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Heavy and Light Beer: A Carbon Isotope Approach To Detect C_4 Carbon in Beers of Different Origins, Styles, and Prices

J. RENÉE BROOKS,*,† NINA BUCHMANN,‡ SUE PHILLIPS,†,+ BRUCE EHLERINGER,§ R. DAVID EVANS,⊥ MIKE LOTT,† LUIZ A. MARTINELLI,|| WILLIAM T. POCKMAN,# DARREN SANDQUIST,⊗ JED P. SPARKS,∇ LYNDA SPERRY,× DAVE WILLIAMS,¶ AND JAMES R. EHLERINGER†

Department of Biology, University of Utah, Salt Lake City, Utah 84112, Max-Planck-Institut für Biogeochemie, Postfach 10 01 64, 07701 Jena, Germany, Washington Group International, Cleveland, Ohio 44113, Department of Biological Sciences, University of Arkansas, Fayetteville, Arkansas 72701, Centro de Energia Nuclear na Agricultura, 13416-000, Piracicaba-SP, Brazil, Department of Biology, University of New Mexico, Albuquerque, New Mexico 87131, Department of Biological Science, California State University, Fullerton, California 92834, Department of Ecology and Evolutionary Biology, Cornell University, Ithaca, New York 14853, Bechtel SAIC Company, LLC, Las Vegas, Nevada 89144, and School of Renewable Natural Resources, The University of Arizona, Tucson, Arizona 85721

The carbon isotope ratios ($\delta^{13}C$) of 160 beers from around the world ranged from −27.3 to −14.9‰, primarily due to variation in the percentage of C_3 or C_4 plant carbon in the final product. Thirty-one percent of beers had a carbon signature of C_3 plants (barley, rice, etc.), whereas the remaining 69% contained some C_3−C_4 mixture (mean of mixtures, 39 ± 11% C_4 carbon). Use of C_4 carbon (corn, cane sugar, etc.) was not confined to beers from any particular region (Pacific Rim, Mexico, Brazil, Europe, Canada, and the United States). However, the $\delta^{13}C$ of European beers indicated mostly C_3 plant carbon. In contrast, U.S. and Canadian beers contained either only C_3 or C_3−C_4 mixtures; Brazilian, Mexican, and Pacific Rim beers were mostly C_3−C_4 mixtures. Among different lagers, U.S.-style lagers generally contained more C_4 carbon than did imported pilsners. Among different ales, those brewed by large high-production breweries contained significant proportions of C_4 carbon, while C_4 carbon was not detected in microbrewery or home-brew ales. Furthermore, inexpensive beers generally contained more C_4 carbon than expensive beers.

KEYWORDS: Carbon isotope ratio; $\delta^{13}C$; beer; adjuncts; C_4; C_3

INTRODUCTION

"It was just an old beer bottle, afloating on the foam,
It was just an old beer bottle, so many miles from home,
And it contained a message with these words writ there on:
Whoever finds this bottle, will find the beer ... all corn."
(modified, author unknown)

Beer is generally comprised of four main ingredients: water, malted barley, hops, and yeast. Other nonessential ingredients are called adjuncts and may include spices, natural flavors, or additional sugars or starch to increase the alcohol content of the final product. The extent to which sugars or starches besides those from malted barley are used is unknown for most beers because beer producers are not required to list ingredients. While many breweries claim to use only the four primary ingredients in the production of beer, others frequently use sugars from sources other than barley, and validating brewery claims can be difficult. The amount of adjuncts used in brewing a particular beer might be of interest to those in the brewing and beer business. In addition, the legal entities of certain countries desire a method for ingredient determination since the use of unlabeled adjuncts is forbidden by law (*1*). With increasing globalization of the world market and the vast number of beers available, a fast, accurate, and inexpensive method for detecting some adjuncts in beer would be useful.

Malted barley extract contains primarily maltose and maltotriose (*2*), generally less fermentable forms of sugar (*3*), while some sugars such as corn sugar are not only less expensive but also mostly sucrose, which is easily metabolized by the yeast

* Corresponding author. Present address: U.S. EPA/NHEERL Western Ecology Division, 200 SW 35th St., Corvallis, OR 97333. Phone: (541) 754-4684. Fax: (541) 754-4799. E-mail: Brooks.ReneeJ@epa.gov.
† University of Utah.
‡ Max-Planck-Institut für Biogeochemie.
§ Washington Group International.
⊥ University of Arkansas.
|| Centro de Energia Nuclear na Agricultura.
University of New Mexico.
⊗ California State University.
∇ Cornell University.
× Bechtel SAIC Co.
¶ The University of Arizona.
+ Present address: USGS Forest and Rangeland Ecosystem Science Center, Canyonlands Field Station, Moab, UT 84532.

