosampler. The samples were analyzed alongside the same laboratory water reference materials as were used during IRIS analysis. The measurement precision for H and O, as defined for the analytical instrumentation used, was 0.5‰ and 0.1‰ for IRIS and 1.2‰ and 0.1‰ for IRMS.

Once all water analyses were complete, unextracted complex beverage sub-samples were analyzed by IRIS. The unextracted complex beverages were deliberately analyzed after the extracted, bottled, and tap water samples as we expected that the water analyzer's vaporization chamber would be dirty with particulates, mainly sugars and lipids, from the injected complex beverages. These deposits, which were visible to the naked eye as a caramel-colored coating in the chamber inlet, could increase instrument memory as molecules of water from one sample injection adhered to the deposits and had an impact on the measured isotopic composition of the following sample. Unextracted complex beverage samples were sorted randomly both between and within beverage types; that is, we did not analyze all the beer samples before analyzing all the soda samples. The unextracted complex beverages were not analyzed by IRMS.

Memory effect calculations and data correction

The isotopic compositions of samples and reference materials measured using IRIS and IRMS were corrected for sample-to-sample memory prior to normalization of the sample data to the reference materials. For memory corrections, the measured isotope value of the previous sample injection (S1) was used to correct for its impact on the measured isotope value of the current injection (S2). Corrected isotope values were calculated using the equation:

$$S2_{corrected} = (S2_{measured} - S1_{corrected} * X)/(1 - X)$$

where X is the memory correction value (fractional contribution) of S1 to the measured isotope value of S2.[18] Data were corrected using a template built in Microsoft® Excel® 2004 for Mac. Fractional memory correction values were manually entered into the data template until the combined average standard deviation of the three reference materials was minimized.

The isotope data generated by IRIS were corrected for memory using three approaches: using all four replicate sample injections, using replicate injections 2–4, and using replicate injections 3 and 4 only. The isotope data generated by IRMS were corrected for sample-to-sample memory once, using replicate sample injections 2 and 3. After memory correction, the sample data were normalized to the water reference materials included in the analysis run using linear

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regression. Only two of the three reference materials, the isotopically heavy and isotopically light waters, were used for data normalization. The assigned values of the intermediate reference material (Evian® bottled water) were compared with its corrected value after standardization. The mean hydrogen and oxygen stable isotope ratios for Evian®, monitored from November 2004 to December 2009, were –73.7‰ for H ($n = 4450$, $1\sigma = 1.7$‰) and –10.29‰ for O ($n = 4411$, $1\sigma = 0.21$‰).

Statistical analysis

The relationship between the δ^2H and δ^{18}O values of the extracted complex beverage waters, bottled waters, and tap waters measured using IRIS was analyzed using ordinary least-squares linear regression. Differences (Δ) between the isotope ratios of bottled, tap, and extracted waters measured by IRMS and IRIS and between the isotopes ratios of paired extracted and unextracted beverages measured by IRIS were tested using two-tailed paired t-tests. Beverage types (beers, sodas, bottled waters, juices, milks, tap waters) were separated for all other statistical analyses. The relationships between the isotope ratios of extracted complex beverage waters, bottled waters, and tap waters measured using both IRMS and IRIS were analyzed using total least-squares linear regression (Deming regression). Deming regression was also used to analyze the relationships between the isotope ratios of paired extracted complex beverage samples and unextracted complex beverage samples. The slopes and the y-intercepts of individual beverage lines were compared with a line of slope = 1 and intercept = 0 using one-way analysis of variance (ANOVA) with beverage type as the factor and a Tukey's post-hoc test to identify differences at $\alpha = 0.01$. All statistical analyses were completed using Prism 5 for Mac OS X (GraphPad Software Inc., La Jolla, CA, USA).

RESULTS

Memory corrections

The memory correction values used for extracted complex beverage waters, bottled waters, and tap waters analyzed by IRIS (0.5–4.0% for H and 0.0–2.7% for O) were smaller than those applied to complex beverage data (1.6–20.0% for H and 0.7–3.7% for O; Table 1). In general, the H memory correction values were larger than those applied during the correction of O isotope compositions. We also observed that the memory correction values applied when using replicate injections 3 and 4 were typically smaller than those needed when either all four injections or replicate injections 2–4 were used. The memory correction values used to correct for the sample-to-sample memory affecting the same waters analyzed via IRMS were relatively small, 2.7% for H and 1.4% for O (Table 1).

