# Correlation of Geographical Location with Stable Isotope Values of Hydrogen and Carbon of Fatty Acids from New Zealand Milk and Bulk Milk Powder

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**ABSTRACT:** The aim of this study was to investigate the correlation of $\delta^2$H and $\delta^{13}$C of bulk milk powder and milk powder fatty acids to their production region. A total of 46 milk powder samples from across New Zealand were collected and analyzed. Principal component analysis (PCA) showed that the $\delta^2$H and $\delta^{13}$C of four fatty acids (C4:0, C14:0, C16:0, C18:1) and bulk milk powder were found to be correlated with regional production area. Linear discriminant analysis (LDA) models were prepared using different combinations of bulk and fatty acid $\delta^2$H and $\delta^{13}$C. All models were effective in discriminating samples from the North and South Islands. The LDA model using just fatty acid $\delta^2$H and $\delta^{13}$C provided the best separation. Therefore, the isotopic composition of the aforementioned fatty acids can be utilized as a good biomarker in milk powder that conveys reliable isotopic information to track milk powders to their regional origin.

**KEYWORDS:** milk powder, food authentication, fatty acid, stable isotopes, multivariate analysis, IRMS

## ■ INTRODUCTION

Milk is an important food commodity that is used as a major or minor ingredient in many processed food products. Freedom of trade in global markets has led to increasing incidents of fraudulent economic practices. Milk fraud and adulteration have long histories. They are economically motivated practices to increase the value of a low-quality milk product by addition of low-cost ingredients such as nitrogen-rich components to boost the apparent protein content. However, as milk quality screening methods and policies become more stringent, the adulteration techniques also become more complex and difficult to detect. Another common fraudulent practice is to pass off product from a low-value producer as that of a high-value producer. Such relabeling of product poses a threat to the genuine producer through loss of sale and potential loss of brand value. The increase in volume and value of global trade in milk provides more opportunity and motivation for fraud[1] and hence the need for robust screening methods to determine the authenticity and origin of the milk products.[2,3]

Milk powder is the most common form of milk being globally traded, with major markets in North America, South America, and the European Community.[4] New Zealand exports 95% of its dairy products and is the largest dairy exporter in the world, accounting for one-third of cross-border dairy trade. In the year ending December 30, 2012, New Zealand dairy export revenues were ca. $14.6 billion and increasing.[5] The global market demand for New Zealand's milk powder quality and acceptability has made it a prime target for fraud. Therefore, novel analytical approaches are required to compliment the traditional traceability systems and provide assurance of origin and safe trade for customers.

Conventional analytical methods that use stable isotope data to determine origin measure the isotopic composition of the bulk sample and hence are applicable to pure (single-source)

products but are unable to distinguish the origin of adulterated dairy products once they are incorporated into mixtures. As most of the milk powder traded internationally is used as an ingredient in other products (e.g., infant formula, confectionary) it is essential to have a system that can provide information on the source of the dairy component of that mixture. Analytical technology enabling the isotopic ratios of specific compounds extracted from mixtures to be determined offers a potential solution to this problem. Some products (e.g., infant formula) add fatty acids (FAs) from other sources, which may confound the application of FA isotope data to determine the origin of the milk. However, the ability to measure specific components may provide a route around this; for example, butyric acid is very unlikely to be added and hence may provide information to test claims of origin.

The isotopic composition of dairy products will reflect the dietary regime of the milk producing animal as well as its metabolism.[6,7] Isotopic fractionation in biochemical and physicochemical reactions lead to wide variation in the $\delta^2$H of individual fatty acid compounds[8] and this variation is important to help in determining milk's region of origin.

Previous studies to determine the geographic origin of milk powder and other food products have demonstrated the potential of stable isotope measurements on the bulk milk powder. Isotope systems used include $^{13}$C, $^{18}$O, $^{15}$N, $^{34}$S,[9,10] and heavy isotopes such as $^{87}$Sr/$^{86}$Sr.[11−14] A number of studies have presented the potential ability of stable isotope techniques to reveal the origin of fresh milk and processed dairy products by measuring bulk and a few fatty acids[10,15−19] or use stable

carbon isotopes to find the relationship between a single fatty acid in milk and fatty acids of the dietary sources.[20]

It has recently been demonstrated that the $\delta^2H$ composition of fatty acids from milk was correlated with a model of the $\delta^2H$ of local precipitation.[21] Therefore, a comprehensive study of fatty acids as specific compounds of milk powder may provide insight and understanding of the extent and robustness of the applicability of this approach for milk powder regional traceability.

