

## RESEARCH ARTICLE

# Carbon and nitrogen stable isotopes in U.S. milk: Insight into production process

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**Rationale:** Stable isotope analysis (SIA), a potential method of verifying the geographic origin and production method of dairy products, has not been applied to United States (U.S.) dairy samples on a national scale. To determine the potential of carbon and nitrogen SIA in authenticity assessment of U.S. dairy products, we analyzed a geographically representative collection of conventional milk samples to determine isotopic variations with (1) Purchase Location and (2) Macronutrient Content.

**Methods:** A total of 136 milk samples spanning five commercially available varieties (3.25% [i.e., 'whole'], 2%, 1%, 0% [i.e., 'skim'] and 1% chocolate) were collected from randomly selected counties across the U.S. as part of the United States Department of Agriculture's (USDA's) National Food and Nutrient Analysis program.  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of bulk samples determined via elemental analysis/isotope ratio mass spectrometry (EA/IRMS) were used to assess the contribution of fat content, added sugar content and census-designated region of collection to isotopic variations within the dataset.

**Results:** There was a negative linear relationship between fat content and  $\delta^{13}\text{C}$  values, with average milk  $\delta^{13}\text{C}$  values decreasing by 0.33‰ for each 8.75% increase in dry weight (1% wet weight) fat content. The average  $\delta^{13}\text{C}$  value of flavored 1% chocolate milk samples, which contain an additional 12 g of added sugar, was 2.05‰ higher than that of 1% unflavored milk (-16.47‰ for chocolate milk vs -18.52‰ for unflavored milk). When controlling for macronutrient content, milk samples collected in West region supermarkets possessed significantly lower  $\delta^{13}\text{C}$  values than samples collected from Midwest, South, and Northeast regions.  $\delta^{15}\text{N}$  values did not vary with macronutrient content or region of collection.

**Conclusions:** Carbon stable isotope ratios in U.S. milk samples varied with macronutrient content and region of purchase, suggesting that SIA can provide insight into production processes within the U.S. dairy industry, with potential applications in national food adulteration and authentication efforts.

## 1 | INTRODUCTION

The origin of dairy products is of interest in the United States (U.S.) given the ethical and ecological implications of modern cattle rearing practices. To meet the growing transparency demands of consumers, dairy distributors have introduced products with specific production method (organic, pasture-raised, etc.) and geographic origin ('Real California Milk' and 'Eat Wisconsin Cheese') claims and priced them higher than their conventional counterparts, making them prime targets for counterfeiting and fraud.<sup>1</sup> Thus, there is a pressing need for simple methods of verifying the geographic origin and production method of U.S. dairy products.

Numerous verification methods have been proposed, including analysis of fatty acids,<sup>2,3</sup> fat soluble vitamins,<sup>3</sup> and ultraviolet-absorbing compounds.<sup>4</sup> While these methods have demonstrated various levels of success in differentiating feeding regimes and geographic origins of animal-based food products, the analysis of these compounds requires complex pre-extraction and purification steps, rendering the methods incompatible with the large-scale sampling efforts that a nationwide authentication effort would entail.

One emerging method for food product origin assessment, which overcomes the above limitations, is Stable Isotope Analysis (SIA) of dairy products. SIA requires no complicated extraction steps and can be run on an automated isotope ratio mass spectrometer. Since stable

isotope ratios of animal tissues reflect the stable isotope composition of their diet.<sup>5,6</sup> SIA has the potential to distinguish the intake of isotopically distinct diets within dairy systems. For example, the stable isotope ratio of carbon ( $^{13}\text{C}/^{12}\text{C}$ ) can be used to determine the intake of  $^{13}\text{C}$ -enriched C4 photosynthetic crops (e.g., maize) relative to C3 crops (e.g., wheat, silage),<sup>7,8</sup> while the nitrogen stable isotope ratio ( $^{15}\text{N}/^{14}\text{N}$ ) can be used to distinguish the fertilization method of feed crops and the proportion of nitrogen-fixing crop species (e.g., soybeans) in the diet.<sup>9</sup> Numerous studies throughout Europe and Australasia have assessed the relationships between light-element stable isotope signatures and the geographic origin<sup>10-17</sup> and feeding/rearing practices<sup>18-24</sup> of dairy products. SIA shows promise as a method of origin authentication, as it was recently used to successfully detect counterfeits of European cheeses with protected designation of origin (PDO).<sup>25,26</sup>