Once the IRIS data had been corrected for memory using the three approaches, there was little variation between the final δ^2H and δ^{18}O values of the secondary reference material Evian® bottled water included in the IRIS water analysis runs (Table 1). The average standard deviation of the final Evian® δ^2H values between the three correction approaches was 0.2‰; for δ^{18}O values, the average standard deviation was 0.02‰. On the other hand, the variations between the final δ^2H and δ^{18}O values of Evian® included in the complex beverage analysis runs and corrected using the three approaches were greater (Table 1). The average standard deviation of the final Evian® δ^2H values was 1.4‰; for δ^{18}O values it was 0.03‰. Based on the results of the three

Table 1. Memory correction values used to account for sample-to-sample memory affecting the measured H and O stable isotope ratios of extracted and unextracted complex beverages analyzed by IRIS and/or IRMS. IRIS data were corrected three times using replicate injections 1–4, 2–4, and 3–4. The standard deviation (SD) of the isotope ratios of the reference materials included in each analysis run was calculated after application of the memory correction (shown in percentages). The average final corrected values of the secondary reference material Evian® bottled water are also presented

Analysis	Material	Run #	Method	Memory correction		Ref. material SD		Corrected Evian*	
				H	O	H (‰)	O (‰)	H (‰)	O (‰)
IRIS	extracted water	Run 1	injections 1-4	3.8%	1.7%	0.75	0.09	−73.7	−10.32
			injections 2-4	1.1%	0.0%	0.61	0.09	−73.8	−10.33
			injections 3-4	0.5%	0.0%	0.63	0.08	−73.8	−10.32
		Run 2	injections 1-4	3.2%	1.6%	0.84	0.08	−74.4	−10.43
			injections 2-4	1.2%	0.8%	0.60	0.06	−74.1	−10.38
			injections 3-4	0.7%	0.0%	0.57	0.07	−73.8	−10.37
		Run 3	injections 1-4	4.0%	2.7%	0.85	0.11	−73.9	−10.34
			injections 2-4	1.6%	1.1%	0.65	0.10	−73.9	−10.36
			injections 3-4	1.0%	1.4%	0.54	0.08	−73.9	−10.35
IRMS	extracted water	Run 1	injections 2-3	2.7%	1.4%	0.79	0.12	−73.8	−10.40
IRIS	complex beverage	Run 1	injections 1-4	7.0%	3.7%	3.94	0.19	−71.2	−10.28
			injections 2-4	3.0%	1.5%	1.82	0.15	−72.5	−10.30
			injections 3-4	1.6%	1.2%	1.29	0.14	−72.9	−10.33
		Run 2	injections 1-4	20.0%	2.5%	7.05	0.19	−78.5	−10.40
			injections 2-4	7.1%	1.7%	3.97	0.15	−76.1	−10.35
			injections 3-4	3.1%	0.7%	2.64	0.13	−74.9	−10.34

* Assigned values: H = −73.7‰, O = −10.29‰

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memory correction approaches, the IRIS data used during statistical analysis and presented graphically are the averages of memory-corrected values from replicate injections 3 and 4 only.

Beverage isotopic analyses

The measured δ^2H and δ^{18}O values of extracted complex beverage waters, bottled waters, and tap waters analyzed using IRIS were correlated (Pearson $r = 0.86$). The correlation was stronger when water extracted from beer samples, which contained alcohol, were excluded (Pearson $r = 0.97$). The δ^2H and δ^{18}O values generally fit the GMWL, defined as $\delta^2H = \delta^{18}O^*8 + 10‰$[1] (Fig. 1), with the notable exceptions of the water extracted from beer samples, which fell above the GMWL, and water extracted from the juices, which fell below the GMWL. Excluding waters extracted from beer and juices, the ordinary least-squares regression line describing the relationship between the δ^2H and δ^{18}O water values ($\delta^2H = 8.2^*\delta^{18}O + 8.4‰$) was not statistically different from the GMWL.

The measured δ^2H values of extracted complex beverages, bottled waters, and tap waters analyzed using IRIS agreed well with data generated from IRMS analysis of those same samples (Pearson $r = 0.999$; Fig. 2(a) and Table 2). Neither the mean $\Delta\delta^2$H value nor the mean $\Delta\delta^{18}$O value of the grouped bottled, tap, and extracted waters measured using both IRMS and IRIS was statistically different from zero when the beer δ^{18}O values were excluded from the analysis. Considering the beverage types individually, the total least-squares regression lines relating the IRIS and IRMS δ^2H measurements of bottled waters, tap waters, and waters extracted from beers and milks were not significantly different from a 1:1 line. On the other hand, the total least-squares regression lines for the waters extracted from sodas and juices (sodas: $\delta^2H_{IRIS} = 0.98^*\delta^2H_{IRMS} - 1.6‰$; juices: $\delta^2H_{IRIS} = 0.94^*\delta^2H_{IRMS} - 4.9‰$) had slopes and intercepts significantly different from 1 and 0, respectively (slopes: $F_{6,137} = 17.47$, $P < 0.001$; intercepts: $F_{6,137} = 57.55$, $P < 0.001$; Table 3). In addition, the y-intercept of the beer regression line was significantly different from 0 ($P < 0.001$).