In this study the capabilities of bulk milk powder hydrogen, carbon, and nitrogen isotopic analysis and compound-specific isotope analysis (CSIA) of hydrogen and carbon of milk powder FAs are investigated to determine their potential for addressing the geographical origin of milk powder produced from different regions. Sampling dairy products in New Zealand offers many advantages for this work. New Zealand dairy practices are fairly uniform; samples from across the country may be obtained from a single company, minimizing differences in production practices; New Zealand has a fairly uniform marine climate but with significant differences in geographical parameters, such as average temperature, that are important for stable isotope composition. Dairy production regions in the North Island of New Zealand have higher average temperatures than in the South Island.

Here we employ a range of short- to long-chain fatty acid isotopic data from milk powder in addition to bulk milk powder isotopic data. Multivariate statistical analysis of the stable isotope data is used to explore the variation in stable isotope ratios within these components of milk to reveal distinctive patterns between production regions.

## ■ MATERIALS AND METHODS

**Sample Collection, Handling, and Preparation.** *Collection of Milk Powder Samples.* A total of 46 skim milk powder samples were collected from 10 large-scale milk processing units prepacked in aluminum-lined sachets. Samples were collected in November 2010 and represented the spring production season when dairy cows would be predominantly pasture fed and there would be negligible supplemental feeding. Each processing unit was supplied milk by local producers in the immediate region. The samples collected represent a range of latitudes from −36 to −46 (Table 1). The

**Table 1. Locations of the Milk Processing Plants across New Zealand from Which Samples Were Obtained**

| dryer region | location | latitude | longitude | n |
|---|---|---|---|---|
| Maungaturoto | North Island | −36.10° | 174.37° | 4 |
| Waitoa | North Island | −37.60° | 175.63° | 4 |
| Morrinsville | North Island | −37.66° | 175.54° | 4 |
| Te Rapa | North Island | −37.72° | 175.22° | 4 |
| Waikato | North Island | −38.01° | 175.31° | 4 |
| Whareroa | North Island | −39.60° | 174.30° | 4 |
| Pahiatua | North Island | −40.45° | 175.82° | 4 |
| Brightwater | South Island | −41.38° | 173.12° | 4 |
| Clandeboye | South Island | −44.21° | 171.38° | 4 |
| Edendale | South Island | −46.31° | 168.79° | 10 |

sampling areas covered a large latitudinal scale across New Zealand as geographical variation and meteorological parameters affect the hydrogen isotope ratios of precipitation.[21] Samples were stored below room temperature in a dark, dry, and ventilated place to prevent the poly- and monounsaturated fatty acids (PUFA and MUFA) undergoing autoxidation and photo-oxidation.[22]

**Analytical Procedures.** *Bulk $\delta^{13}C$ and $\delta^{15}N$ Analysis.* Bulk $\delta^{13}C$ and $\delta^{15}N$ were measured on 0.8 ± 0.1 mg aliquots of dry milk powder by combustion in an elemental analyzer to $CO_2$ and $N_2$. The isotopic composition of the sample gases was measured by a Delta Advantage isotope ratio mass spectrometer (Thermo-Finnigan, Bremen, Germany) operating in continuous flow mode. Raw delta values were normalized and reported against the international scales for carbon and nitrogen, VPDB and AIR, respectively. Normalization was made by three-point calibration with two glutamic acid international reference materials and a laboratory EDTA (Elemental Microanalysis Ltd., UK) standard for carbon (USGS-40 = −26.2‰, USGS-41 = 37.8‰, EDTA = −38.52‰) and nitrogen (USGS-40 = −4.52‰, USGS-41 = 47.57‰, EDTA = −0.73‰). Time-based drift correction was calculated from the laboratory standard analyzed at regular intervals with the samples. Analytical precision based on the replicate analyses of the QC standard (EDTA, *n* = 12) was 0.2‰ for $\delta^{13}C$ and 0.3‰ for $\delta^{15}N$.

