Research Article

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Effect of baking and fermentation on the stable carbon and nitrogen isotope ratios of grain-based food

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RATIONALE: Isotope ratio mass spectrometry (IRMS) is used extensively to reconstruct general attributes of prehistoric and modern diets in both humans and animals. In order to apply these methods to the accurate determination of specific intakes of foods/nutrients of interest, the isotopic signature of individually consumed foods must be constrained. For example, 86% of the calories consumed in the USA are derived from processed and prepared foods, but the relationship between the stable isotope composition of raw ingredients and the resulting products has not been characterized.
METHODS: To examine the effect of common cooking techniques on the stable isotope composition of grain-based food items, we prepared yeast buns and sugar cookies from standardized recipes and measured bulk $\delta^{13}C$ and $\delta^{15}N$ values of samples collected throughout a 75 min fermentation process (buns) and before and after baking at 190°C (buns and cookies). Simple isotope mixing models were used to determine if the isotopic signatures of 13 multi-ingredient foods could be estimated from the isotopic signatures of their constituent raw ingredients.
RESULTS: No variations in $\delta^{13}C$ or $\delta^{15}N$ values were detected between pre- and post-baked yeast buns (pre: –24.78‰/2.61‰, post: –24.75‰/2.74‰), beet-sugar cookies (pre: –24.48‰/3.84‰, post: –24.47‰/3.57‰), and cane-sugar cookies (pre: –19.07‰/2.97‰, post: –19.02‰/3.21‰), or throughout a 75 min fermentation process in yeast buns. Using isotopic mass balance equations, the $\delta^{13}C/\delta^{15}N$ values of multi-ingredient foods were estimated from the isotopic composition of constituent raw ingredients to within 0.14 ± 0.13‰/0.24 ± 0.17‰ for gravimetrically measured recipes and 0.40 ± 0.38‰/0.58 ± 0.53‰ for volumetrically measured recipes.
CONCLUSIONS: Two common food preparation techniques, baking and fermentation, do not substantially affect the carbon or nitrogen isotopic signature of grain-based foods. Mass-balance equations can be used to accurately estimate the isotopic signature of multi-ingredient food items for which quantitative ingredient information is available.

Knowledge of the stable isotope composition of food sources allows scientists to make numerous distinctions concerning the dietary patterns of animals and humans. Based on the premise that isotopic signatures of foods are transferred to consumer's tissues upon ingestion and assimilation, stable isotope analysis (SIA) of bodily tissues can be used in paleo and modern human diet reconstruction to determine variations in food consumption patterns along regional and socioeconomic lines.[1–7] For instance, analysis of the stable isotope ratio of nitrogen ($\delta^{15}N$ value) is used to determine meat intake of individuals and populations due to the elevated $\delta^{15}N$ values of animal foods,[8–10] while analysis of the stable isotope ratio of carbon ($\delta^{13}C$ value) can be used to determine dietary introduction of agriculturally important C4 grasses due to their distinct isotope signatures.[11] Recent developments involve the use of stable isotopes as intake biomarkers of specific foods relevant to public health research, including fish[12,13] and added sugars[14–18] (See Jahren *et al.*[19] for a review of the added sugar biomarker). These biomarkers show promise for future clinical research application, as preliminary trials have utilized Red Blood Cell $\delta^{13}C$ and $\delta^{15}N$ values to assess the impact of added sugar intake and fish-derived long-chain fatty acid (EPA/DHA) intake on chronic disease risk factors such as blood pressure, blood lipids, and inflammatory markers.[20–22]

For the most accurate interpretations of results in stable isotope dietary reconstruction studies, three things must be known: the isotopic signature of dietary components, the isotopic signature of bodily tissues, and potential modifications of the stable isotope signature during incorporation of food biomass into tissues, known as diet-tissue fractionation patterns. Tissue isotope signatures are relatively easy to attain via non-invasive tissue samples, such as hair,[23] nail,[6] and fingerstick blood,[15] and factors affecting diet-tissue fractionation patterns, such as energy availability,[24] dietary macronutrient composition,[25] pregnancy,[26] and certain disease states,[27] have been well characterized. However, accurate calculation of whole-diet isotope signatures remains difficult for modern isotope researchers.

Numerous prior studies have estimated whole-diet isotope signatures of individuals and populations from self-reported dietary intake data[4,6,7,28–30] or controlled feeding trials.[31–33]

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In order to derive representative whole-diet signatures, researchers need to know the isotopic composition of all foods consumed by the individual/population. This is relatively simple to accomplish for study populations who consume a short list of staple foods, such as hunter-gatherers, sustenance farmers, or modern subjects consuming a fixed diet in the context of controlled feeding trials,[31,34] but the immense complexity of the "supermarket diet" of developed nations complicates these calculations in free-living subjects. Via modern food-processing techniques, a few commodity raw ingredients are combined to produce the thousands of food items present in average supermarkets. While a majority of raw ingredients have been subjected to stable isotope analysis, comparatively little work has been conducted on the multi-ingredient prepared and processed foods derived from these raw ingredients, yet these foods comprise 86% of calories consumed in the American diet.[35]