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(*3*, *4*). Stern et al. (*4*) demonstrated that brewer's yeast fermented corn sugar more rapidly than more complex barley sugars. Thus, adding corn sugar to beer can greatly accelerate the brewing process and increase the alcohol content with a minimal amount of ingredients. Since decreasing brewing time can increase production rate, the use of corn sugar would decrease the cost of brewing beer. Corn is also less expensive than malted barley, creating an additional incentive to decrease brewing costs through the use of corn instead of barley.

Within the plant kingdom, there exist plants with three different photosynthetic pathways: the C_3, C_4, and crassulacean acid metabolism (CAM) pathways. Many grains, including barley, are C_3 plants. However, C_4 plants such as corn, sorghum, and sugar cane produce the most common inexpensive sugars available on the world market. These highly fermentable C_4 sugars are common additives to certain alcoholic beverages (*5*−*7*). Each photosynthetic pathway discriminates differently against the heavier carbon isotope (^{13}C) present in atmospheric CO_2. The isotopic ratio of organic carbon ($\delta^{13}C$) in sugars used to produce alcohol reflects the photosynthetic pathway of the plant producing the substrate. For example, the factors that determine the $\delta^{13}C$ of C_3 plants can be described by the following equation (*8*):

$$\delta^{13}C_{C_3\ \mathrm{plant}} = \delta^{13}C_{\mathrm{air}} - \left(a + (b - a)\left(\frac{c_i}{c_a}\right)\right) \quad (1)$$

where $\delta^{13}C_{air}$ reflects the source CO_2 in air for photosynthesis, and *a* and *b* are constants representing the discrimination against the heavier isotope of carbon due to diffusion of CO_2 into the leaf (a = 4.4‰ (parts per thousand)) and the discrimination due to carboxylation (b = 27‰). The ratio c_i/c_a is the ratio of the CO_2 concentration inside and outside the leaf. Plants that utilize the C_4 pathway have specialized internal anatomical structures and utilize an additional enzyme (phosphoenolpyruvate or PEP carboxylase) during the process of photosynthesis, which allows CO_2 to be concentrated inside of cells known as the bundle sheath. These specialized leaf structures and the PEP carboxylase enzyme add additional factors that affect discrimination against the heavier isotope of carbon during photosynthesis (*9*):

$$\delta^{13}C_{C_4\ \mathrm{plant}} = \delta^{13}C_{\mathrm{air}} - \left(a + (b_4 + b_3\phi - a)\left(\frac{c_i}{c_a}\right)\right) \quad (2)$$

where b_4 is the discrimination by PEP carboxylase against bicarbonate (−5.7‰), b_3 is the discrimination by ribulose-1,5-bisphosphate (RuBP) carboxylase (29‰), and ϕ is the leakage rate of CO_2 out of the bundle sheath cells. Thus, these two pathways create distinct $\delta^{13}C$ differences between C_3 and C_4 plants, and these differences can be used to track the relative contribution of C_4 and C_3 carbon sources in organic carbon mixtures (*10*−*13*) such as alcoholic beverages. This means that the $\delta^{13}C$ of an alcoholic beverage is an indicator of the amount of C_3 and C_4 ingredients used to produce this beverage. Carbon isotope ratios of alcohol have been used successfully to detect adjunct ingredients in other alcoholic beverages such as wine (*14*), brandy (*5*, *7*, *15*), and whiskey (*5*, *6*). However, this approach has not yet been applied to beer. The analysis of $\delta^{13}C$ is relatively simple with modern isotope ratio mass spectrometers and inexpensive at many commercial laboratories.

The objectives of this study were to determine if C_4 carbon was present in beers widely sold in the United States of America and Brazil, to explore the impact of the brewing process on the $\delta^{13}C$ of the product, and to explore whether differences in the C_4 carbon content were related to the origin, price, or style of the beers.

MATERIALS AND METHODS

A total of 160 beers were sampled in the summer of 1994, covering a wide range of beer styles and many regions of the world. The sample size reflects the variation and availability of beer in the U.S. market (n = 129), with an additional selection of beers purchased in Brazil (n = 31). For each beer sampled, the price, style, brewery, and location of purchase were recorded. Some samples were made available from a private donor, and price data were not available. From each beer sample, 25 mL of beer was placed into a glass Petri dish and dried at 60 °C until no liquid remained. The residues were then ground with a mortar and pestle to a fine powder. A 2-mg subsample of the residue was combusted in an elemental analyzer (model 1108, Carlo Erba, Milano, Italy) which was connected directly to an isotope ratio mass spectrometer (IRMS, Delta S, Finnigan MAT, Bremen, Germany), so the resulting CO_2 was analyzed for $\delta^{13}C$ in the IRMS through continuous flow between the instruments. All samples were analyzed at either the Stable Isotope Ratio Facility for Ecological Research, University of Utah, or the Stable Isotope Laboratory of CENA, University of São Paulo, Brazil. The isotope ratio ($\delta^{13}C$) is reported as