*Bulk $\delta^2H$ Measurement.* The nonexchangeable portion of the hydrogen in the milk powder was determined by a dual steam equilibration process as described previously.[21,23] Briefly, hexatuplicate aliquots of 0.6 ± 0.1 mg of milk powder samples were equilibrated at 110 °C with water of known isotopic composition ($\delta^2H$ = −262.7 or +60‰). The dried samples were analyzed using a Costech zero blank autosampler connected to a TC/EA coupled to a Delta V Advantage via a ConFlo-III interface (Thermo-Finnigan). The TC/EA pyrolyzed the samples to $H_2$ and CO gases in the reduction furnace at 1400 °C.

To monitor the reproducibility of the measurement, the same offline steam equilibration was used on quadruplicates of each of BWB (Bowhead Whale Baleen), CBS (Caribou Hoof Standard), and KHS (Kudu Horn Standard) keratin sample provided by Len Wassenaar, Stable Isotope Hydrology and Ecology Research Laboratory, Gatineau, QC, Canada. The measured $\delta^2H$ values[21] were in close agreement with reported values from previous studies.[23,24]

Equilibration with steam of known isotopic composition was regarded as the preferred method that provided useable precision, at the time of this experimental work. This technique may cause exchange of protons that would not be readily exchanged at room temperature. Room temperature equilibration has more recently been used successfully,[25,26] and comparable results using either method have been obtained in these authors' laboratory. Moreover, performing hydrogen equilibration in ambient conditions on milk may encourage the activity of milk's naturally occurring lipase that might have survived pasteurization (temperature = 72−78 °C) and may lead to hydrolysis of milk triglycerides into free fatty acids. Therefore, the faster, higher temperature approach was considered more suitable for the present study.

**Extraction and Esterification of Milk Powder Fatty Acids.** Milk fat is predominantly composed of triglycerides.[27] These were extracted from milk powder using a modified Bligh and Dyer cold extraction procedure.[21,28] Fatty acids were extracted in 90 × 12 mm Kimax tubes using chloroform/methanol (BDH, Analar) 1:2 followed by a second extraction using chloroform. Triplicate extractions of milk fat were made for each sample. The extracted fatty acids were derivatized by immediately adding 0.54 M $H_2SO_4$ (J. T. Baker USA) in 3 mL of isopropanol (Ajax Finechem Pty Ltd.) to the extracted lipid aliquot.

The Kimax tubes were sealed and heated to 100 °C for 60 min, promoting the esterification of fatty acids into their respective fatty acid isopropyl ester (FAIPE). The isopropyl esters have advantages over the methyl esters as the FAIPEs are less volatile, and therefore extracts are less likely to suffer loss of the short-chain fatty acids (SCFAs).[29] The lower volatility of the FAIPEs also improves chromatographic resolution of SCFAs from the solvent front, for example, butyric acid, C4:0.[21] FAIPEs were extracted into 2 mL of hexane (Merck, Darmstadt, Germany) and transferred to amber GC vials before storage at −20 °C until analysis.[17]