With prepared and processed foods representing the majority of food sources in developed nations, knowledge of their isotopic composition is of vital importance to dietary reconstruction studies. Central to this knowledge is an understanding of how combining and cooking raw ingredients affects the isotopic compositions of the resultant food products. Nutritional food databases, such as the USDA's National Nutrient Database for Standard Reference,[36] include extensive entries of food items subjected to various cooking methods, as cooking is known to alter the nutritional composition of certain foods.[37] However, the lack of a central database of food stable isotope values has limited the breadth of food isotope knowledge, with current collections rarely containing both raw and cooked forms of the same item. As a result, the effect of cooking on food isotope signatures is largely unknown. While recent studies have analyzed the effect of cooking on the carbon and nitrogen stable isotope compositions of terrestrial[38] and marine[39] animal products, results have been inconclusive. Furthermore, the effect of cooking on isotope ratios of grain-based desserts and yeast breads, the two largest sources of calories in the American diet, are yet to be reported.[40]

To prioritize sampling efforts for future food isotope databases, it is important to determine how cooking affects the isotopic composition of foods and whether the isotopic signatures of multi-ingredient foods can be estimated from their constituent raw ingredients. Thus, the objectives of this study were to (a) determine if the stable isotope compositions of grain-based desserts and yeast breads are altered by common cooking processes, and (b) test the accuracy with which isotopic mass-balance equations can estimate the isotopic signature of multi-ingredient foods. To accomplish these objectives, samples of various baked goods, confectioneries, and preserves were prepared using both gravimetric (i.e., gram weight) and volumetric (i.e., cup, tablespoon) ingredient measurement techniques.

EXPERIMENTAL

Ingredient acquisition and sample handling

Raw ingredients were collected from local grocery store chains (Safeway Hawaii USA, Costco Hawaii USA, Kroger Virginia USA) between January and June 2014. Ingredients requiring refrigeration were stored at 4°C until preparation. All perishable samples (milk, butter, eggs) were used within 1 week of purchase. Aliquots of raw ingredients and prepared foods were stored at –40°C for 1–4 months prior to stable isotope analysis. For the analysis of eggs, a single egg from each dozen was reserved for isotopic analysis, as the isotopic signatures of eggs from the same production system have been shown to be similar.[41]

Gravimetric and volumetric sample preparation

To determine the precision with which mass-balance calculations can estimate the isotope signature of multi-ingredient foods, four food items (yeast buns, beet/cane sugar cookies, and fudge) were prepared using gravimetric measurement techniques and 11 food items (2 types of biscuit, 5 types of cake, muffins, jam, brownies, and fudge) were prepared using volumetric measurement techniques. Fudge was prepared using both gravimetric and volumetric measuring techniques to compare the isotopic signatures obtained via the two preparation methods. All recipes were derived from an instructional food preparation laboratory manual,[42] with the exception of yeast buns, which were prepared from a recipe designed to replicate commercially available hamburger buns. Weight/volume conversions from the USDA's National Nutrient Database for Standard Reference[36] were used to convert volumetric ingredient measurements to the nearest gram weight for the preparation of gravimetrically prepared samples. Preliminary trials were conducted prior to actual experiments and existing recipes were modified (e.g., baking sheets lined with parchment paper instead of cooking oil) in order to establish optimal cooking conditions and prevent external inputs of carbon and/or nitrogen. Ingredient profiles are provided in Tables 1 and 2 for gravimetric and volumetric samples, respectively.

All recipes were prepared in kitchens equipped with natural gas ranges and ovens between January and June 2014. Samples of final products were taken from three locations (e.g., 3 different buns, 3 corners of cake) and combined to ensure a homogenous sample. Gravimetric recipes were prepared in triplicate with similar ingredients to determine the inter-experiment precision. Volumetric results are based on a single preparation.

Cooking methods

To determine the effect of common cooking techniques on the isotopic signature of grain-based foods, samples from the gravimetrically prepared yeast buns, beet sugar cookies, and cane sugar cookies detailed above were collected at various points throughout the cooking process as follows:

Yeast buns

Buns were prepared via the 'straight dough' method of rising: Active-dry yeast was mixed in 45°C water and activated for 10 min. Milk, egg, oil, sugar, and salt were combined and incorporated into the activated yeast mixture with a wire whisk. Flour was subsequently added

Table 1. Isotopic composition of raw ingredients, pre-cooked samples, and final products for gravimetrically prepared samples