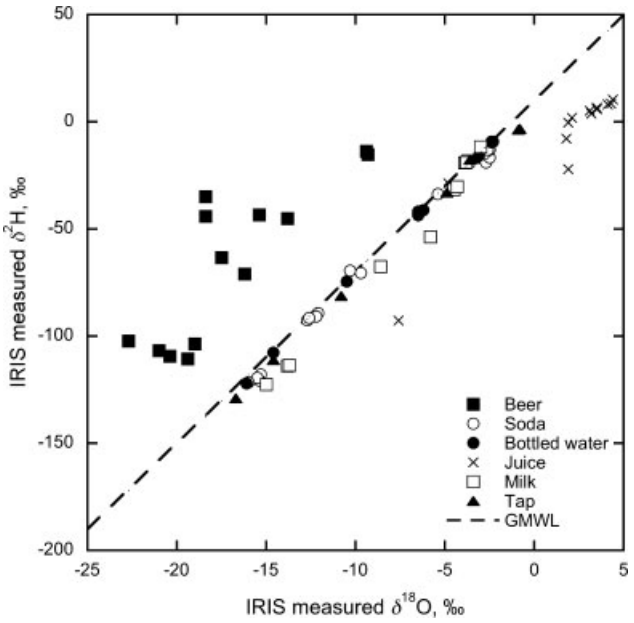

Figure 1. Cross plot of the δ^2H and δ^{18}O values of bottled and tap waters as well as water extracted from beer, sodas, fruit juices, and milk measured using isotope ratio infrared spectroscopy (IRIS), specifically wavelength-scanned cavity ring-down spectroscopy (WS-CRDS). The δ^2H and δ^{18}O values were correlated (Pearson $r = 0.86$). The Global Meteoric Water Line (GMWL, defined as $\delta^2H = 8^*\delta^{18}O + 10‰$) is shown for reference by the dashed black line.

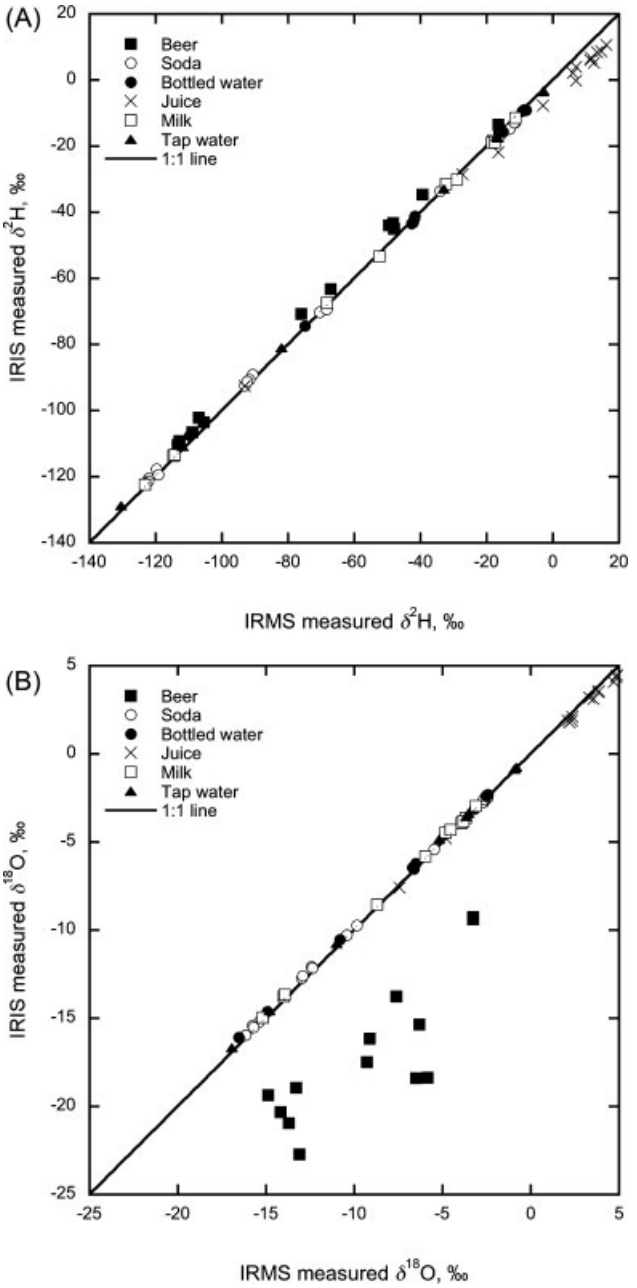

Figure 2. Cross plot of the δ^2H (A) and δ^{18}O (B) values of extracted complex beverages, bottled waters, and tap waters measured using both isotope ratio mass spectrometry (IRMS) and isotope ratio infrared spectroscopy (IRIS). The 1:1 line is shown for reference by the solid black line in each panel.