**Milk Powder FAIPE Separation and $\delta^2H$ and $\delta^{13}C$ Analysis.** Milk FAIPEs were initially quantified with an Agilent 6890N gas chromatography−mass spectrometry system (GC-MS) equipped with a TR-225 (60 m × 0.25 mm × 0.25 μm, Thermo Scientific) GC column with stationary phase of mid polarity coupled to 5975B inert XL EI/CI MSD.
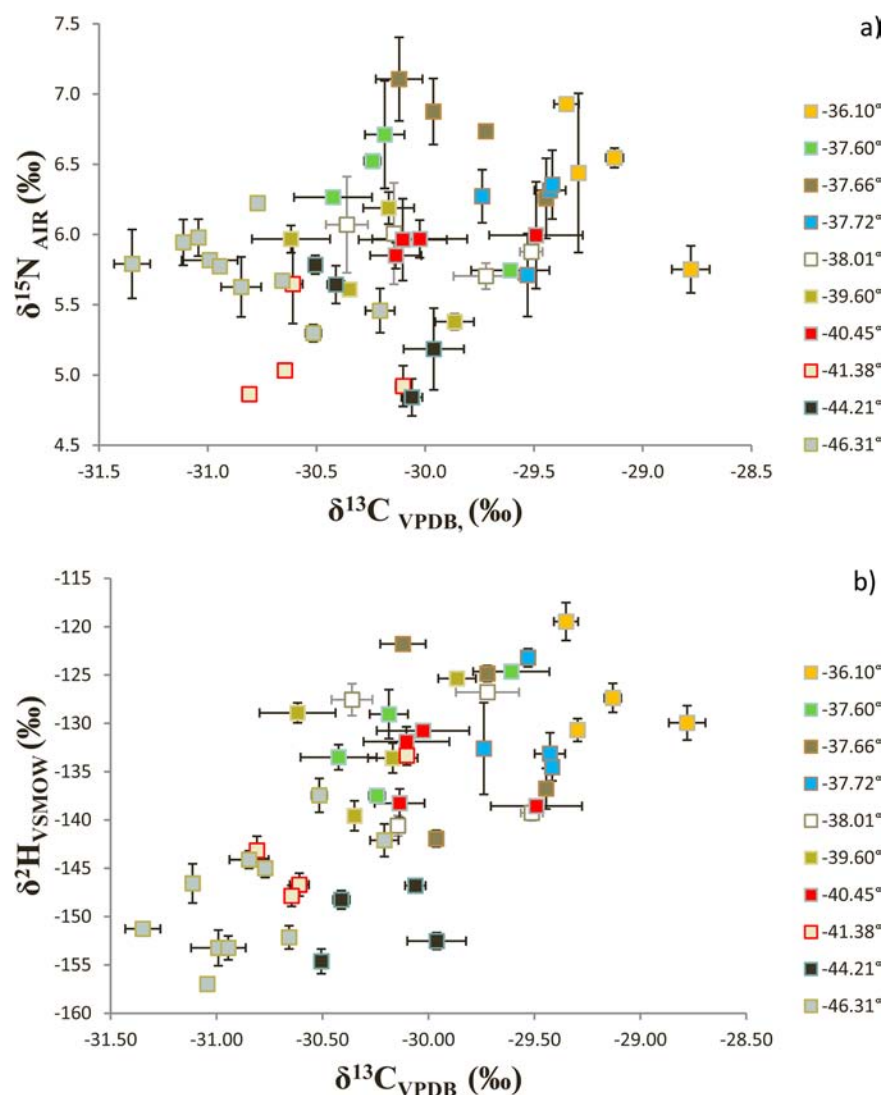
**Figure 1.** Bivariate plots of (a) $\delta^{13}C$ versus $\delta^{15}N$ and (b) $\delta^{13}C$ versus $\delta^2H$ of bulk milk powder samples from 10 processing plants across New Zealand. The latitude of each milk processing plant is given in the legend.

A Thermo Trace GC Ultra gas chromatograph coupled to a Delta Plus XP isotope ratio mass spectrometer (Thermo-Finnigan) was used for measuring $\delta^2H$ values of FAIPEs. The GC was equipped with a TG-225MS (60 m × 0.32 mm × 0.5 $\mu$m, Thermo Scientific) column with stationary phase of mid polarity. Samples were injected by GC-PAL autosampler. The inlet port temperature and carrier gas (helium) were maintained at 260 °C and 2.2 mL/min, respectively, during the entire batch. The GC operating conditions were as follows: splitless injection with initial oven temperature of 45 °C held for 4 min, ramping at 20 °C/min to 170 °C, holding for 0.5 min, ramping at 4 °C/min to the final temperature of 238 °C, and holding for 10 min. This chromatographic program provided very good separation even on low-abundance FAIPEs and FAIPEs isomers and minimized peak coelution and memory effect especially important for successive compounds with large $\delta^2H$ differences.[31]

**Data Normalization.** To measure the isotopic effect of esterification with isopropyl alcohol, an in-house standard of heptanoic acid C7:0 (NuCheck Prep, Elysian, MN, USA) was esterified in triplicate with each batch as described previously.[30] The $\delta^2H$ and $\delta^{13}C$ values of unesterified C7:0 were measured in bulk using injection with a GC-PAL autosampler to the TC/EA-IRMS and by placing 1 mg of the sample in sealed tin capsules into a Carlo Erba NA1500 elemental analyzer (Carlo Erba, Milan, Italy), respectively. This enables the $\delta^2H$ and $\delta^{13}C$ values of FAIPEs to be corrected by mass balance calculation

for the effect of the isopropyl group attached to the free fatty acids in the esterification process.

$\delta^2H$ and $\delta^{13}C$ values of FAIPEs of the samples were measured using GC-IRMS. To correct the instrumental drift as a function of time and to verify that the measured and true isotopic values agree, a blend of three commercially available esters, isopropyl nonanoate C9:0, isopropyl pentanoate C5:0 (Penta Manufacturing, Livingston, NJ, USA), and methyl heneicosanoate C21:0 (NuCheck Prep), was co-injected with samples as internal standards.