Recipe	Avg Wet Weight[a] (g)	Avg Carbon Content[a] (g)	Avg Nitrogen Content[a] (g)	$\delta^{13}C/\delta^{15}N$ Values (‰)		
				Trial #1	Trial #2	Trial #3
Buns						
Buns 2% Milk	121±1.00	6.18±0.05	0.64±0.01	−18.17/5.54	−18.17/5.54	−18.17/5.54
Large Egg	52±3.51	6.89±0.51	1.00±0.05	−16.34/4.55	−18.92/5.19	−18.92/5.19
White Sugar	26±0.00	10.89±0.07	0.00	−12.14/n.a.	−26.26/n.a.	−26.26/n.a.
All Purpose Flour	420±0.00	166.91±0.00	8.32±0.00	−24.99/2.82	−24.99/2.82	−24.99/2.82
Soybean Oil	26±0.00	19.84±0.00	0.00±0.00	−31.27/n.a.	−31.27/n.a.	−31.27/n.a.
Active Dry Yeast	10±0.29	4.31±0.13	0.72±0.00	−15.87/−1.04	−15.87/−1.04	−15.87/−1.04
0 Min Fermentation				−24.26/2.56	−24.92/2.64	−24.93/2.75
45 Min				−24.39/2.55	−24.89/2.77	−24.92/2.59
75 Min (Raw Bun)				−24.49/2.57	−24.90/2.60	−24.94/2.66
Baked Bun				−24.45/2.92	−24.87/2.62	−24.93/2.69
Baked Bun (est)				−23.95/2.90	−25.06/2.95	−25.06/2.93
Beet Sugar Cookies (p. xxi)						
All Purpose Flour	210±0.00	83.45±0.00	4.16 ±0.00	−24.99/2.82	−24.99/2.82	−24.99/2.82
Unsalted Butter	52±0.00	31.27±0.00	0.00	−24.45/n.a.	−24.45/n.a.	−24.45/n.a.
Pure Vanilla Extract	5±0.00	0.90±0.00	0.00	−14.71/n.a.	−14.71/n.a.	−14.71/n.a.
Baking Soda	3±0.00	0.42±0.00	0.00	−2.83/n.a.	−2.83/n.a.	−2.83/n.a.
Cream of Tartar	3±0.00	0.75±0.00	0.00	−22.19/n.a.	−22.19/n.a.	−22.19/n.a.
Light Corn Syrup	20±0.00	6.41±0.00	0.00	−10.92/n.a.	−10.92/n.a.	−10.92/n.a.
White **Beet** Sugar	180±0.00	75.60±0.00	0.00	−26.26/n.a.	−26.26/n.a.	−26.26/n.a.
Large Egg	53±1.73	7.04±0.23	1.05±0.03	−18.92/5.19	−18.92/5.19	−18.92/5.19
Raw Cookie					−24.51/3.95	−24.46/3.73
Baked Cookie				−24.49/3.08	−24.49/3.74	−24.45/3.39
Baked Cookie (est)				−24.63/3.29	−24.63/3.29	−24.62/3.31
Cane Sugar Cookies (p. xxi)						
All Purpose Flour	210±0.00	82.94±0.00	4.27±0.00	−24.80/2.91	−24.80/2.91	−24.80/2.91
Unsalted Butter	52±0.00	32.24±0.07	0.00	−21.13/n.a.	−21.13/n.a.	−21.13/n.a.
Pure Vanilla Extract	5±0.00	0.90±0.00	0.00	−14.71/n.a.	−14.71/n.a.	−14.71/n.a.
Baking Soda	3±0.00	0.42±0.00	0.00	−2.83/n.a.	−2.83/n.a.	−2.83/n.a.
Cream of Tartar	3±0.00	0.75±0.00	0.00	−22.19/n.a.	−22.19/n.a.	−22.19/n.a.
Light Corn Syrup	21±0.58	6.48±0.18	0.00	−10.69/n.a.	−10.69/n.a.	−10.69/n.a.
White **Cane** Sugar	180±0.00	75.63±0.01	0.00	−12.55/n.a.	−12.55/n.a.	−12.33/n.a.
Large Egg	52±6.43	6.72±0.68	1.12±0.21	−16.72/4.82	−16.72/4.82	−20.32/4.72
Raw Cookie				−18.95/2.91	−19.24/3.50	−19.02/2.50
Baked Cookie				−18.98/2.91	−19.08/3.71	−19.01/3.01
Baked Cookie (est)				−18.93/3.34	−18.92/3.35	−18.95/3.03

(Continues)

Table 1. (Continued)

Recipe	Avg Wet Weight[a] (g)	Avg Carbon Content[a] (g)	Avg Nitrogen Content[a] (g)	$\delta^{13}C/\delta^{15}N$ Values (‰)		
				Trial #1	Trial #2	Trial #3
Fudge (p. 173)						
Whole (3.25%) Milk	162±1.15	9.51±0.09	0.82±0.00	−19.67/4.70	−19.67/4.70	−20.70/4.70
Unsalted Butter	26±0.00	16.12±0.03	0.00	−21.13/n.a.	−21.13/n.a.	−21.13/n.a.
Pure Vanilla Extract	4±0.58	0.66±0.10	0.00	−14.71/n.a.	−14.71/n.a.	−14.71/n.a.
100% Chocolate Bar	56±0.00	34.29±0.00	1.29	−30.37/4.68	−30.37/4.68	−30.37/4.68
Light Corn Syrup	42±0.00	13.17±0.00	0.00	−10.69/n.a.	−10.69/n.a.	−10.69/n.a.
White Cane Sugar	416±0.00	174.78±0.02	0.00	−12.55/n.a.	−12.55/n.a.	−12.33/n.a.
Fudge				−15.80/n.a.	−15.84/n.a.	−15.66/n.a.
Fudge (est)				−15.75	−15.75	−15.63

n.a.: Nitrogen concentration below detection limit of IRMS when samples weighed at 2 mg
[a]Average wet weight and C/N content of three trials provided as mean ± 1 SD
Cookie and fudge recipes derived from *Food Selection and Preparation: A Laboratory Manual*, (2nd edn.)[42] (page numbers provided in parentheses).

and the resulting dough was hand-kneaded for 10 min on a clean countertop. A sample of dough was collected using a 15 mL centrifuge tube (Table #1 Label: 0 min Fermentation) and immediately stored at –20°C to inactivate yeast, while the remaining dough was covered and allowed to rise for 45 min at room temperature. Following the first rise, another sample was taken (45 min) and the remaining dough was split into eight buns. The buns were transferred to a baking sheet lined with parchment paper and allowed to rise for another 30 min before another sample was taken from one bun (75 min (Raw Bun)). Finally, the remaining seven buns were cooked at 190°C for 16 min and a cross-sectional sample was taken from three buns (Baked Bun).