Despite the success of SIA in dairy product authentication within the international literature, the stable isotope composition of U.S. dairy products has not been well explored. In international analyses of butter<sup>27</sup> and milk,<sup>11</sup> the  $\delta^{13}\text{C}$  values of U.S. dairy products were 5–8‰ higher than their European and Australian counterparts. However, due to the limited sample size and geographic scope of the U.S. sample sets ( $n < 7$  for both cases), these studies were unable to determine the sources of the isotopic variations within the U.S. dairy supply. Such variations, which could arise from differences in the isotopic composition of the source cattle's diet (e.g., ratio of C3/C4 grains and silage) or in the amount of isotopically conspicuous macronutrients in the final product (e.g., fat and/or added sugar content of the milk), may provide valuable information about the origin of milk within the U.S. food supply.

To determine the utility of SIA as a tool for verifying the production process of U.S. dairy products, constraining the sources and extent of isotopic variability within the U.S. dairy supply is essential. Here, we characterize the variability in carbon and nitrogen stable isotope composition of conventional milk by purchase location and macronutrient content via analysis of a geographically representative collection of commercially available unflavored (skim, 1%, 2% and whole) and flavored (1% chocolate) milk varieties provided by the USDA's National Food and Nutrient Analysis Program.

## 2 | EXPERIMENTAL

### 2.1 | USDA food sampling

Conventional (non-organic) milk samples spanning four fat contents (3.25% [i.e., 'whole'], 2%, 1%, and 0% [i.e., 'skim']), as well as flavored 1% chocolate milk, were collected by the United States Department of Agriculture (USDA) Nutrient Data Lab (NDL) as part of the National Food and Nutrient Analysis Program (NFNAP). Full details of the NFNAP sampling plan can be located in the USDA's food sampling protocol.<sup>28</sup> Briefly, 24 counties within the U.S. were randomly selected for sample collection from the four census-designated geographic regions using a probability-proportional-to-size sampling plan based on regional consumption data. In each selected county, 24 milk samples of each fat content were collected from randomly selected

supermarkets and shipped on dry ice to one of the NDL's satellite laboratories for processing. Brand selection was based on current market share data at each sampling location. Upon arriving at the USDA's food analysis laboratory, the 24 samples were homogenized to produce a 'composite' sample for each county. Thus, the sample set represents an extensive, geographically representative sampling of milk within the U.S. food supply, with a 'theoretical'  $n$  of 576 (24 counties  $\times$  24 samples/county) for each milk variety.

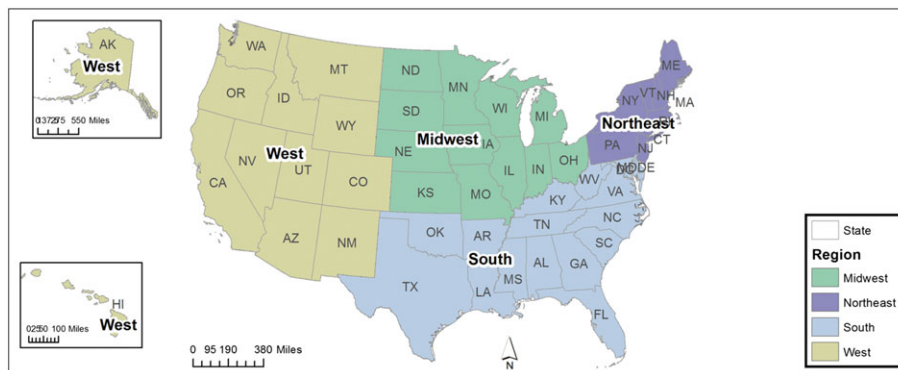
Samples were collected and processed between 2008 and 2013 and stored at  $-80^\circ\text{C}$  at the USDA's Food Analysis Laboratory Control Center in Blacksburg, Virginia. Neither freezing nor cold storage affects the stable carbon or nitrogen isotope composition.<sup>29</sup> The samples were shipped on dry ice in 60-mL glass vials to the University of Hawaii at Manoa and stored at  $-40^\circ\text{C}$  prior to stable isotope analysis.