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Table 2. The δ^2H and δ^{18}O values of some commonly imbibed bottled beverages measured as extracted water by IRIS and IRMS, and measured as complex, unextracted beverages by IRIS

		Extracted water		Extracted water		Complex beverages	
		IRIS		IRMS		IRIS	
Beverage	State	δ^2H, ‰	δ^{18}O, ‰	δ^2H, ‰	δ^{18}O, ‰	δ^2H, ‰	δ^{18}O, ‰
Apple juice	UT	−92.7	−7.56	−93.0	−7.35	−88.9	−7.83
Coca-cola	CA	−89.2	−12.11	−90.6	−12.40	−90.9	−12.35
Coca-cola	CA	−90.8	−12.16	−91.5	−12.37	−89.5	−12.28
Coca-cola	FL	−18.9	−2.73	−17.4	−2.63	−12.3	−2.53
Coca-cola	FL	−16.7	−2.52	−15.7	−2.45	−13.6	−2.48
Coca-cola	FL	−33.7	−5.42	−33.9	−5.46	−29.3	−5.24
Coca-cola	UT	−117.8	−15.25	−119.7	−15.40	−115.0	−15.30
Coca-cola	UT	−120.6	−15.42	−122.0	−15.74	−117.4	−15.42
Coca-cola	UT	−119.5	−15.53	−119.1	−15.70	−119.2	−15.47
Coca-cola	WA	−70.4	−9.75	−70.4	−9.83	−65.2	−9.34
Coors beer	FL	−15.3	−9.27	−16.0	−3.24	−14.0	−9.32
Coors beer	FL	−45.2	−13.79	−47.8	−7.59	−44.6	−14.61
Coors beer	FL	−13.6	−9.41	−16.3	−3.23	−10.7	−9.61
Coors beer	UT	−109.4	−20.35	−112.7	−14.17	−107.6	−20.05
Coors beer	WA	−106.7	−20.98	−108.8	−13.69	−104.7	−19.66
Dasani	FL	−9.2	−2.36	−8.0	−2.47	–	–
Dasani	FL	−9.1	−2.34	−8.6	−2.42	–	–
Dasani	FL	−9.5	−2.41	−9.1	−2.51	–	–
Dasani	UT	−122.0	−16.11	−123.2	−16.53	–	–
Dasani	WA	−74.5	−10.54	−74.9	−10.80	–	–
Diet Coke	CA	−92.6	−12.75	−92.9	−12.97	−95.2	−12.75
Diet Coke	CA	−91.4	−12.60	−92.3	−12.90	−94.4	−12.71
Diet Coke	FL	−14.9	−2.76	−13.0	−2.72	–	–
Diet Coke	FL	−13.0	−2.54	−11.3	−2.56	−13.1	−2.47
Diet Coke	FL	−13.0	−2.58	−11.6	−2.63	−13.0	−2.35
Diet Coke	UT	−121.5	−15.98	−122.2	−16.13	−124.7	−16.08
Diet Coke	WA	−69.5	−10.28	−68.2	−10.40	−70.4	−10.22
Fiji	FL	−41.2	−6.23	−41.6	−6.50	–	–
Fiji	FL	−41.9	−6.49	−41.7	−6.61	–	–
Fiji	UT	−43.2	−6.46	−42.2	−6.68	–	–
Fiji	WA	−43.5	−6.54	−42.6	−6.60	–	–
Grapefruit juice	FL	6.6	3.48	11.7	3.85	9.3	3.44
Grapefruit juice	FL	8.2	4.06	13.5	4.74	11.7	4.24
Grapefruit juice	UT	3.9	3.16	7.1	3.34	13.5	3.11
Local beer	CA	−43.4	−15.39	−48.1	−6.29	−38.6	−14.77
Local beer	CA	−34.8	−18.39	−39.3	−5.81	−32.3	−18.50
Local beer	CA	−70.9	−16.17	−75.9	−9.10	−67.9	−16.38
Local beer	CA	−63.4	−17.51	−67.0	−9.24	−59.5	−16.18
Local beer	CA	−44.1	−18.42	−49.3	−6.46	−38.5	−15.72
Local beer	UT	−103.7	−18.97	−105.3	−13.26	−99.3	−18.92
Local beer	UT	−110.6	−19.38	−113.3	−14.87	−112.5	−20.81
Local beer	WA	−102.2	−22.73	−106.8	−13.08	−100.6	−22.75
Local bottled water	CA	−107.7	−14.63	−109.6	−14.90	–	–
Local bottled water	FL	−15.8	−3.13	−15.0	−3.16	–	–
Milk	CA	−67.5	−8.58	−68.1	−8.67	−66.1	−8.80
Milk	CA	−31.6	−4.49	−32.1	−4.80	−29.3	−4.51
Milk	CA	−30.2	−4.32	−28.9	−4.51	−25.0	−4.47
Milk	CA	−53.6	−5.85	−52.3	−5.94	−52.0	−6.24
Milk	FL	−18.3	−3.66	−17.9	−3.66	−14.7	−3.61
Milk	FL	−19.1	−3.93	−18.2	−3.90	−15.2	−3.79
Milk	FL	−18.9	−3.82	−17.5	−3.78	−18.1	−3.65
Milk	FL	−11.6	−2.98	−11.1	−3.10	−3.1	−2.89
Milk	UT	−113.8	−13.78	−114.4	−13.98	−114.5	−13.86
Milk	UT	−113.5	−13.69	−114.1	−13.92	−112.6	−13.60
Milk	WA	−122.5	−14.98	−123.1	−15.17	−119.3	−14.99
Orange juice	FL	8.5	4.34	14.8	4.87	16.1	4.56
Orange juice	FL	−0.3	1.87	7.1	2.36	2.1	1.73
Orange juice	FL	5.2	3.08	12.6	3.57	–	–
Orange juice	FL	10.5	4.43	16.2	4.91	14.6	4.44
Orange juice	FL	−28.6	−4.81	−27.1	−4.78	−21.7	−4.81
Orange juice	UT	2.0	2.08	6.2	2.38	6.7	1.91