$$\delta^{13}C = (R_{\mathrm{sample}}/R_{\mathrm{standard}} - 1) \times 1000 \quad (3)$$

where R_{sample} and $R_{standard}$ are the $^{13}C/^{12}C$ ratios of the sample and the standard (Pee Dee Belemnite limestone), respectively (*8*). The overall precision of organic carbon isotope measurements was ±0.11‰.

To test the effect of the brewing process itself on the carbon isotope ratio of beer, samples were collected at several steps through a controlled home-brewing process of an English-style ale. Malted barley (1 kg of English 2 Row) was ground and mixed with 4 L of 66 °C water to convert starch into sugar. The resulting wort was mixed with additional barley sugars (1.5 kg of medium peat malt) and hops (Nugget, 14.67 alpha, 28 g), which were added to 20 L of boiling water and boiled for 1 h. After boiling, another 28 g of hops (Williamette, 4.5 alpha) was added, and the mixture was cooled rapidly to 27 °C. The mixture was strained and transferred to a glass carboy for fermentation. Yeast (Muntons) was added, and the carboy was sealed with an airlock. The mixture was allowed to ferment for 2 weeks and was then bottled for carbonation. Samples were taken of the raw ingredients (malted barley, hops, yeast), of the wort produced after the barley starch was converted to sugar, after boiling the wort when the hops were added, and after fermenting. These samples were prepared and analyzed for $\delta^{13}C$ as described above.

To calculate the percentage C_4 carbon in a beer, we assumed that the carbon in the beer residue was from two possible sources: C_3 plants (barley, rice, wheat) or C_4 plants (corn, sugar cane, sorghum). The initial masses of the hops and yeast ingredients are much smaller than that of the carbon from barley contained in the wort, and much of the yeast and hops is removed later in the brewing process. Consequently, the $\delta^{13}C$ signatures of the hops and yeast are not quantitatively important compared to that of barley. We determined the $\delta^{13}C$ values of a variety of malted barley used for beer brewing and the $\delta^{13}C$ of corn and cane sugar, common C_4 adjuncts (**Table 1**). Our measured values for these ingredients were similar to those of other studies with a more extensive sampling of sugars (*12*, *16*). We then used a linear two-ended mixing model to calculate the percentage of C_4 carbon in each beer sample (eq 4):

$$\%\ C_4\ \mathrm{carbon} = \frac{(\delta^{13}C_{\mathrm{beer}} - \delta^{13}C_{C_3})}{(\delta^{13}C_{C_4} - \delta^{13}C_{C_3})} \times 100 \quad (4)$$

where $\delta^{13}C_{C_3}$ = −25.2‰, the measured mean of barley + 1 standard deviation (see below), and $\delta^{13}C_{C_4}$ = −12.5. The mixing model approach to determining the relative amount of C_4 and C_3 carbon is highly robust and has been successfully used in a variety of circumstances, including animal diet (*17*, *18*), soil organic mater composition (*10*, *19*−*21*),

Table 1. Variation in $\delta^{13}C$ Values of Various Carbo-Containing Ingredients of Beer

ingredient	style	$\delta^{13}C$ (‰)
barley	English 2 row	–25.9
barley	English 2 row toasted 10 min	–25.6
barley	English crystal malt	–26.5
barley	German, Munich	–25.8
barley	imported Belgian—Munich style	–26.5
barley	American 2 row	–24.2
barley	medium peat malt	–25.9
barley	Briess traditional dark malt	–26.0
barley	Dewolf-Cosyn's special	–26.8
barley	Briess chocolate malt	–25.9
barley	black patent	–25.8
	average ± SD	–25.9 ± 0.7
hops	Northern Brewers 7.8 alpha	–23.6
hops	Nugget 14.7 alpha	–24.1
hops	Kent Golding 5.2 alpha	–24.6
hops	Chinook 12.1 alpha	–27.5
hops	Cascade 8.1 alpha	–24.7
hops	Williamette 4.5 alpha	–24.9
	average ± SD	–24.9 ± 1.4
yeast	Munton's	–24.0
corn sugar		–13
cane sugar		–12
	average ± SD	–12.5 ± 0.3

paleoecology (*11*, *12*) and food authentication and characterization (*5*, *22*, *23*). Because sucrose is preferentially fermented before other sugars (*4*), this percentage of C_4 carbon represents the C_4 carbon in beer after brewing.