### 2.2 | Stable isotope analysis

Milk samples were freeze-dried for 48 h in a Freezone 4.5-L freeze-dryer (Labconco, Kansas City, MO, USA) and homogenized with a mortar and pestle, using liquid nitrogen when necessary. Two milligrams of each sample were loaded into high-purity tin capsules (EA Consumables, Pennsauken, NJ, USA) and analyzed for carbon and nitrogen stable isotope composition using an ECS 4010 elemental analyzer (Costech Analytical Technologies Inc., Valencia, CA, USA) coupled with a DeltaV stable isotope mass spectrometer (Thermo Scientific, Bremen, Germany) via a Conflo IV interface (Thermo Scientific). Stable isotope ratios are reported in standard  $\delta$ -notation [ $\delta = R_{\text{sample}}/R_{\text{standard}} - 1$ ], with  $R$  representing the isotope ratio ( $^{13}\text{C}/^{12}\text{C}$ ,  $^{15}\text{N}/^{14}\text{N}$ ) in the sample or international standard (VPDB for carbon, AIR for nitrogen). Post-run off-line blank-, size-, and drift-corrections for assigning the final  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values on the VPDB and AIR scales, respectively, were performed according to Werner and Brand.<sup>30</sup> The internal laboratory standards used in normalizing data were L-glutamic acid (Thermo Fisher, Grand Island, NY, USA;  $\delta^{13}\text{C} = -13.43\text{‰}$ ) and glycine (Thermo Fisher;  $\delta^{13}\text{C} = -43.51\text{‰}$ ) for carbon and glycine (Thermo Fisher;  $\delta^{15}\text{N} = 1.24\text{‰}$ ) and glycine (Brian Popp Lab;  $\delta^{15}\text{N} = 11.25\text{‰}$ ) for nitrogen. Values are reported in units of permil (‰) and represent the mean of three analyses (standard deviation (SD) of three replicates never exceeded 0.1‰ for  $\delta^{13}\text{C}$  values and 0.2‰ for  $\delta^{15}\text{N}$  values).

### 2.3 | Statistical analysis

Descriptive statistics (means, SDs, sample size) are reported for each fat level. To determine the effect of fat content on milk stable isotope composition, we conducted simple linear regressions between the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of unflavored milk samples and fat content as % dry mass. The water contents of milk varieties for dry mass calculations were derived from the USDA's National Nutrient Database for Standard Reference.<sup>31</sup> One-way analysis of variance (ANOVA) was used to assess whether the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of USDA milk samples differed between purchase regions. When a significant  $F$ -value was found, the means were separated using Tukey's Honestly Significant Difference (HSD) test at a confidence level of 95%. The four census-designated regional divisions used in our



**FIGURE 1** Census-designated regions of the United States as defined by the U.S. Census Bureau<sup>32</sup>

study were the West, Midwest, South, and Northeast (Figure 1), as defined by the U.S. Census Bureau.<sup>32</sup> Statistical analyses were conducted using GraphPad Prism 6 (Graphpad Software Inc., La Jolla, CA, USA).

### 3 | RESULTS

Summary statistics of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values for each milk variety (0%, 1%, 2%, 3.25%, and 1% chocolate) are presented in Table 1. The complete dataset is available in Table S1 (supporting information).

For unflavored samples, there was a significant negative linear relationship between fat content and measured  $\delta^{13}\text{C}$  values (Figure 2), with average milk  $\delta^{13}\text{C}$  values decreasing by 0.33‰ for each 8.75% increase in dry weight (1% wet weight) fat content ( $y = -0.0376x - 18.15$ ,  $p < 0.0001$ ). The calculated carbon isotope discrimination between the lipid and lipid-free portions of milk ( $\Delta^{13}\text{C}_{\text{lipid-lipid free}}$ ) was  $-3.76\text{‰}$ . There was a larger range of  $\delta^{13}\text{C}$  values for whole milk (4.01‰) than for the 2% fat milk (3.58‰), 1% (3.65‰), and skim milk (3.25‰) samples, although the variances were not significantly different (Figure 3). Milk  $\delta^{15}\text{N}$  values did not vary with fat level, with all flavored and unflavored samples fitting within a narrow range of 4.5–6.2‰ (Figure 4). The average  $\delta^{13}\text{C}$  value of 1% chocolate milk was  $-16.47\text{‰}$ , which was 2.05‰ higher than that of 1% unflavored milk ( $-18.52\text{‰}$ ).