Beet and cane sugar cookies

Wet ingredients were mixed together in a large metal bowl with a Sunbeam Heritage™ 4.6-quart stand mixer (Sunbeam Products Inc., Boca Raton, FL, USA) until well incorporated. Dry ingredients were subsequently added to the wet ingredient mixture and beaten until homogenous. A sample of the resulting dough was collected in a 15 mL centrifuge tube (Raw Cookie) and the remaining dough was formed into 16 two-inch diameter balls and placed on a baking sheet lined with parchment paper. Cookies were baked at 190°C for 4 min on the bottom rack and 4 min on the top rack. Immediately following baking, a cross-section of three cookies was taken with 15 mL centrifuge tubes (Baked Cookie).

Processing and analysis

Aliquots of raw ingredients and prepared foods were freeze-dried for 72 h in a Freezone 4.5-L freeze-dryer (Labconco, Kansas City, MO, USA) and homogenized with a mortar and pestle, including liquid nitrogen when necessary. Samples (2 mg) were loaded into high-purity tin capsules (EA Consumables, Collingswood, NJ, USA) and analyzed for their carbon and nitrogen stable isotope compositions using a ECS 4010 elemental analyzer (Costech, Valencia, CA, USA) configured with a Delta-V stable isotope ratio mass spectrometer (Thermo Scientific, Bremen, Germany). Oil samples were prepared by pipetting 2 μL into tin capsules and analyzing as described above. The stable isotope values are reported in standard δ-notation [$\delta = R_{sample}/R_{standard} - 1$, where R is the isotope ratio ($^{13}C/^{12}C$, $^{15}N/^{14}N$) 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 $\delta^{13}C/\delta^{15}N$-values on the VPDB/AIR scales were performed according to Werner and Brand.[43] Internal laboratory standards used to normalize data were L-glutamic acid (Thermo Fisher, Rockford, IL, USA, $\delta^{13}C$ = –13.43‰) and glycine (Thermo Fisher, $\delta^{13}C$ = –43.51‰) for carbon, and glycine (Thermo Fisher, $\delta^{15}N$ = 1.24‰) and glycine (Brian Popp Lab, University of Honolulu; $\delta^{15}N$ = 11.25‰) for nitrogen. The values are reported in units of permil (‰) and represent the mean of three analyses (the standard deviation of three replicates never exceeded 0.2‰ for $\delta^{13}C$ values and 0.25‰ for $\delta^{15}N$ values).

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Table 2. Isotopic composition of raw ingredients and final products for volumetrically prepared samples

Recipe	Volumetric Measurement	Carbon Content (g)	Nitrogen Content (g)	$\delta^{13}C/\delta^{15}N$ Values (‰)
Basic Biscuit (p. 40)				
All Purpose Flour	2 Cups	102.40	4.90	−26.74/3.48
Double Acting Baking Powder	1 Tbsp	1.84	0.00	−13.09/n.a
Unsalted Butter	5 Tbsp	42.19	0.00	−20.12/n.a.
2% Milk	¾ Cup	8.18	0.91	−16.93/5.76
Basic Biscuit				−24.41/3.73
Basic Biscuit (est)				−25.61/3.84
Muffin (Basic Recipe) (pp. 38–39)				
All Purpose Flour	2 Cup	102.40	4.90	−26.74/3.48
Double Acting Baking Powder	1 tsp	1.84	0.00	−13.09/n.a.
White Sugar	1 Tbsp	10.51	0.00	−12.38/n.a.
Canola Oil	2 Tbsp	20.99	0.00	−29.72/n.a.
Large Egg	1 egg	16.85	2.72	−16.71/4.73
2% Milk	1 Cup	10.91	1.22	−16.93/5.76
Muffin				−23.70/3.49
Muffin (est)				−24.36/4.18
Lowfat Buttermilk Biscuit (p. 41)				
All Purpose Flour	2 Cups	102.40	4.90	−26.74/3.48
Baking Powder	2 tsp	1.67	0.00	−9.73/n.a.
Baking Soda	¼ tsp	0.16	0.00	−6.72/n.a.
Margarine (80% Vegetable Oil)	3 Tbsp + 1 tsp	28.10	0.00	−20.12/n.a.
Cultured Lowfat Buttermilk	¾ Cup	7.09	0.87	−16.88/5.86
Buttermilk Biscuit				−24.23/4.26
Buttermilk Biscuit (est)				−24.68/3.84
Milk Chocolate Cake (pp. 53–54)				
Cake Flour	1 Cup	54.28	1.95	−27.86/3.62
Unsalted Butter	¼ Cup	33.75	0.00	−20.12/n.a.
White Sugar	¾ Cup	63.06	0.00	−12.38/n.a.
Cultured Lowfat Buttermilk	½ Cup	4.73	0.58	−16.88/5.86
Baking Powder	1¼ tsp	1.05	0.00	−9.73/n.a.
Pure Vanilla Extract	½ tsp	0.37	0.00	−14.98/n.a.
Large Egg	1 egg	16.85	2.72	−16.71/4.73
Unsweetened Cocoa Powder	¼ Cup	10.18	1.00	−27.72/5.65
Milk Chocolate Cake				−19.52/4.50
Milk Chocolate Cake (est)				−19.71/4.64
Devils Food Cake (pp. 53–54)				
Cake Flour	1 Cup	54.28	1.95	−27.86/3.62
Unsalted Butter	¼ Cup	33.75	0.00	−20.12/n.a.
White Sugar	¾ Cup	63.06	0.00	−12.38/n.a.
Cultured Lowfat Buttermilk	½ Cup	4.73	0.58	−16.88/5.86
Baking Soda	¾ tsp	0.47	0.00	−6.72/n.a.
Pure Vanilla Extract	½ tsp	0.37	0.00	−14.98/n.a.
Large Egg	1 egg	16.85	2.72	−16.71/4.73
Unsweetened Cocoa Powder	¼ Cup	10.18	1.00	−27.72/5.65
Devils Food Cake				−19.59/3.84
Devils Food Cake (est)				−19.73/4.64
Dark Chocolate Cake (pp. 53–54)				
Cake Flour	1 Cup	54.28	1.95	−27.86/3.62
Unsalted Butter	¼ Cup	33.75	0.00	−20.12/n.a.
White Sugar	¾ Cup	63.06	0.00	−12.38/n.a.
Whole (3.25%) Milk	½ Cup	6.83	0.55	−17.71/6.24
Baking Soda	1¼ tsp	0.79	0.00	−6.72/n.a.