All statistical analyses were conducted using SPSS (Version 10, SPSS Inc., Chicago, IL). Because of nonhomogeneity of variance, we used nonparametric statistical tests. For categorical differences (e.g., among regions of origin or beer styles), one-way ANOVA was used. For the trend in price with $\delta^{13}C$ values, a simple generalized linear model was used. Variance is reported throughout the paper as standard deviation (SD) unless otherwise noted (e.g., SE for standard error).

RESULTS AND DISCUSSION

The carbon isotope ratios of all 160 beer samples ranged from −27.3‰ to −14.9‰, with a mean of −21.9 ± 2.8‰. The distribution of the $\delta^{13}C$ values for beer was bimodal (**Figure 1B,C**), with one peak centered around −25‰ and the other around −19‰. The bimodality was pronounced for North American beers (**Figure 1B**), while beers from Europe, Brazil, Mexico, and the Pacific Rim tended to fall in only one peak (**Figure 1C**). The bimodal distribution closely resembled that found in the plant kingdom, where $\delta^{13}C$ can be used to clearly separate C_3 from C_4 plants (**Figure 1A**) (*12*, *24*). European beers had an average $\delta^{13}C$ value of −25.6 ± 1.5‰), significantly more negative isotopically ($P < 0.001$) than all other regions included in this study (mean, −21.4 ± 2.6‰). Brazilian beers had the highest isotopic ratio (−19.7 ± 2.4‰), significantly higher than those of Canadian and European beers ($P < 0.001$).

Average $\delta^{13}C$ values for C_3 plants are typically about −26 ‰, whereas the average $\delta^{13}C$ for C_4 plants is about −12‰ (**Figure 1A**). Generally, the main carbon source in beer is malted barley, a C_3 plant with an average carbon isotope ratio of −25.9 ± 0.7‰) (**Table 1**). Other typical carbon-containing ingredients in beer are hops (−24.9 ± 1.4‰) and yeast (−24‰), although their contribution of carbon to the carbon in beer is generally very small compared to that supplied by barley. Thus, the peak for the beer samples at −25 ‰ corresponds very well with what we would expect if all the carbon in beer were from a C_3 carbon source (**Figure 1**). The second peak in the $\delta^{13}C$ values of beer, at −19‰, could be explained either by fractionation during the brewing process or by mixing C_3 and C_4 carbon sources.

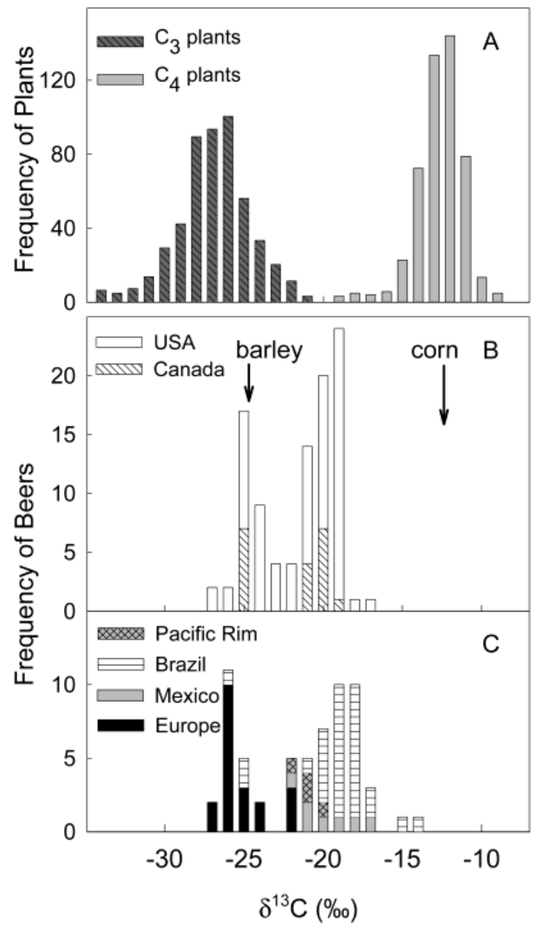

Figure 1. (A) Absolute frequencies of carbon isotope ratios ($\delta^{13}C$) of C_3 and C_4 plants ($n = 1000$; adapted from Cerling et al. (*12*)). (B) Absolute frequencies of carbon isotope ratios ($\delta^{13}C$) of beers brewed in the United States of America and in Canada. (C) Absolute frequencies of carbon isotope ratios ($\delta^{13}C$) of beers brewed in Europe, Mexico, Brazil, and the Pacific Rim (Japan, Australia). Arrows indicate average $\delta^{13}C$ values for two main beer ingredients, malted barley and corn sugar (see **Table 1**).