Milk samples collected in West region supermarkets possessed significantly lower  $\delta^{13}\text{C}$  values than samples collected from the Midwest, South, and Northeast regions (Figure 2), with average  $\delta^{13}\text{C}$  values of West region milk samples being 1–2.1‰ lower than those of other regions. Within the West region, milk samples collected

**TABLE 1** Mean USDA milk carbon and nitrogen stable isotope ratios by nutrient content\*

Variety	Fat content (% dry weight)	$\delta^{13}\text{C}$ values* (‰)	$\delta^{15}\text{N}$ values* (‰)
Skim	0.96	$-18.08 \pm 0.84$ (27)	$5.21 \pm 0.40$ (27)
1%	8.75	$-18.53 \pm 0.89$ (27)	$5.24 \pm 0.44$ (27)
2%	17.50	$-18.91 \pm 0.97$ (28)	$5.17 \pm 0.39$ (28)
Whole	29.41	$-19.19 \pm 1.06$ (28)	$5.09 \pm 0.41$ (28)
1% Chocolate	8.75	$-16.49 \pm 1.03$ (26)	$5.27 \pm 0.40$ (26)

\*Mean  $\pm$  1 SD (number of samples)

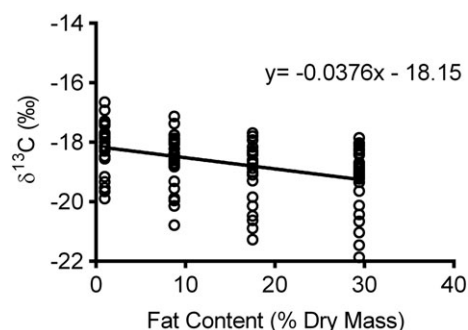
from the Pacific coast states (California and Washington) possessed the five lowest  $\delta^{13}\text{C}$  values in all fat levels tested. For skim milk, the mean  $\delta^{13}\text{C}$  value of these 'Pacific west' samples ( $-19.51 \pm 0.28\text{‰}$ ) was significantly lower than that of the 'mountain west' samples from Colorado and Arizona ( $-17.76\text{‰} \pm 0.52$ ). The highest  $\delta^{13}\text{C}$  values ( $-16.65\text{‰}$  for skim milk) were found in milk samples from North Carolina. Mean regional  $\delta^{15}\text{N}$  values were not significantly different.

### 4 | DISCUSSION

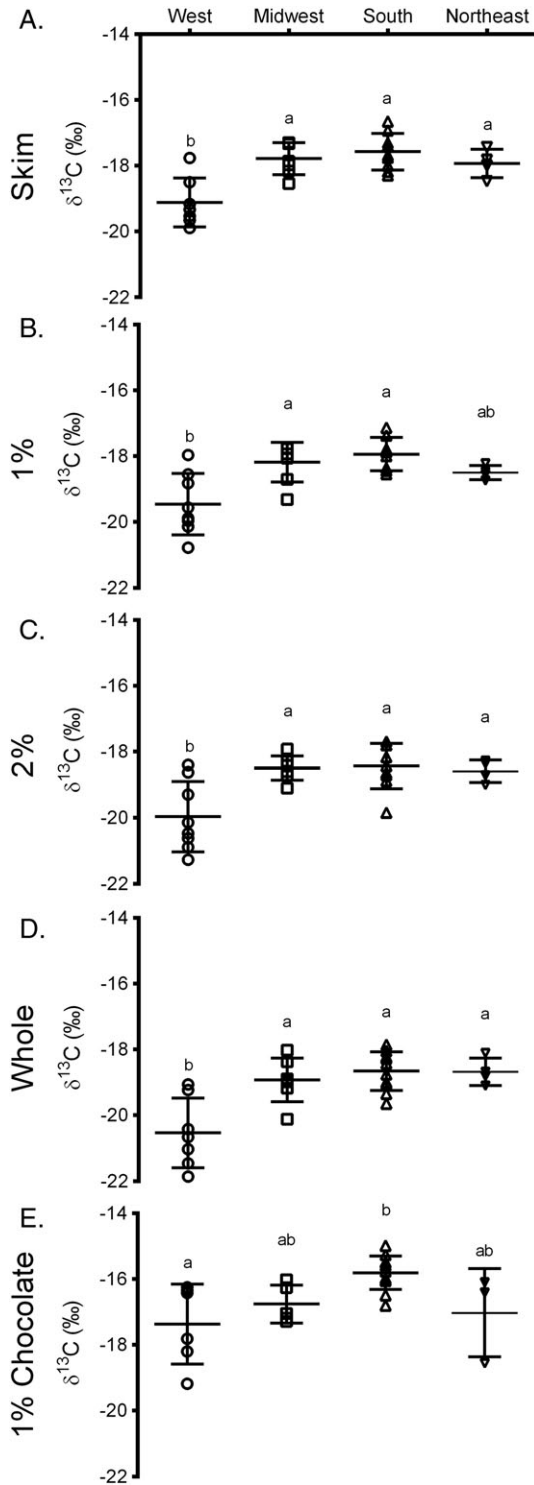
Our study represents the first nationwide characterization of carbon and nitrogen stable isotope signatures for commercially available milk varieties within the United States food supply. Our analyses revealed variations in the carbon stable isotope signatures of milk with (1) Macronutrient Content and (2) Region of Collection.