(Continues)

Table 2. (Continued)

Recipe	Volumetric Measurement	Carbon Content (g)	Nitrogen Content (g)	$\delta^{13}C/\delta^{15}N$ Values (‰)
Dark Chocolate Cake (pp. 53–54)				
Pure Vanilla Extract	½	0.37	0.00	−14.98/n.a.
Large Egg	1 egg	16.85	2.72	−16.71/4.73
Unsweetened Cocoa Powder	¼ Cup	10.18	1.00	−27.72/5.65
Dark Chocolate Cake				−19.61/4.67
Dark Chocolate Cake (est)				−19.71/4.48
Yellow Cake (pp. 50–51)				
Cake Flour	1 Cup	54.28	1.95	−27.86/3.62
White Sugar	2/3 Cup	56.33	0.00	−12.38/n.a.
Double Acting Baking Powder	1½ tsp	0.92	0.00	−13.09/n.a.
Unsalted Butter	¼ Cup	33.75	0.00	−20.12/n.a.
2% Milk	½ Cup	5.45	0.61	−16.93/5.76
Pure Vanilla Extract	½ tsp	0.37	0.00	−14.98/n.a.
Large Egg	1 egg	16.85	2.72	−16.71/4.73
Yellow Cake				−19.55/4.82
Yellow Cake (Estimated)				−19.53/4.44
Jelly Roll Cake (p. 57)				
Cake Flour	1 Cup	54.28	1.95	−27.86/3.62
Double Acting Baking Powder	1 tsp	0.61	0.00	−13.09
Large Egg	3 eggs	50.56	8.17	−16.71/4.73
White Sugar	1 Cup	84.07	0.00	−12.38/n.a.
Pure Vanilla Extract	1 tsp	0.73	0.00	−14.98/n.a.
Confectioner's Sugar	½ Cup	20.87	0.00	−11.82/n.a.
Strawberry Jam (See Below)	¾ Cup	59.08	0.00	−12.67/n.a.
Jelly Roll Cake				−16.36/3.15
Jelly Roll Cake (est)				−16.33/3.79
Strawberry Jam (p. 17)				
Fresh Strawberries (Sliced)	2½ Cups	15.03	0.00	−25.71/n.a.
White Sugar	2½ Cups	210.19	0.00	−12.38/n.a.
Premium Fruit Pectin	3 Tbsp + ½ tsp	17.88	0.00	−12.75/n.a.
Strawberry Jam				−12.67/n.a.
Strawberrry Jam (est)				−13.23/n.a.
Prune-Raisin Brownie (p. 76)				
California Raisins	¼ Cup	11.86	0.00	−25.77/n.a.
Dried Prunes	¾ Cup	27.90	0.00	−27.28/n.a.
Cake Flour	1½ Cups	81.42	2.93	−27.86/3.62
White Sugar	1 1/3 Cups	111.80	0.00	−12.38/n.a.
Unsweetened Cocoa Powder	1 Cup	40.71	3.99	−27.72/5.65
Instant Coffee	1 tsp	0.43	0.04	−26.99/2.53
Baking Powder	2 tsp	1.59	0.00	−9.73/n.a.
Baking Soda	½ tsp	0.32	0.00	−6.72/n.a.
Large Egg	1 egg	16.85	2.72	−16.71/4.73
Egg Whites	2 egg whites	1.86	0.52	−15.31/5.30
Canola Oil	2 Tbsp	20.99	0.00	−29.72/n.a.
Pure Vanilla Extract	2 tsp	1.47	0.00	−14.98/n.a.
Prune-Raisin Brownie				−22.34/2.93
Prune-Raisin Brownie (est)				−21.54/4.24
Fudge (p. 173)				
Whole (3.25%) Milk	2/3 Cup	9.15	0.74	−17.71/6.24
Unsalted Butter	2 Tbsp	17.06	0.00	−20.12/n.a.
Pure Vanilla Extract	1 tsp	0.87	0.00	−14.98/n.a.
100% Chocolate Bar	2 ounces	34.13	1.32	−30.12/4.84