The process of brewing beer with all C_3 ingredients did not significantly change the isotopic ratio from the raw ingredients to the final product. The malted barley used in the controlled brewing was a mixture of English 2 row and medium peat malt with a $\delta^{13}C$ value of −25.9‰, which is similar to that of raw barley, so the malting process did not alter the isotopic ratio. The hops were an equal combination of Nugget (bittering hops 14.67 alpha) and Williamette hops (finishing hops, 4.5 alpha) with an average $\delta^{13}C$ value of −24.3‰. We used Munton's yeast with a $\delta^{13}C$ value of −24.0‰. Converting the malted barley starch to sugar during mashing resulted in a $\delta^{13}C$ similar to that of the original malted barley (−25.6‰). The next step of boiling and adding the hops had no effect on the $\delta^{13}C$ value of the intermediate product (−25.4‰). The final step of fermenting also produced no shift in the $\delta^{13}C$ (−25.9‰). Others have also found that fermentation does not cause a significant fractionation of carbon isotopes (*4*, *25*). On the basis of these results, we concluded that the large variation in $\delta^{13}C$ we observed in the beers could not have been due to isotopic shifts of the C_3 signature during brewing, but was related to the amount of C_4 and C_3 ingredients in the final beer product.

We calculated the contribution of C_4 carbon to the total carbon content (see eq 4), assuming that malted barley had a $\delta^{13}C$ value

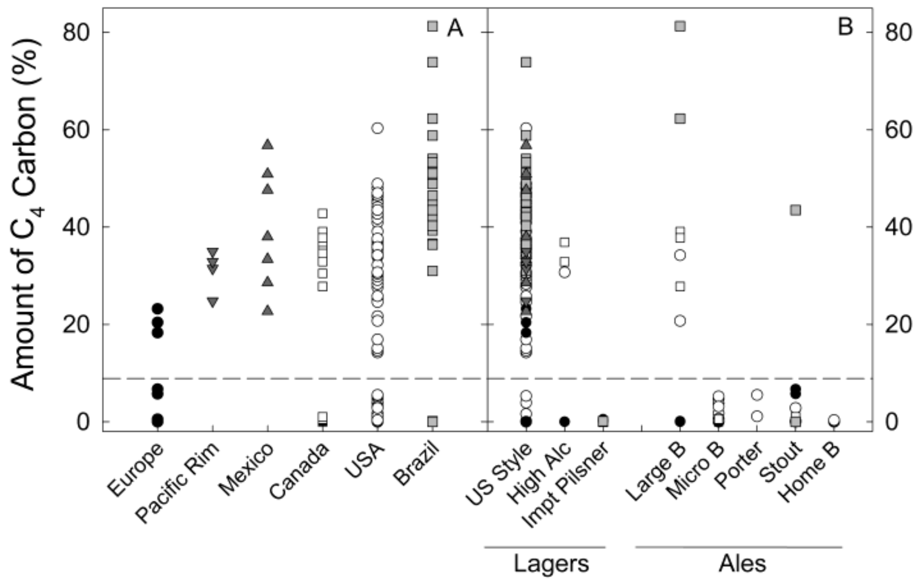

Figure 2. (A) Amounts of C_4 carbon in beers from around the world. Different symbols are used to represent the different regions. (B) Amount of C_4 carbon in different types of lagers and ales (High Alc, high alcohol lagers; Impt Pilsner, imported pilsner; Large B, large brewery ales; Micro B, microbrewery ales; home B, home-brewed ales). Note, 35 beers have a δ^{13}C value of 0% C_4 carbon and, thus, are not all visible along the *x*-axis. The dashed line represents our most conservative threshold between beers with only C_3 carbon and beers with a mixture of C_3 and C_4 carbon. See text for details.

Table 2. Regional Summary of Beers Using C_4 Carbon and the Average Percentage of C_4 Carbon Used in Those Mixtures (±SD)

	n_{total}	$n_{mixture}$[a]	% beers using C_4	% C_4 in mixtures[b]	
Europe	20	3	15	20.7 ± 2.5	a
Pacific Rim	4	4	100	31.1 ± 4.4	ab
Mexico	7	7	100	39.7 ± 12.5	bc
Canada	19	12	63	35.3 ± 4.0	abc
USA	79	56	71	36.6 ± 9.8	bc
Brazil	31	28	90	48.7 ± 10.8	c
total	160	110	69	39.1 ± 11.4	

[a] $n_{mixture}$ represents the number of beers with a percent C_4 carbon content greater than 8% (see details in text). [b] Different letters indicate significant differences among the percentage of C_4 carbon used in mixtures from different regions.