#### 4.1 | Fat/added sugar content alters milk $\delta^{13}\text{C}$ signatures

The stable isotope signatures of milk products are influenced by the unique compositions of naturally occurring and added nutrients. Of the naturally occurring components, the difference in carbon stable isotope composition between the lipid (triacylglycerols/sterols) and lipid-free (protein/carbohydrate) components ( $\Delta^{13}\text{C}_{\text{lipid-lipid free}}$ ) is particularly conspicuous, with lipids possessing lower  $\delta^{13}\text{C}$  values due to enzymatic fractionations during formation.<sup>5</sup>  $\Delta^{13}\text{C}_{\text{lipid-lipid free}}$

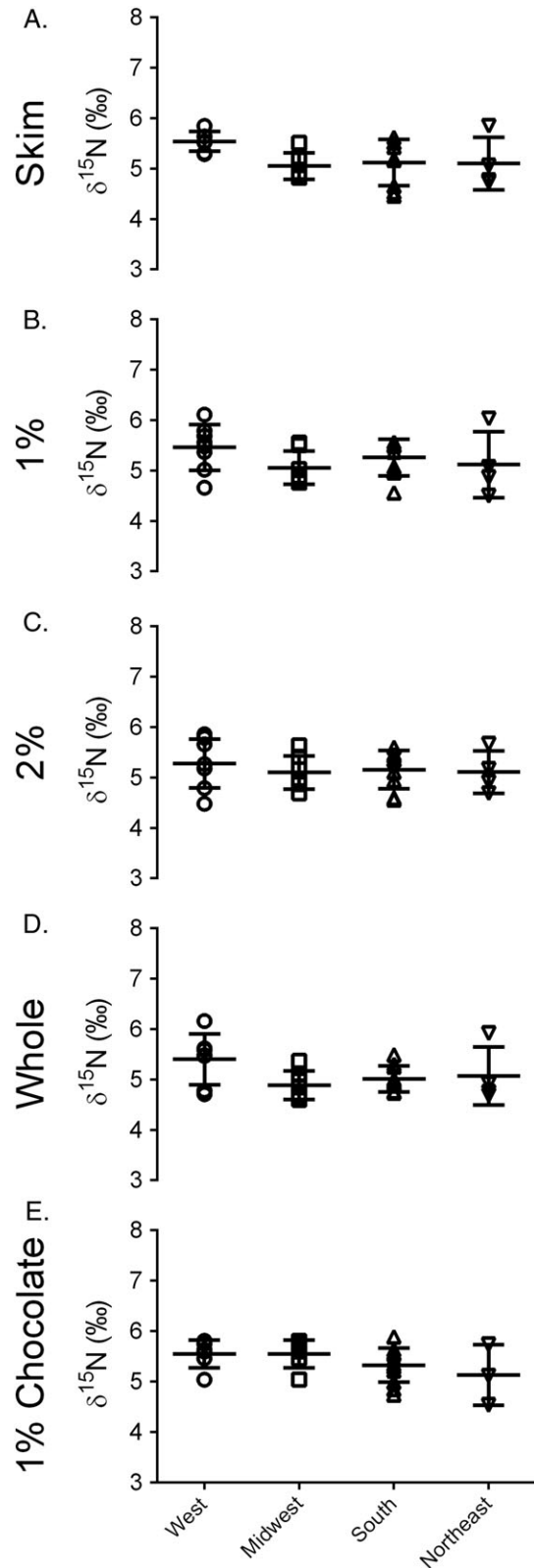


**FIGURE 2** Relationship between fat content and  $\delta^{13}\text{C}$  values of unflavored milk samples



**FIGURE 3**  $\delta^{13}\text{C}$  values of (A) skim, (B) 1%, (C) 2%, (D) whole, and (E) 1% chocolate milk samples by census-designated region of purchase. Different lowercase letters indicate that regional mean values are significantly different according to Tukey's HSD test

values of  $-3.3$  and  $-3.5$ ‰ were determined for stall- and pasture-fed cows, respectively, on long-term, isotopically consistent diets.<sup>33</sup> The similar offset between the  $\Delta^{13}\text{C}_{\text{lipid-lipid free}}$  value ( $-3.76$ ‰) calculated in our study and the values from controlled feeding studies with homogeneous diets suggests that dairy cattle on conventional U.S. dairy farms are close to isotopic equilibrium, i.e., their diets are homogeneous over a long period of time, and that the effect of varying