The $\delta^2H$ and $\delta^{13}C$ values of C5:0 and C9:0 used as an in-house standard were determined as described for C7:0. Because C21:0 is a solid compound at room temperature, its $\delta^2H$ value was measured using an offline equilibration procedure.[21,23] Raw data were normalized against a standard polyethylene sheet (IAEA-CH-7, −100.3‰) that was coanalyzed with samples. The $\delta^2H$ values were reported in units of per mil (‰) with respect to Vienna Standard Mean Ocean Water (VSMOW).

The $\delta^2H$ and $\delta^{13}C$ precisions of internal standards were 1.6 and 0.3‰, respectively, when calculated according to the equation[32]

$$\text{mean precision (\%)} = \sqrt{\sum_{i=1}^{i=n} \sigma_i^2/n} \tag{1}$$

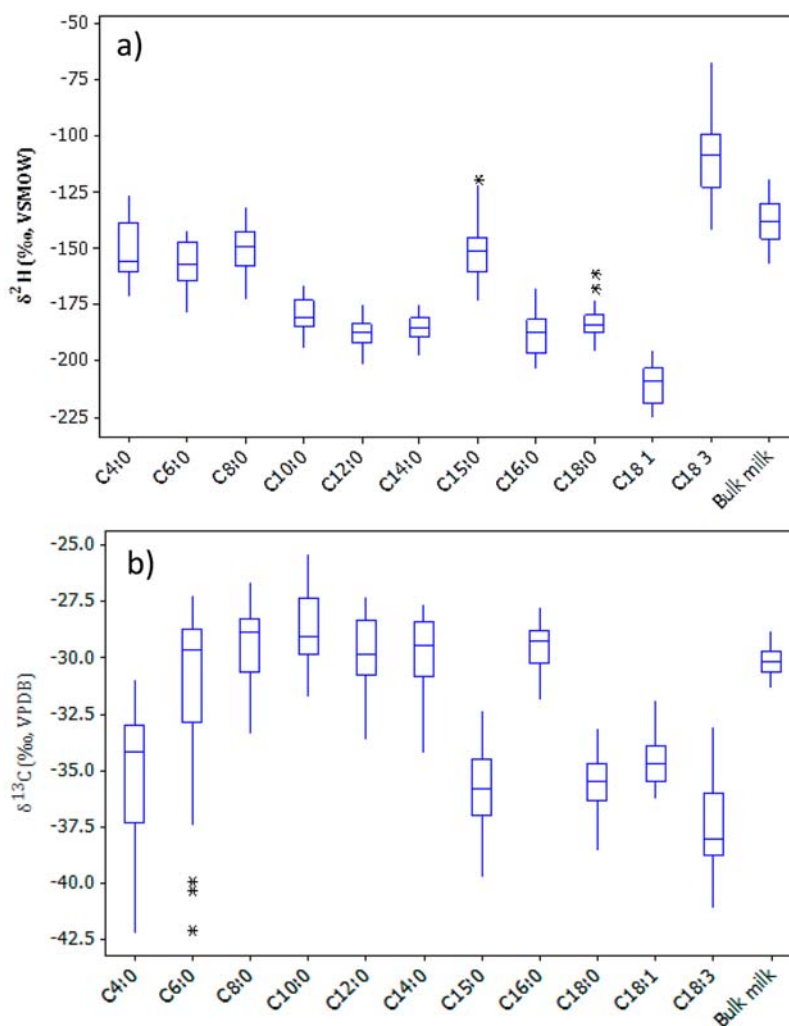where $\sigma$ is the standard deviation of delta values and $n$ is the total number of esters involved in the calculation.

**Figure 2.** Box plots of (a) $\delta^2$H and (b) $\delta^{13}$C of bulk milk powder and 11 milk-derived fatty acids from samples collected across New Zealand.

Comparison of the measured $\delta^2$H and $\delta^{13}$C values of stable isotopes of C5:0, C9:0, and C21:0 standards analyzed by TC/EA-IRMS and GC-IRMS presented Pearson correlations of 0.99 and 0.98. Therefore, a linear calibration for the three IS compounds (raw vs actual) was used to correct for instrumental drift during batches.