of −25.2‰ and C_4 sugars had a δ^{13}C value of −12.5‰. The value of −25.2‰ (mean for malted barley + 1 SD) was used instead of −25.9‰ (mean of malted barley) for the C_3 carbon source in order to give a conservative estimate of the C_4 carbon percentage in the beer samples. In addition, we established a threshold C_4 carbon percentage, below which beers were considered to be from C_3 ingredients in order to avoid “false positives” of C_4 carbon detection. Using −25.2‰ in our calculations, 8% C_4 carbon use translates to a δ^{13}C of −24.2‰, which was the highest δ^{13}C value of malted barley measured (**Table 1**). If we had used the mean (−25.9‰), then −24.2‰ would have been calculated as 12.7% C_4 carbon and potentially provided a “false positive” for the presence of C4 ingredients. In the beer sampled in this study, a gap exists between 6.7% and 14.2% C_4 carbon. We interpreted this gap to be a distinguishing point between C_3 beers and beers that contain a C_3 and C_4 mixture. Thus, using an 8% threshold, 31% of all beers analyzed indicated all C_3 ingredients in the final product. The remaining 69% of all beers were a mixture of C_3 and C_4 carbon, with an average of 39 ± 11% C_4 carbon (**Table 2**).

Separating the beers according to their regions of origin, we found C_4 carbon in beers from many regions around the world (**Figure 2A**, **Table 2**). Only 3 of the 20 European beers contained C_4 carbon, and these mixtures were generally lower in C_4 carbon than those from other regions (21% vs 40%). For Canadian beers, 63% contained C_4 carbon, and 71% of American beers used some C_4 ingredients. Ninety percent of Brazilian beers were C_3 and C_4 mixtures, and the fraction of C_4 carbon used was the highest of any region (49 ± 11%). All the beers from the Pacific Rim and Mexico contained some amount of C_4 ingredients. The low occurrence of C_4 products in the European samples may reflect that some European countries have strict laws that regulate the ingredients of beer. For example, German breweries must strictly follow the German Beer Law that is based on the Bavarian Purity Law (Reinheitsgebot) from 1516. This law restricted the ingredients of beer to water, barley, and hops. In 1516, yeast addition to start fermentation was unknown, and infection occurred naturally by air-borne yeasts. Therefore, yeast was not a recognized ingredient in beer. All German beers in our study had δ^{13}C values more negative than −25.5‰, indicating only C_3 carbon in these beers. In addition, historically Europeans did not have easy access to C_4 plants because these plants generally do not occur at high latitudes (*26*). Corn sugar originated in Central and South America, and cane sugar is grown extensively in these regions, so it is not surprising that these regions utilize native C_4 sugar sources.

The use of C_4 carbon appeared to be prevalent in certain styles of beer, most notably the common U.S.-style lagers ($n = 105$) and ales ($n = 9$) brewed by large breweries in the United States, Canada, Brazil, Mexico, and the Pacific Rim (**Figure 2B**). Although 7 of the 105 U.S.-style lagers we sampled use only C_3 carbon, the other 98 beers contained an average of 39 ± 11% C_4 carbon. Lager beers that were brewed to enhance the alcohol content (i.e., malt liquors and ice beers, $n = 4$) also contained a high percentage of C_4 carbon (34 ± 3%), with the exception of the only German malt liquor, which showed no C_4 carbon contribution in the final beer product. European-style lagers, e.g., import pilsners ($n = 10$) from Germany and The Netherlands, as well as specialty ales, e.g., porters ($n = 2$) and stouts ($n = 11$), fashioned after the English style of ale brewing, did not contain significant C_4 carbon amounts, with the exception of one Brazilian stout which contained 43.5% C_4