**FIGURE 4**  $\delta^{15}\text{N}$  values of (A) skim, (B) 1%, (C) 2%, (D) whole, and (E) 1% chocolate milk samples by census-designated region of purchase

lipid concentrations on the isotopic composition of milk may be predictable. However, further studies on the temporal variations in milk  $\Delta^{13}\text{C}_{\text{lipid-lipid free}}$  values from individual milk suppliers are necessary before a universal relationship between lipid content and  $\delta^{13}\text{C}$  values can be established for U.S. milk products.

Flavored milk products, with chocolate milk being the most common, account for approximately 25% of the fluid milk consumed in America<sup>34</sup> and over 60% of milk consumed within the U.S. school system.<sup>35</sup> Flavored milk contains approximately 12 g of added sugar per 8 oz serving.<sup>31</sup> This added sugar, which is primarily derived from the C4 plant sugarcane in the U.S., probably explains the 2.05‰ higher  $\delta^{13}\text{C}$  values of 1% chocolate milk samples than those of unflavored 1% milk samples observed in our study. Using the average measured  $\delta^{13}\text{C}$  values of cane sugar (-12.16‰,  $n = 3$ , unpublished data) and unflavored 1% milk (-18.53‰,  $n = 28$ ), along with proportional carbon contributions of 0.31 and 0.69 for sugar and milk, respectively, we calculated the  $\delta^{13}\text{C}$  value of 1% chocolate milk to be -16.56‰, which is within 0.1‰ of the average measured  $\delta^{13}\text{C}$  value for 1% chocolate milk in our study (-16.47‰). These data provide further evidence of the conspicuous and predictable  $\delta^{13}\text{C}$  signatures of added-sugar-containing foods in the American diet, supporting the potential utility of a  $\delta^{13}\text{C}$  biomarker of added sugar intake.<sup>36</sup>

## 4.2 | Regional estimates of maize feeding in U.S. dairy products

In our study, we observed a largely homogeneous stable isotope composition within each milk variety, with  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  value ranges of  $\approx 3.5\text{‰}$  and  $\approx 1.5\text{‰}$ , respectively, suggesting relatively similar feeding regimens for conventional U.S. dairy cattle. To estimate the contributions of C3 and C4 crops to dairy cattle diets, we inserted the  $\delta^{13}\text{C}$  value of whole (3.25%) milk, which reflects the  $\delta^{13}\text{C}$  value of the diet,<sup>7</sup> into a mass balance equation, with  $\delta^{13}\text{C}$  values of -27‰ (average value for C3 plants) and -12‰ (average value for C4 plants) representing milk from cows consuming 0% and 100% maize, respectively. The average whole milk  $\delta^{13}\text{C}$  value of -19.19‰ measured in our study suggests that the source dairy cattle consumed a diet containing approximately 50% C4 plants (e.g., maize), while none consumed a diet containing less than 35% C4 plants. The homogeneous, corn-based diet of U.S. dairy cattle suggested by our results reflects the recent movement of the dairy industry towards regional concentrated animal feeding operations (CAFOs), large feedlot systems in which cattle are raised on homogenous, maize-based rations.<sup>37</sup>

Despite the low overall variation in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signatures of U.S. milk products, we observed regional patterns within the  $\delta^{13}\text{C}$  dataset, with skim milk samples from the West region exhibiting mean  $\delta^{13}\text{C}$  values approximately 1.34‰ lower than those of skim milk collected in other census regions. Assuming an increase in milk  $\delta^{13}\text{C}$  value of 1.43–1.52‰ for each 10% increase in dietary maize content,<sup>8,9</sup> the source cattle of west-coast dairy samples consumed a diet containing approximately 8–9% less maize than dairy cattle in other regions of the country. Jahren and Kraft<sup>38</sup> noted a similar difference of 1.5‰ in the carbon stable isotope signatures of fast-food beef samples collected from west-coast and east-coast restaurants, suggesting distinct feeding patterns for the beef and dairy cattle supplying western retail markets. This may result from (a) higher rates of pasture-feeding for west-coast dairies or (b) higher proportions of C3 grains (e.g., wheat and barley) in the diets of western cattle due to regional availability. Although the source dairies/feedlots of the

samples collected in our study and those of Jahren and Kraft are not available, the results are indicative of distinct regional supply chains for terrestrial animal proteins in America.