(*Continues*)

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Table 2. (Continued)

		Extracted water		Extracted water		Complex beverages	
		IRIS		IRMS		IRIS	
Beverage	State	δ^2H, ‰	δ^{18}O, ‰	δ^2H, ‰	δ^{18}O, ‰	δ^2H, ‰	δ^{18}O, ‰
Orange juice	UT	5.9	3.54	11.5	3.90	14.2	3.48
Orange juice	WA	−7.8	1.77	−2.9	2.23	11.5	3.51
Tap water	CA	−81.2	−10.79	−81.8	−10.98	–	–
Tap water	CA	−33.0	−4.87	−32.8	−5.16	–	–
Tap water	FL	−3.3	−0.78	−2.7	−0.78	–	–
Tap water	FL	−17.7	−3.59	−16.5	−3.64	–	–
Tap water	FL	−17.2	−3.39	−16.8	−3.51	–	–
Tap water	FL	−3.6	−0.90	−2.8	−0.90	–	–
Tap water	WA	−111.0	−14.58	−111.7	−14.78	–	–
Tap water	WA	−128.9	−16.69	−130.3	−16.91	–	–

The δ^{18}O values of extracted complex beverages, bottled waters, and tap waters measured using IRIS were positively correlated with the δ^{18}O values measured using IRMS (Pearson $r = 0.90$). Most paired oxygen isotope data points fit an IRIS versus IRMS 1:1 line (Fig. 2(b) and Table 2), with the exception of the beer samples. Excluding the extracted beer samples from consideration, neither the slope nor the y-intercept of any beverage total least-squares regression line was different from the 1:1 line (Table 3).

Finally, we observed that the δ^2H and δ^{18}O values of waters extracted from the beer, sodas, juices, and milk and analyzed using IRIS were correlated with the δ^2H and δ^{18}O values of the same unextracted beverages analyzed using IRIS (Pearson $r = 0.998$ and 0.997, respectively). The mean $\Delta\delta^2$H value of paired unextracted and extracted beverages measured using IRIS was significantly different from 0 ($t_{50} = 5.93$, $P < 0.0001$) but the mean $\Delta\delta^{18}$O value of paired unextracted and extracted beverages was not different from 0. The slopes of the total least-squares regression lines describing the relationship between unextracted and extracted beverage δ^2H values were not different from 1 for the beers, sodas, juices, or milk (Fig. 3(a)); however, the y-intercepts of the beer, soda, juice, and milk regression lines were all different from 0 ($F_{4,118} = 17.87$, $P < 0.0001$; Table 3). The total least-squares regression lines describing the relationship between the δ^{18}O values of beer, soda, juice, and milk analyzed as both unextracted beverages and extracted waters using IRIS (Fig. 3(b)) were not statistically different from the 1:1 line (Table 3).