(Continues)

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Table 2. (Continued)

Recipe	Volumetric Measurement	Carbon Content (g)	Nitrogen Content (g)	$\delta^{13}C/\delta^{15}N$ Values (‰)
Fudge (p. 172)				
Light Corn Syrup	2 Tbsp	12.57	0.00	−11.33/n.a.
White Sugar	2 Cups	168.15	0.00	−12.38/n.a.
Fudge				−15.74/n.a.
Fudge (est)				−15.59/n.a.

n.a.: Nitrogen concentration below detection limit of IRMS when samples weighed at 2 mg.
Recipes derived from *Food Selection and Preparation: A Laboratory Manual*, (2nd edn.)[42] (page numbers provided in parentheses).

Estimated food calculations

The delta values of gravimetric and volumetric foods were estimated using the following isotopic mass-balance calculation:

$$\delta_{est} = \frac{\left(\delta_1 \times \frac{C}{N_1} + \delta_2 \times \frac{C}{N_2} + \ldots + \delta_n \times \frac{C}{N_n}\right)}{\left(\frac{C}{N_1} + \frac{C}{N_2} + \ldots + \frac{C}{N_n}\right)}$$

where δ_{est} is the estimated $\delta^{13}C/\delta^{15}N$ value of the final prepared food, δ_n is the measured $\delta^{13}C/\delta^{15}N$ value of ingredient n used in the recipe, and C/N_n is the carbon or nitrogen content of ingredient n. The carbon/nitrogen contents of raw ingredients were calculated by multiplying the initial wet weights by the fractional dry mass and fractional % carbon/nitrogen. Water contents of ingredients used in the calculation of fractional dry masses were acquired from the USDA National Nutrient Database for Standard Reference.[36]

RESULTS

The $\delta^{13}C$ and $\delta^{15}N$ values for individual ingredients, raw food, and cooked food products from the gravimetric experiments are listed in Table 1. The $\delta^{13}C$ and $\delta^{15}N$ values for ingredients and food products from the volumetric experiments are listed in Table 2.

$\delta^{13}C/\delta^{15}N$ values of raw ingredients

The $\delta^{13}C$ values of animal products varied by ≈3–4‰ (–16.34‰ to –20.32‰ for eggs, –17.71‰ to –20.70‰ for whole milk, and –20.12‰ to –24.45‰ for butter). The $\delta^{13}C$ value of whole milk has been shown to approximate that of the animal's diet.[44,45] Using approximate values of –26‰ for C3 plants and –11‰ for C4 plants, we have calculated that the source dairy cows in this study were fed a diet consisting of approximately 40–55% C4 plants (e.g., maize). The $\delta^{13}C$ and $\delta^{15}N$ values of the other raw ingredients were consistent with prior studies (Tables 1 and 2).[14,38,46]

Effect of cooking treatments on isotopic values

Two cooking processes were analyzed in our study: Oven baking and yeast fermentation, representing the principle preparation methods used in commercial baked good production. Table 1 shows a comparison of raw and cooked $\delta^{13}C$ and $\delta^{15}N$ values of yeast buns and cookies.

The effect of baking on $\delta^{13}C$ and $\delta^{15}N$ values was evaluated by comparing the means between pre- and post-baked beet sugar cookies, cane sugar cookies, and yeast buns using two-tailed paired t-tests assuming equal variance between samples. The mean $\delta^{13}C/\delta^{15}N$ values of pre- and post-baked yeast buns (pre: –24.78‰/2.61‰, post: –24.75‰/2.74‰), beet-sugar cookies (pre: –24.48‰/3.84‰, post: –24.47‰/3.57‰), and cane-sugar cookies (pre: –19.07‰/2.97‰, post: –19.02‰/3.21‰) were not significantly different ($p > 0.05$).

To test the effect of food-based fermentation processes on the $\delta^{13}C$ and $\delta^{15}N$ values of baked goods, samples were taken from yeast dough prior to fermentation and at two time points during the fermentation process. The mean values at each time point (0 min: –24.71‰/2.65‰, 45 min: –24.73‰/2.63‰, 75 min: –24.78‰/2.61‰) were not significantly different ($p > 0.05$).

Estimated values

The estimated $\delta^{13}C$ and $\delta^{15}N$ values of gravimetric foods are listed in Table 1. Figure 1 compares estimated and measured $\delta^{13}C/\delta^{15}N$ values of gravimetrically and volumetrically prepared foods. The average absolute differences between the estimated and the average $\delta^{13}C$ / $\delta^{15}N$ values ($\Delta\delta^{13}C_{est \rightarrow actual}$ / $\Delta\delta^{15}N_{est \rightarrow actual}$) were 0.14 ± 0.13‰/0.24 ± 0.17‰ for gravimetrically measured recipes and 0.40 ± 0.38‰/0.58 ± 0.53‰ for volumetrically measured recipes.