A reference mixture (F8) containing eight ethyl and methyl fatty acid esters was purchased from Arndt Schimmelmann, Indiana University. Components in this standard have a range of $\delta^{13}$C values from −23.24 to −31.41‰. The standard was used to assess the precision and accuracy of the GC-IRMS instrument. Regression between measured and reported values for repeated 3 $\mu$L injections ($n$ = 3) was $r$ = 0.96, $P$ < 0.05, with a slope of 1.02 for $\delta^2$H. The regression parameters for $\delta^{13}$C values were $r$ = 0.97, $P$ < 0.05, with a slope of 0.87.

**Statistical Analysis.** Preliminary data exploration was done with box plots prepared using Minitab Statistical software. Principal component analysis (PCA) was performed on the $\delta^2$H and $\delta^{13}$C data using the Unscrambler X Statistical package (CAMO Inc., Oslo, Norway). Prior to performance of the PCA, the data were mean centered. Linear discriminant analysis (LDA) models were determined using the R Statistical Programming Platform.[33]

## ■ RESULTS AND DISCUSSION

**Milk Powder Bulk $\delta^{13}$C, $\delta^{15}$N, and $\delta^2$H.** Bulk carbon and nitrogen isotope ratio values ($n$ = 46) revealed variation of 2.57‰ within a range of −30.14 ± 0.58‰ (mean ± SD) and a

variation of 2.27‰ within a range of 5.93 ± 0.53‰ (mean ± SD) for $\delta^{13}$C and $\delta^{15}$N, respectively.

In general, it has been shown that the carbon isotopic ratio of an animal's tissue is closely related to its diet's isotopic composition.[34] An average carbon isotope enrichment with respect to diet of 0.2‰ and a nitrogen isotope enrichment of 4‰ in cow's milk have been reported by Petersen and Fry.[35] This makes it possible to make an assessment of the type of feed the animal has been provided with by measurement of carbon and nitrogen isotopes of their milk.[7]

Bulk $\delta^{13}$C and $\delta^{15}$N of milk powder showed a weak, linked trend of depletion as the sample origin shifts toward southern (colder) regions (bivariate plot in Figure 1a). This may be explained due to differences in climatic conditions; for example, temperature and humidity are known to promote shifts in $\delta^{13}$C isotopic values in plants from different regions due primarily to the effects of evapotranspiration and photosynthesis.[36]

The slightly more positive values of $\delta^{13}$C for northern (warmer) areas could be the indication of silage fermentation, a byproduct of the bacterial activity of which can be volatile SCFAs such as acetic acid (C2:0), butyric acid (C4:0), and caproic acid (C6:0).[37] One possibility for the observed lower $\delta^{13}$C in southern regions is the use of grass silage as a supplementary feed. $\delta^{13}$C of grass silage (−29.6 ± 0.3‰) contrasts that of maize silage (−11.8 ± 0.1‰) and that of

**Table 2. Pearson Correlation and $P$ Value for the Fatty Acid and Bulk Milk Powder $\delta^{13}C*$ and $\delta^2H**$ Values[a]**