DISCUSSION

In this study we measured the stable isotope ratios of complex beverages – beers, sodas, juices, and milk – using isotope ratio infrared spectroscopy (specifically, wavelength-scanned cavity ring-down spectroscopy) without first extracting water. We found that analyses of unextracted complex beverages using IRIS increased the instrument memory to values greater than the memory observed during analyses of extracted complex beverages, bottled waters, and tap waters. However, the negative impact of sample-to-sample memory could be corrected by using replicate injections 3 and 4 of a 4-injection IRIS analysis sequence. With the exception of the sodas and juices, the measured δ^2H and δ^{18}O values of extracted complex beverages, bottled waters, and tap waters measured by IRIS were statistically indistinguishable from those same waters analyzed via IRMS. In addition, the isotope ratios of the unextracted and extracted complex beverages were highly correlated when analyzed using IRIS, although the y-intercepts of the lines describing the relationships between the measured δ^2H values of soda, beer, juice, and milk were statistically different from a 1:1 line with an intercept of 0.

Beverage water δ^2H and δ^{18}O values measured via IRIS generally fit the GMWL

As documented previously for the IRMS analysis of bottled water[3,4,7,9] and water extracted from beer, soda,[3] and milk,[2] we expected the δ^2H and δ^{18}O values of most beverage waters analyzed by IRIS to fit the GMWL (Fig. 1). We observed that the not-from-concentrate orange, grapefruit, and apple juices fell below the GMWL, indicative of the evaporative enrichment of water within the fruit during growth.[20,21] All the citrus juices claimed to have originated in the state of Florida, USA, where both the precipitation and the tap water available for orange and grapefruit tree irrigation are relatively isotopically homogeneous throughout the year.[6] Thus, we expected – and observed – that the measured δ^2H and δ^{18}O values of water from orange and grapefruit juices produced in Florida generally clustered together on a H and O isotope cross plot (Fig. 1).

We found that the extracted water from beer samples analyzed by IRIS fell above the GMWL due to the presence of an ethanol or extremely small methanol contaminant in the extract, which act as spectral interferences.[12] It appears the alcohol interference in the IRIS analysis of water extracted from beers affected the O isotope ratios more than the H isotope ratios. The same water samples analyzed by IRMS had near identical measured δ^2H values (Fig. 2(a) and Table 2) while the measured δ^{18}O values did not fit the 1:1 regression line of IRIS on IRMS (Fig. 2(b)).

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Table 3. The slopes and intercepts (in units of ‰) for the total least squares regression lines describing the relationship between the hydrogen (top) and oxygen (bottom) isotope ratios of samples measured using both IRIS and IRMS (left), and measured as both extracted and un-extracted complex beverages (right). Slopes and intercepts were tested against a line of slope 1 and intercept 0 using ANOVA with a Tukey's post-hoc test

	Hydrogen							
	IRIS vs. IRMS				Complex beverages vs. extracted water			
	Slope	Significant?	Intercept	Significant?	Slope	Significant?	Intercept	Significant?
Beer	1.001		3.375	***	1.017		3.661	**
Soda	0.977	**	−1.620	***	1.029		3.110	**
Bottled water	0.980		−1.087		n/a		n/a	
Juice	0.942	***	−4.941	***	1.022		6.894	***
Milk	0.986		−1.030		1.033		4.611	***
Tap water	0.983		−0.089		n/a		n/a	

	Oxygen							
	IRIS vs. IRMS				Complex beverages vs. extracted water			
	Slope	Significant?	Intercept	Significant?	Slope	Significant?	Intercept	Significant?
Beer	0.985		−7.889	***	0.977		−0.112	
Soda	0.980		−0.071		1.017		0.205	
Bottled water	0.979		0.026		n/a		n/a	
Juice	0.969		−0.308		1.028		0.096	
Milk	0.988		0.033		1.005		0.006	
Tap water	0.989		0.055		n/a		n/a	

$^{**}P < 0.01$ $^{***}P < 0.001$

It may not be necessary to extract water from beverages prior to stable isotope analysis

It has been previously documented that the measured isotopic composition of pure waters analyzed using IRIS, specifically WS-CRDS, matched those same waters analyzed using IRMS.[12] Disregarding extracted water samples containing alcohol, the isotopic compositions of extracted complex beverages, bottled waters, and tap waters analyzed by IRIS in this study generally agreed well with those same samples analyzed by IRMS (Figs. 2(a) and 2(b)). However, the lines describing the relationship between the δ^2H values of extracted soda and juice waters analyzed using both IRIS and IRMS were significantly different from a line with a slope of 1 and an intercept of 0. The difference between the slopes and intercepts of the 1:1 line and the soda/juice lines were relatively small, suggesting that the phenomenon affecting the measured δ^2H values of the sodas and juices had a minor – albeit statistically significant – impact.