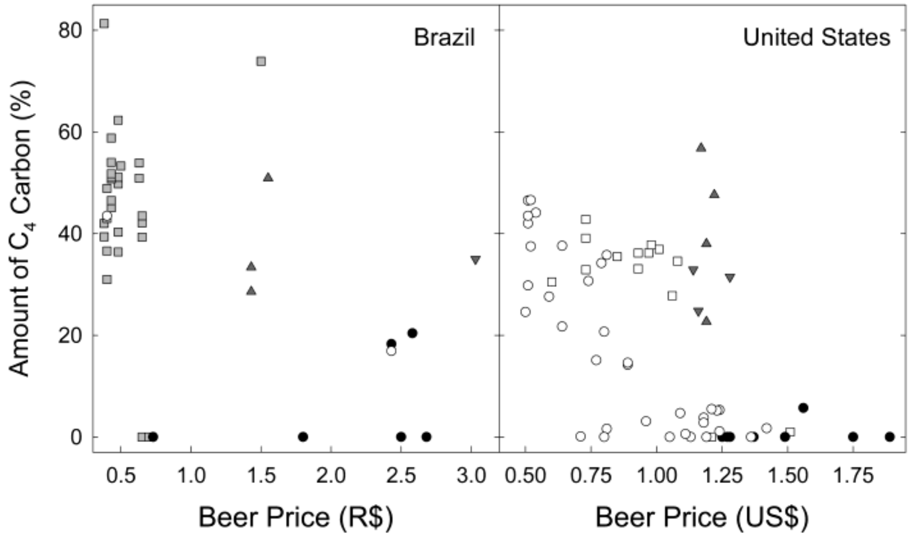

Figure 3. Relationship between percentage of C_4 carbon and price of 0.35 L of beer sold in Brazil or the United States. Symbols are the same as in **Figure 2**. Prices for beers purchased in Brazil are given in the Brazilian Real (R$), and those purchased in the United States in U.S. dollars (US$).

carbon. All microbrewery ($n = 15$) and home-brew beers ($n = 4$) did not contain significant amounts of C_4 carbon.

Relating the percentage C_4 carbon of beers in our study to the price of 0.35 L of beer (prices were normalized to a standard volume of beer), we found that the C_4 content increased as beer prices decreased (**Figure 3**). This trend was significant in both locations where beers were purchased, Brazil (adj $R^2 = 0.23$, $P < 0.001$) and the United States (adj $R^2 = 0.38$, $P < 0.001$). This price trend in the beer samples is interesting since the price of raw ingredients is only a small component of the cost of beer. Beer prices are highly influenced by the cost of labor, processing, packaging, and marketing in addition to the ingredient cost. Nevertheless, the trend exists although there is variation in the data. Two contributing factors could be that using C_4 sugars accelerates the brewing process (thereby decreasing the indirect costs outlined above) and that C_4 ingredients are less expensive than malted barley. Adding corn or cane sugar results in higher alcohol content with minimal time and ingredients. Excluding imported beer, which is more expensive due to other factors (transport, import taxes, customs, etc.), the price trend is significant within groups with large sample sizes, for instance, U.S. domestic beers (**Figure 3**). A similar price trend was noted with Brazilian brandies, where prices increased with decreasing C_4 content (*7*). In contrast, European beers did not follow this trend since they mostly use C_3 ingredients, independent of their price. Mexican beers were unusual because they generally had high prices along with high C_4 contents. In the United States, many of the more expensive beers were brewed by microbreweries that typically advertise the purity of their ingredients, whereas the less expensive beers were brewed by much larger high-volume production breweries.

Brewing beer with a large proportion of grist other than barley is not illegal in most regions. However, many beer companies advertise the purity of their products, and thus misrepresent their product if they are adding ingredients not traditionally used in brewing beer. Traditionally, beer is a fermented alcoholic beverage made from malted barley and flavored with hops. Like wine drinkers who expect a product made from grapes, beer drinkers expect their beer is made from barley unless clearly labeled otherwise, such as wheat beer. Analysis of the carbon isotope ratio of beer allows for accurate detection of C_4 carbon in beer, which can comprise over 40% of the carbon in beer even after fermentation. While $\delta^{13}C$ analysis cannot detect all adjuncts in beer (for example rice, wheat, and beet sugar, all C_3 carbon additives), the analysis provides a powerful tool for detecting ingredients and testing the claims of brewers. In addition, breweries may be interested in the ingredients used by their competitors. Moreover, providing brewing ingredients on the labels can help consumers make informed choices about the products they purchase.

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LITERATURE CITED

(1) BGBl. Bundesgesetzblatt Teil I. Preliminary German Beer Law Part 1. 1993, pp 1399−1401.

(2) Hough, J. S. *The biotechnology of malting and brewing*; Cambridge University Press: Cambridge, 1985; p 168.

(3) Phillips, A. W. Utilization by yeasts of the carbohydrates wort. *J. Inst. Brewing* **1955**, *61*, 1−122.

(4) Stern, L. A.; Chamberlain, C. P.; Blum, J. D. Isotopic lessons in a beer bottle. *J. Geosci. Educ.* **1997**, *45*, 157−161.

(5) Simpkins, W. A.; Rigby, D. Detection of the illicit extension of potable spirituous liquors using ^{13}C:^{12}C ratios. *J. Sci. Food Agric.* **1982**, *33*, 898−903.

(6) Parker, I. G.; Kelly, S. D.; Sharman, M.; Dennis, M. J.; Howie, D. Investigation into the use of carbon isotope ratios ($^{13}C/^{12}C$) of Scotch whisky congeners to establish brand authenticity using gas-chromatography-combustion-isotope ratio mass spectrometry. *Food Chem.* **1998**, *63*, 423−428.