DISCUSSION

In this study, we demonstrated that two common cooking techniques, fermentation and baking, did not affect the stable carbon or nitrogen isotope composition of a grain-based dessert or yeast bread. We also validated an accurate method of estimating $\delta^{13}C$ and $\delta^{15}N$ values of multi-ingredient prepared foods from the isotopic signatures of their raw ingredients.

This is the first published evidence demonstrating the effect of cooking on grain-based food products. The average differences between the $\delta^{13}C$ and $\delta^{15}N$ values of pre- and post-cooked food were ≤0.10‰ and ≤0.41‰, and were not significantly different for any of the recipes or preparation methods tested ($p > 0.05$). Only two prior studies have specifically analyzed the effect of cooking on stable isotope

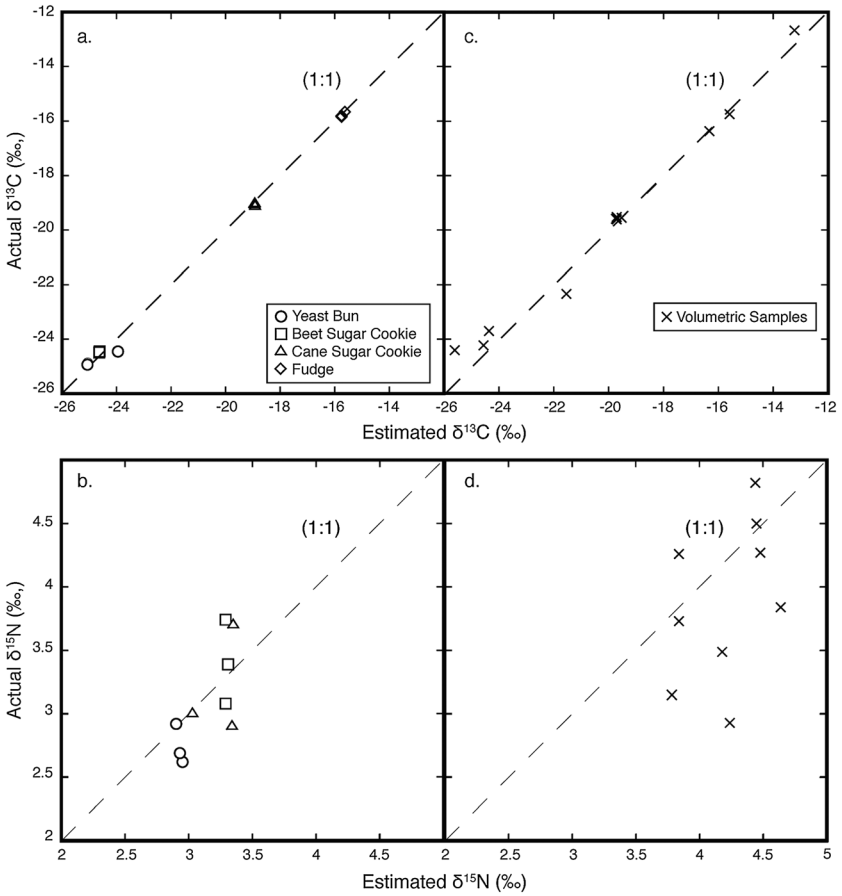

Figure 1. Comparison of estimated and actual $\delta^{13}C$ and $\delta^{15}N$ values of gravimetrically (a, b) and volumetrically (c, d) measured samples. Estimated values are derived from isotope signatures and elemental compositions of raw ingredients using mass-balance equations as outlined in the Experimental section. Data points are superimposed upon a 1:1 line representing no difference between estimated and actual isotope values. c, Fudge $\delta^{15}N$ not available (nitrogen concentration below detection limit of IRMS when samples weighed at 2 mg); d, Fudge & Srawberry Jam $\delta^{15}N$ not available (nitrogen concentration below detection limit of IRMS when samples weighed at 2 mg).

ratios, with both focusing solely on animal products. In agreement with our findings, Huelsemann *et al*. reported that cooking did not result in any significant changes in the $\delta^{15}N$ values of meat samples, although the type of meat(s) and cooking method(s) analyzed were not reported.[38] Similar results were obtained by Fernandes *et al*., who noted no differences in the $\delta^{13}C$ and $\delta^{15}N$ values in bulk flesh of two teleost fish species following steaming, baking, and boiling.[39] However, elevations in $\delta^{13}C$ and $\delta^{15}N$ values of ≈1‰ have been reported in marine animal samples subjected to oven drying at 60°C for 48 h, suggesting that heating may alter the isotopic composition of animal tissues under certain conditions.[47,48]

Our estimation of multi-ingredient prepared food $\delta^{13}C$ and $\delta^{15}N$ values using the isotope values of their raw ingredients offers a proof of principle for future dietary reconstruction studies. Using publicly available weight/volume conversion charts and water content data from the USDA,[36] we cross-validated the accuracy and precision of a simple mass-balance calculation using both gravimetric and volumetric methods of ingredient measurement. The mean differences between the estimated and actual $\delta^{13}C$ and $\delta^{15}N$ values for gravimetrically measured samples, 0.14 ± 0.13‰ and 0.24 ± 0.17‰, respectively, are within the instrumental uncertainty of the measurement. Recipes prepared volumetrically exhibited higher mean differences of 0.40 ± 0.38‰ and 0.58 ± 0.53‰ for the $\delta^{13}C$ and $\delta^{15}N$ values, respectively, possibly due to the inherent inaccuracy associated with volumetric measuring techniques. The range of estimated to actual offsets was also larger for volumetrically prepared samples, suggesting that the precision in volumetric ingredient measuring techniques varied between experiments. Given that different individuals prepared each of the volumetrically prepared samples, these results probably reflect the range that would be found in foods and meals prepared by free-living subjects.