We assume, as did West and colleagues,[22] a source of error in the IRIS-measured δ^2H values. That is, we assume that the IRMS-measured δ^2H values are correct and the comparison of IRIS measurements with them is demonstrating an inaccuracy of the WS-CRDS analyzer for these samples. Similar to the waters extracted from plant leaves and soils by West *et al.*,[22] the waters extracted from sodas and juices presumably included some trace amounts of organic contaminants that may have introduced a spectral interference and had an impact on the IRIS measurements. For sodas, these were probably the colorings and flavorings that give the carbonated soft drinks their distinctive appearance and taste. For the juices, fruits probably contain similar organic contaminants to those observed in extracted plant leaf waters.[22] However, if such components did cause spectral interference in our IRIS measurements of soda and juice waters, these effects were much smaller than those observed in the study of plant and soil waters. In fact, the IRIS/IRMS lines for the extracted sodas and juices suggest that the differences between the two measurement methods are smaller than both the stated IRIS machine precision and the calculated standard deviation of Evian® bottled water included in our analysis runs across the range of values measured here. The differences between the two methods would only be significantly greater than the instrument precision for beverage waters that have extremely high or low δ^2H values.

We observed that the δ^2H and δ^{18}O values of extracted complex beverages measured by IRIS generally matched the δ^2H and δ^{18}O values of the beverages analyzed without water extraction by IRIS (Figs. 3(a) and 3(b)), albeit with some variation in measured δ^2H values. This suggests that it may not be necessary to extract water from beverages prior to isotope analysis, although unextracted complex beverage δ^2H values may need to have a beverage-specific offset correction applied post-analysis (Table 2). Despite this, these results demonstrate that it may be possible to reduce the time and manpower required between beverage collection and stable isotope ratio analysis by simply analyzing a complex beverage without prior water extraction using WS-CRDS.

Caution is needed when analyzing complex beverages 'as is' using IRIS

Before we can recommend the analysis of complex beverages without water extraction using the WS-CRDS analyzers

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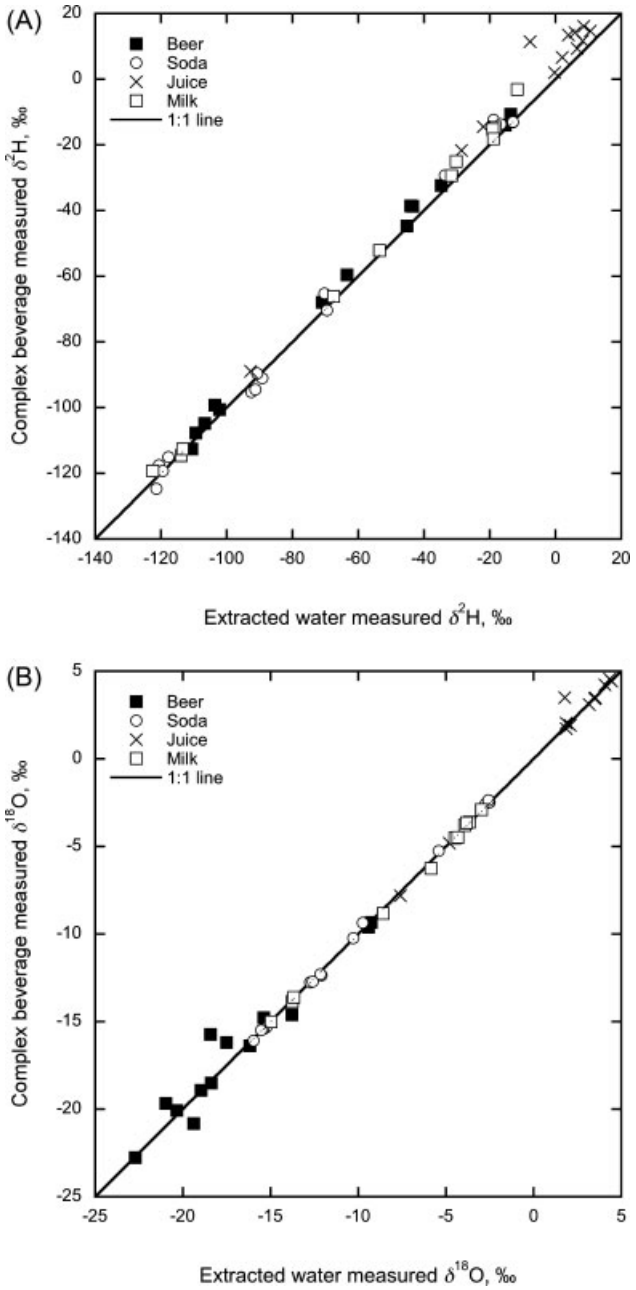

Figure 3. Cross plot of the δ^2H (A) and δ^{18}O (B) values of extracted complex beverages and those same complex beverages analyzed without water extraction using IRIS. The 1:1 line is shown for reference by the solid black line in each panel.