## 5 | CONCLUSIONS

Here we report the first nationwide carbon and nitrogen stable isotope analysis of commercially available milk in the United States. Both fat and added sugar concentration, which altered the carbon stable isotope composition in our sample set, must be accounted for when comparing milk samples with heterogeneous macronutrient compositions. In samples with similar macronutrient content, the measured stable isotope signatures in our study were largely homogeneous, supporting the consolidation of feeding regimens and production processes that has characterized the U.S. dairy industry in recent decades. However, the distinct  $\delta^{13}\text{C}$  signature of milk collected in the West region suggests that regional variations in feed composition still persist among conventional dairy farms. While further studies are needed to verify regional and production method variations in stable isotope signatures, the representative values determined in this study can be used as a foundation for interpretation of dairy product stable isotope ratios in the American food supply.

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## REFERENCES

1. Johnson R. Food fraud and economically motivated adulteration of food and food ingredients. *Congressional Research Service Report*; 2014.
2. Kraft J, Collomb M, Möckel P, Sieber R, Jahreis G. Differences in CLA isomer distribution of cow's milk lipids. *Lipids*. 2003;38(6):657-664.
3. Butler G, Nielsen JH, Slots T, et al. Fatty acid and fat-soluble antioxidant concentrations in milk from high- and low-input conventional and organic systems: seasonal variation. *J Sci Food Agric*. 2008;88(8):1431-1441.
4. Besle J, Viala D, Martin B, et al. Ultraviolet-absorbing compounds in milk are related to forage polyphenols. *J Dairy Sci*. 2010;93(7):2846-2856.
5. DeNiro MJ, Epstein S. Influence of diet on the distribution of carbon isotopes in animals. *Geochim Cosmochim Acta*. 1978;42(5):495-506.
6. Deniro MJ, Epstein S. Influence of diet on the distribution of nitrogen isotopes in animals. *Geochim Cosmochim Acta*. 1981;45(3):341-351.
7. Minson DJ, Ludlow MM, Troughton JH. Differences in natural carbon isotope ratios of milk and hair from cattle grazing tropical and temperate pastures. *Nature*. 1975;256:602-602.
8. Metges C, Kempe K, Schmidt H-L. Dependence of the carbon-isotope contents of breath carbon dioxide, milk, serum and rumen fermentation products on the  $\delta^{13}\text{C}$  value of food in dairy cows. *Br J Nutr*. 1990;63(02):187-196.