This is the first study to report the accuracy of estimating isotope values for multi-ingredient foods from the measured values of their raw ingredients. A similar approach, used by Huelsemann *et al*. to calculate the isotopic signature of meals, involved determining the contribution of each ingredient to the total carbon and nitrogen content of the meal from the percentage carbon and nitrogen of each ingredient as specified by a nutritional analysis software package.[31] Although the reported estimated and experimental values

for the $\delta^{13}C$ and $\delta^{15}N$ values were within the limits of error of the measurement, they did not report the dishes subjected to these cross-validation studies or the values obtained.

As noted in the introduction, analysis of all food samples consumed by free-living subjects over the course of a long-term clinical or observational study would be nearly impossible due to the wide variety of foods consumed and methods of preparation utilized in developed nations. However, the multi-ingredient processed food items that make up a significant proportion of our food choices are primarily composed of a short list of commodity ingredients (wheat flour, soybean oil, sugar, etc.). Thus, knowing the effect of various cooking methods on stable isotope composition and being able to estimate the $\delta^{13}C$ and $\delta^{15}N$ value of multi-ingredient food items from raw ingredients may serve to increase the accuracy of estimated dietary isotope signatures while minimizing the quantity of food samples required for analysis.

While the results of our study demonstrate that cooking does not alter the $\delta^{13}C$ and $\delta^{15}N$ values of grain-based food items, several limitations must be taken into account. First, our findings are only applicable to bulk isotope analysis of foods subjected to baking and fermentation. In addition to alterations in bulk isotope signatures resulting from loss or gain of carbon and nitrogen, alterations in the isotopic signatures of specific compounds may result from chemical reactions occurring throughout the cooking process. Such compound-specific changes were noted by Fernandes *et al.*,[39] who found a $\delta^{15}N$ increase of ≈0.5‰ in water-extracted fish samples subjected to various cooking methods despite there being no change in bulk flesh values. Food-processing techniques involving chemical or physical alteration may also produce compound-specific isotope effects, as Scampicchio *et al.*[49] found alterations in the $\delta^{13}C$ and $\delta^{15}N$ values of whey and lipid fractions of milk subjected to high-temperature heat-processing. Further compound-specific testing, along with analyses of a wider variety of commonly consumed prepared and processed foods, is warranted to fully understand the effects of cooking on food stable isotope composition for dietary reconstruction studies.

One limitation of mass-balance equations for estimating isotopic signatures of processed foods is the absence of quantitative information regarding the amount of each ingredient contained in packaged foods, known as quantitative ingredient declaration (QUID),[50] from American food labels. Since American food manufacturers are not required to list the percentage weight of most ingredients, it would be difficult to determine the fractional proportion of carbon and nitrogen contributed by each ingredient to the total pool from food labels alone. Faced with similar difficulties, the USDA frequently works with companies to obtain quantitative ingredient profiles for estimation of nutrient composition in foods not subjected to individual analysis. Should stable isotope values be incorporated into a standard reference database, similar information could be obtained for use in isotopic mass-balance equations. While this bars the immediate use of mass-balance equations for packaged foods in America, these concepts could be applied in other countries where QUID is mandated, such as the EU and Australia, or to calculate the isotopic ratio of multi-ingredient foods prepared in test kitchens as part of controlled-feeding studies. In the latter example, analysis of a few raw ingredients could be used in calculating the isotopic signatures of multiple complex foods and meals.

Finally, mass-balance equations are limited by the variable isotopic signatures of certain raw ingredients, particularly animal products and added sugars. As demonstrated in this study and others, the isotopic signatures of animal-derived raw ingredients vary considerably as a result of variable feeding practices of American livestock. Even larger variability is found in sugar-based raw ingredients, as both C3 beet sugar ($\delta^{13}C \approx -26‰$) and C4 cane sugar ($\delta^{13}C \approx -11‰$) are prevalent in the U.S. food supply.[51] As seen in our results on beet sugar vs cane sugar cookies, the type of sugar used can alter the $\delta^{13}C$ value of prepared foods. In the United States, food companies and sugar distributors are not required to differentiate the type of sugar used in products, and many distributors frequently switch sources depending on market prices.[52–55] Thus, while representative values of most raw ingredients could be used to reasonably estimate the $\delta^{13}C$ values of processed and prepared foods, animal- and sugar-based ingredients may need to be individually analyzed if these ingredients make up a large percentage of the carbon and/or nitrogen pools.

CONCLUSIONS

We have demonstrated that the $\delta^{13}C$ and $\delta^{15}N$ values of grain-based foods did not change with cooking and can be estimated from the isotopic signatures of their respective raw ingredients. We also demonstrated the similarities in isotopic composition of foods prepared using gravimetric and volumetric measuring techniques, suggesting that mass-balance calculations could be applied to a variety of multi-ingredient foods for which the ingredient profiles are known. This knowledge may help shape future food isotope analysis projects by allowing researchers to calculate the isotopic composition of a wide variety of prepared foods from analyses of commodity raw ingredients.

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