| | C4:0 butyric | C6:0 caproic | C8:0 caprylic | C10:0 capric | C12:0 lauric | C14:0 myristic | C15:0 pentadecanoic | C16:0 palmitic | C18:0 stearic | C18:1 oleic | C18:3 α-linolenic |
|---|---|---|---|---|---|---|---|---|---|---|---|
| C6:0 | 0.847* | | | | | | | | | | |
| | 0.000 | | | | | | | | | | |
| | 0.78** | | | | | | | | | | |
| | 0.000 | | | | | | | | | | |
| C8:0 | 0.786 | 0.913 | | | | | | | | | |
| | 0.000 | 0.000 | | | | | | | | | |
| | 0.53 | 0.79 | | | | | | | | | |
| | 0.000 | 0.000 | | | | | | | | | |
| C10:0 | 0.605 | 0.666 | 0.758 | | | | | | | | |
| | 0.000 | 0.000 | 0.000 | | | | | | | | |
| | 0.73 | 0.82 | 0.79 | | | | | | | | |
| | 0.000 | 0.000 | 0.000 | | | | | | | | |
| C12:0 | 0.683 | 0.713 | 0.783 | 0.877 | | | | | | | |
| | 0.000 | 0.000 | 0.000 | 0.000 | | | | | | | |
| | 0.39 | 0.72 | 0.82 | 0.76 | | | | | | | |
| | 0.007 | 0.000 | 0.000 | 0.000 | | | | | | | |
| C14:0 | 0.731 | 0.704 | 0.668 | 0.772 | 0.947 | | | | | | |
| | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | | | | | | |
| | 0.46 | 0.49 | 0.45 | 0.78 | 0.55 | | | | | | |
| | 0.001 | 0.000 | 0.002 | 0.000 | 0.000 | | | | | | |
| C15:0 | 0.582 | 0.485 | 0.414 | 0.753 | 0.64 | 0.653 | | | | | |
| | 0.000 | 0.001 | 0.006 | 0.000 | 0.000 | 0.000 | | | | | |
| | 0.31 | 0.68 | 0.76 | 0.64 | 0.76 | 0.33 | | | | | |
| | 0.034 | 0.000 | 0.000 | 0.000 | 0.000 | 0.026 | | | | | |
| C16:0 | 0.723 | 0.683 | 0.646 | 0.613 | 0.741 | 0.813 | 0.419 | | | | |
| | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.005 | | | | |
| | 0.33 | 0.03 | −0.17 | 0.32 | −0.14 | 0.68 | −0.2 | | | | |
| | 0.024 | 0.849 | 0.263 | 0.030 | 0.346 | 0.000 | 0.177 | | | | |
| C18:0 | 0.82 | 0.645 | 0.588 | 0.62 | 0.663 | 0.711 | 0.549 | 0.714 | | | |
| | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | | | |
| | 0.31 | 0.48 | 0.4 | 0.44 | 0.53 | 0.18 | 0.7 | −0.005 | | | |
| | 0.034 | 0.001 | 0.006 | 0.002 | 0.000 | 0.213 | 0.000 | 0.971 | | | |
| C18:1 | 0.366 | 0.27 | 0.118 | 0.000 | −0.009 | 0.046 | 0.025 | 0.265 | 0.661 | | |
| | 0.014 | 0.076 | 0.446 | 0.998 | 0.955 | 0.766 | 0.871 | 0.082 | 0.000 | | |
| | 0.55 | 0.24 | −0.06 | 0.38 | −0.08 | 0.46 | −0.06 | 0.83 | 0.33 | | |
| | 0.000 | 0.101 | 0.684 | 0.009 | 0.606 | 0.001 | 0.682 | 0.000 | 0.024 | | |
| C18:3 | −0.186 | −0.323 | −0.319 | −0.008 | −0.304 | −0.328 | 0.249 | −0.127 | −0.007 | 0.231 | |
| | 0.243 | 0.045 | 0.042 | 0.961 | 0.053 | 0.036 | 0.122 | 0.436 | 0.964 | 0.147 | |
| | 0.41 | 0.53 | 0.4 | 0.46 | 0.44 | 0.17 | 0.68 | 0.04 | 0.78 | 0.3 | |
| | 0.004 | 0.000 | 0.006 | 0.001 | 0.002 | 0.267 | 0.000 | 0.794 | 0.000 | 0.041 | |
| bulk milk | 0.629 | 0.668 | 0.642 | 0.298 | 0.372 | 0.323 | 0.123 | 0.63 | 0.608 | 0.491 | −0.187 |
| | 0.000 | 0.000 | 0.000 | 0.055 | 0.015 | 0.028 | 0.426 | 0.000 | 0.000 | 0.001 | 0.255 |
| | 0.4 | 0.1 | −0.16 | 0.3 | −0.16 | 0.47 | −0.17 | 0.86 | 0.13 | 0.89 | 0.22 |
| | 0.007 | 0.521 | 0.294 | 0.043 | 0.279 | 0.001 | 0.264 | 0.000 | 0.403 | 0.000 | 0.150 |

[a]$P$ value <0.05 is significant.

concentrate supplements that have been reported by other authors.[38,39] A trend similar to that in $\delta^{13}C$ was observed for $\delta^{15}N$ (Figure 1a). Soil nitrogen is affected by soil microbial flora and fertilizers being used for plant nutrition, which depends on agricultural practices. This will consequently affect the $\delta^{15}N$ of the feed and animal as has been previously observed.[40,41] A few studies have reported inefficient use of dietary nitrogen in milk production.[42] In addition, the incorporation of body reserve nitrogen into the product[43] is an extra factor that increases the $\delta^{15}N$ isotopic fractionation on the biosynthesis pathway to milk and is thus a further complication for the employment of $\delta^{15}N$ to discriminate the origin of milk.