9. Schwertl M, Auerswald K, Schäufele R, Schnyder H. Carbon and nitrogen stable isotope composition of cattle hair: ecological fingerprints of production systems? *Agric Ecosyst Environ.* 2005;109:153-0165.
10. Silva AV, Hélié JF, Caxito de Andrade F, et al. Multi-stable isotope analysis as a tool for assessing the geographic provenance of dairy products: A case study using buffalo's milk and cheese samples from the Amazon basin, Brazil. *Int Dairy J.* 2014;35(2):107-110.
11. Luo D, Dong H, Luo H, et al. Multi-element (C, N, H, O) stable isotope ratio analysis for determining the geographical origin of pure milk from different regions. *Food Anal Methods.* 2016;9(2):437-442.
12. Manca G, Franco MA, Versini G, et al. Correlation between multielement stable isotope ratio and geographical origin in Peretta cows' milk cheese. *J Dairy Sci.* 2006;89(3):831-839.
13. Crittenden RG, Andrew AS, LeFournour M, et al. Determining the geographic origin of milk in Australasia using multi-element stable isotope ratio analysis. *Int Dairy J.* 2007;17(5):421-428.
14. Ehtesham E, Hayman AR, McComb KA, Van Hale R, Frew RD. Correlation of geographical location with stable isotope values of hydrogen and carbon of fatty acids from New Zealand milk and bulk milk powder. *J Agric Food Chem.* 2013;61(37):8914-8923.
15. Kornel BE, Werner T, Rossmann A, Schmidt H-L. Measurement of stable isotope abundances in milk and milk ingredients – a possible tool for origin assignment and quality control. *Zeitschrift für Lebensmitteluntersuchung und -Forschung A.* 1997;205(1):19-24.
16. Scampicchio M, Mimmo T, Capici C, et al. Identification of milk origin and process-induced changes in milk by stable isotope ratio mass spectrometry. *J Agric Food Chem.* 2012;60(45):11268-11273.
17. Camin F, Wietzerbin K, Cortes AB, et al. Application of multielement stable isotope ratio analysis to the characterization of French, Italian, and Spanish cheeses. *J Agric Food Chem.* 2004;52(21):6592-6601.
18. Ehtesham E, Hayman A, Van Hale R, Frew R. Influence of feed and water on the stable isotopic composition of dairy milk. *Int Dairy J.* 2015;47:37-45.
19. Molkentin J, Giesemann A. Follow-up of stable isotope analysis of organic versus conventional milk. *Anal Bioanal Chem.* 2010;398(3):1493-1500.
20. Chung I-M, Park I, Yoon J-Y, Yang Y-S, Kim S-H. Determination of organic milk authenticity using carbon and nitrogen natural isotopes. *Food Chem.* 2014;160(0):214-218.
21. Molkentin J, Giesemann A. Differentiation of organically and conventionally produced milk by stable isotope and fatty acid analysis. *Anal Bioanal Chem.* 2007;388(1):297-305.
22. Molkentin J. Applicability of organic milk indicators to the authentication of processed products. *Food Chem.* 2013;137(1-4):25-30.
23. Molkentin J. Authentication of organic milk using  $\delta^{13}\text{C}$  and the  $\alpha$ -linolenic acid content of milk fat. *J Agric Food Chem.* 2009;57(3):785-790.
24. Kaffarnik S, Schröder M, Lehnert K, Baars T, Vetter W.  $\delta^{13}\text{C}$  values and phytanic acid diastereomer ratios: Combined evaluation of two markers suggested for authentication of organic milk and dairy products. *Eur Food Res Technol.* 2014;238(5):819-827.
25. Camin F, Wehrens R, Bertoldi D, et al. H, C, N and S stable isotopes and mineral profiles to objectively guarantee the authenticity of grated hard cheeses. *Anal Chim Acta.* 2012;711:54-59.
26. Capici C, Mimmo T, Kerschbaumer L, Cesco S, Scampicchio M. Determination of cheese authenticity by carbon and nitrogen isotope analysis: stelvio cheese as a case study. *Food Anal Methods.* 2015;8:2157-2162.
27. Rossmann A, Haberhauer G, Hölzl S, et al. The potential of multielement stable isotope analysis for regional origin assignment of butter. *Eur Food Res Technol.* 2000;211(1):32-40.
28. Pehrsson PR, Haytowitz DB, Holden JM, Perry CR, Beckler DG. USDA's national food and nutrient analysis program: Food sampling. *J Food Compos Anal.* 2000;13(4):379-389.
29. Sweeting CJ, Polunin NV, Jennings S. Tissue and fixative dependent shifts of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in preserved ecological material. *Rapid Commun Mass Spectrom.* 2004;18(21):2587-2592.
30. Werner RA, Brand WA. Referencing strategies and techniques in stable isotope ratio analysis. *Rapid Commun Mass Spectrom.* 2001;15(7):501-519.
31. Agricultural Research Service. USDA Nutrient Database for Standard Reference. Release 28; 2015.
32. United States Census Bureau. Census Regions and Divisions of the United States. 2011.
33. Schneider S, Auerswald K, Bellof G, Schnyder H.  $^{13}\text{C}$  discrimination between diet, faeces, milk and milk components. *Isotopes Environ Health Stud.* 2014;51:31-45.
34. Sebastian RS, Goldman JD, Enns CW, LaComb RP. Fluid milk consumption in the United States. In: *What We Eat In America, NHANES 2005-2006.* Food Surveys Research Group; 2010.
35. Fayet-Moore F. Effect of flavored milk vs plain milk on total milk intake and nutrient provision in children. *Nutr Rev.* 2016;74(1):1-17.
36. Jähren AH, Bostic JN, Davy BM. The potential for a carbon stable isotope biomarker of dietary sugar intake. *J Anal At Spectrom.* 2014;29:795-816.
37. Peterson HH. *Geographic changes in US dairy production.* Long Beach, California: Annual Meeting of the American Agricultural Economics Association; 2002:28-31.
38. Jähren AH, Kraft RA. Carbon and nitrogen stable isotopes in fast food: Signatures of corn and confinement. *Proc Natl Acad Sci U S A.* 2008;105(46):17855-17860.

## SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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