produced by Picarro Inc., we note that some precautions are needed both when analyzing samples and when correcting data. First, the syringe used for injection of complex beverages into the vaporization chamber can quickly become clogged. This most frequently occurred after the injection of juices, which contained high concentrations of sugars. Soaking the syringe in warm water dissolved the most persistent obstructions, and routine cleaning (at least every 8 h) of the syringe with a series of ethanol and water rinses generally prevented sugar crystals from accumulating. We note that we used the standard injection method protocol as programmed on the Picarro model L1102-*i* water analyzer, which did not include a procedure to solvent-clean the syringe between sample injections. It would be relatively straightforward to modify this preset program to include such a cleaning step, thereby reducing the likelihood of obstructions forming in the syringe due to sugars.

Secondly, beverage particulates, such as dissolved sugars, were directly injected into the vaporization chamber, coating it with sticky, caramel-colored residue that was easily visible within the injection inlet and difficult to remove by scraping with a metal spatula. This residue was probably the cause of the increased memory seen during the IRIS analysis of complex beverages. In fact, two batches of complex beverages were analyzed in sequence and the memory correction values determined for the second analysis run of complex beverages were higher than those applied to the first analysis run (Table 1), implying that the memory increased as the amount of residue increased. This suggests that (1) the vaporization chamber should be cleaned regularly to remove residue, preventing the increase of machine memory; and (2) as the memory increases, it may be necessary to increase the number of sample injections. Alternatively, the system could be modified to include a surface for particulates and residue to accumulate before the vaporization chamber. This is the preferred solution, as it does not require machine downtime for maintenance or increase the amount of time (i.e., injection replicates) needed to analyze a sample. Picarro Inc. recently introduced a high-throughput vaporizer (model A0212) that includes a removable inlet liner for this purpose although this liner was not developed in time for use in this study.

Finally, the stable oxygen isotope analysis of alcohol-containing samples using IRIS is not currently possible. While the δ^2H values of alcohol-containing beer samples measured by IRIS matched those measured by IRMS, we found that the measured δ^{18}O values did not agree between the two measurement methods. It may be possible to correct for the interference of ethanol and methanol, as suggested by Brand and colleagues.[12] However, this requires that users know or measure the concentration of ethanol and methanol within their samples *a priori* so that appropriate corrections are applied.

At the time of this study, there were no options in the WS-CRDS water analyzer software to apply an alcohol contaminant correction or to identify those samples that probably contained ethanol and/or methanol and thus required correction. Picarro Inc. has recently introduced ChemCorrect™, software that can screen for spectroscopic irregularities and compare those irregularities against a library of known, common water contaminants. To date, we have not had the opportunity to use ChemCorrect™ when analyzing beverages containing alcohol in order to identify suspect analytical results. From this work, we found that comparing the δ^2H and δ^{18}O values of samples measured using IRIS with the GMWL was a simple way to identify extracted water samples from beer that contained 3–9% alcohol by volume (ABV) as those samples fell above the GMWL. More work is needed to determine if the measured isotope ratios of other beverages containing alcohol at higher (e.g., wine with ~10–15% ABV) or lower (e.g., kombucha, or fermented tea, with 0.5–1.0% ABV) concentrations fit the same pattern.

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CONCLUSIONS

The use of stable isotope analysis, among other analytical techniques, for tracking and tracing the origin of foods and beverages is rapidly expanding, for example through coordinated efforts such as the recently completed TRACE Project,[23] a 5-year program sponsored by the European Union to develop methods to trace the origin of food.[24–26] As consumers demand more information about food items in their diet, including how and where foods and beverages were produced, the need for food traceability using rapid techniques like stable isotope analysis grows.

In this study we have demonstrated that it is possible to quickly and efficiently analyze several types of beverages 'as is', without an initial time-consuming extraction of water, using isotope ratio infrared spectroscopy, specifically wavelength-scanned cavity ring-down spectroscopy. For most beverages under consideration, the stable isotope analysis of extracted water using either IRIS or IRMS returned matching or near-to-matching results. Demonstrating even more promise, the results of the IRIS measurement of the oxygen isotope ratios of extracted beverages waters were statistically indistinguishable from the $\delta^{18}O$ values obtained from the IRIS analysis of unextracted complex beverage; the hydrogen isotope ratios fit 1:1 lines with some evidence of a beverage-specific offset. However, the small differences in the $\delta^{2}H$ values of waters extracted from sodas and juices measured using both IRIS and IRMS cause us to recommend, as did West et al.,[22] that investigators cross-check the IRIS-measured $\delta^{2}H$ and $\delta^{18}O$ values of waters (and beverages) under consideration using IRMS before beginning the wholesale analysis of waters using isotope ratio infrared spectroscopy.

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