(7) Pissinato, L.; Martinelli, L. A.; Victoria, R. L.; Camargo, P. B. Using stable carbon isotopic analyses to access the botanical origin of ethanol in Brazilian brandies. *Food Res. Int.* **1999**, *32*, 665−668.

(8) Farquhar, G. D.; Ehleringer, J. R.; Hubick, K. T. Carbon isotope discrimination and photosynthesis. *Annu. Rev. Plant Physiol. Mol. Biol.* **1989**, *40*, 503−537.

(9) Farquhar, G. D. On the nature of carbon isotope discrimination in C4 plants. *Aust. J. Plant Physiol.* **1983**, *10*, 205−226.

(10) Boutton, T. W. Stable carbon isotope ratios of soil organic matter and their use as indicators for vegetation and climate change. In *Mass Spectrometry of Soils*; Boutton, T. W., Yamasaki, S., Eds.; Marcel Dekker: New York, 1996; pp 47−82.

(11) Cerling, T. E.; Wang, Y.; Quade, J. Expansion of C4 ecosystems as indicators of global ecological change in the late Miocene. *Nature* **1993**, *361*, 344−345.

(12) Cerling, T. E.; Harris, J. M.; MacFadden, B. J.; Leakey, M. G.; Quade, J.; Eisenmann, V.; Ehleringer, J. R. Global vegetation change through the Miocene/Pliocene boundary. *Nature* **1997**, *389*, 152−158.

(13) Buchmann, N.; Ehleringer, J. R. CO_2 concentration profiles and carbon and oxygen isotopes in C_3 and C_4 crop canopies. *Agric. For. Meteorol.* **1998**, *89*, 45−58.

(14) Weber, D.; Rossmann, A.; Schwarz, S.; Schmidt, H.-L. Correlations of carbon isotope ratios of wine ingredients for the improved detection of adulterations I. Organic acids and ethanol. *Z. Lebensm. Unters. Forsch. A* **1997**, *205*, 158−164.

(15) Hogben, R.; Mular, M. Major congeners of Australian and imported brandies and other spirits as indicators of authenticity. *J. Sci. Food Agric.* **1976**, *27*, 1108−1114.

(16) Carro, O.; Hillaire-Marcel, C.; Gagnon, M. Sugars and sugar products: Detection of adulterated maple products by stable carbon isotope ratio. *J. Assoc. Off. Anal. Chem.* **1980**, *63*, 840−845.

(17) Phillips, D. L. Mixing models in analyses of diet using multiple stable isotopes: a critique. *Oecologia* **2001**, *127*, 166−170.

(18) Steuter, A. A.; Steinauer, E. M.; Hill, G. L.; Bowers, P. A.; Tieszen, L. L. Distribution and diet of bison and pocket gophers in a sandhills prairie. *Ecol. Appl.* **1995**, *5*, 756−766.

(19) Boutton, T. W.; Archer, S. R.; Midwood, A. J.; Zitzer, A. F.; Bol, R. $\delta^{13}C$ values of soil organic carbon and their use in documenting vegetation change in a subtropical savanna ecosystem. *Geoderma* **1998**, *82*, 5−41.

(20) Balesdent, J.; Mariotti, A. Measurement of soil organic matter turnover using ^{13}C natural abundance. In *Mass Spectrometry of Soils*; Boutton, T. W., Yamasahi, S., Eds.; Marcel Dekker: New York, 1996; pp 83−111.

(21) Bird, M. I.; Pousai, P. Variations in $\delta^{13}C$ in the surface soil organic carbon pool. *Global Biogeochem. Cycles* **1997**, *11*, 313−322.

(22) Martin, G. G.; Martin, Y. L.; Naulet, N.; McManus, H. J. D. Application of H-2 SNIF-NMR and C-13 SIRA-MS analyses to maple syrup: Detection of added sugars. *J. Agric. Food Chem.* **1996**, *44*, 3206−3213.

(23) Simpkins, W. A. Detection of Illicit Spirits. In *Wine Analysis*; Linskens, H.F., Jackson, J.F., Eds.; Springer-Verlag: Berlin, 1988; pp 317−338.

(24) O'Leary, M. H. Carbon isotopes in photosynthesis: fractionation technique may reveal new aspects of carbon dynamics in plants. *BioSci* **1988**, *38*, 328−336.

(25) DeNiro, M. J.; Epstein, S. Mechanism of carbon isotope fractionation associated with lipid synthesis. *Science* **1977**, *197*, 261−263.

(26) Ehleringer, J. R.; Sage, R. F.; Flanagan, L. B.; Pearcy, R. W. Climate change and the evolution of C_4 photosynthesis. *Trends Ecol. Evol.* **1991**, *6*, 95−99.

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