Bulk values of $\delta^2H$ and $\delta^{13}C$ show a more pronounced north−south depletion gradient (Figure 1b) than that observed in the $\delta^{13}C/\delta^{15}N$ bivariate plot (Figure 1a). This can be described mainly by isotopic fractionation of hydrogen, such that the hydrogen in water vapor becomes gradually depleted in deuterium as it travels away from its source and undergoes progressive depletion of the heavier isotopes during rainout.[44] Therefore, the hydrogen isotopes are more intimately linked to the rainfall processes and hence are expected to provide a more robust geographical pattern than is found for the C and N isotope systems.

**Milk Powder Fatty Acid $\delta^2H$ and $\delta^{13}C$ Values.** Figure 2a presents a box plot that illustrates the $\delta^2H$ variation of 11 fatty

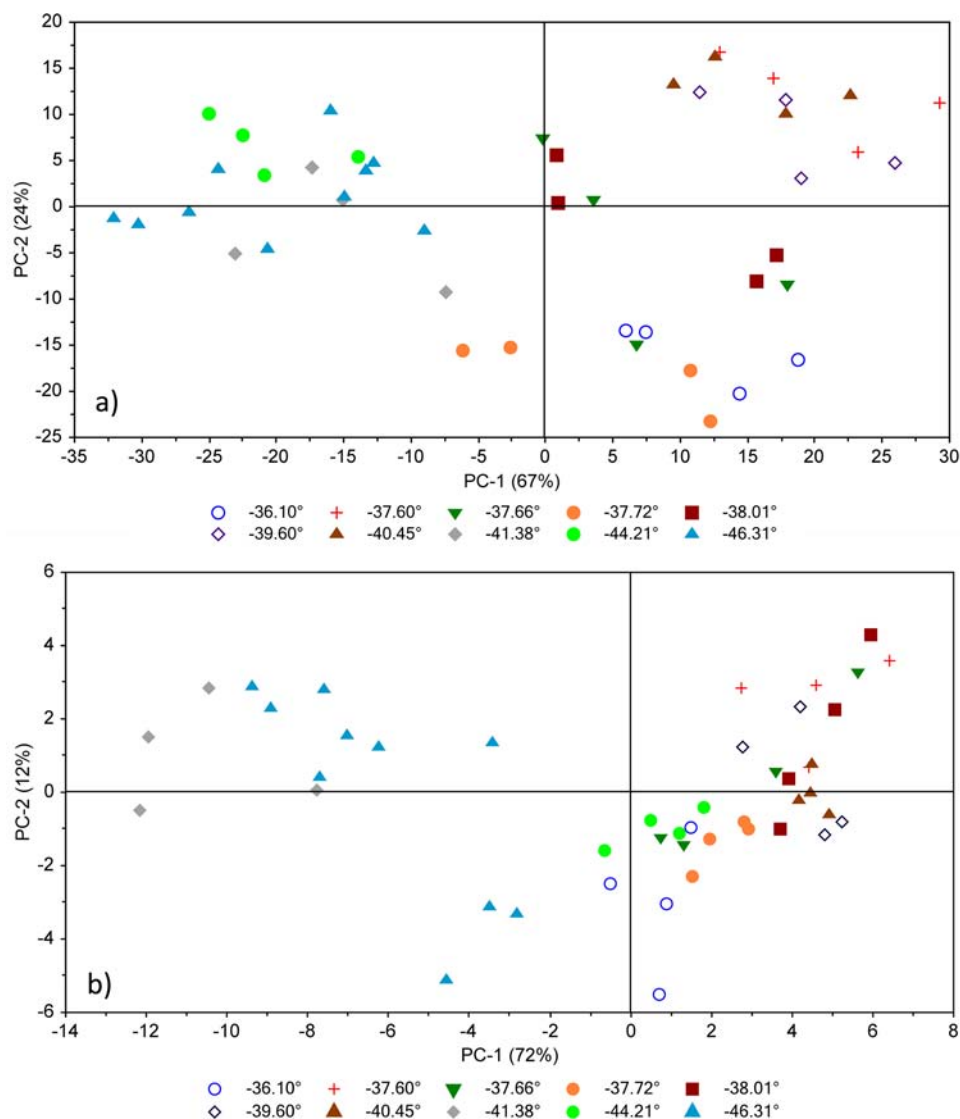**Figure 3.** PCA score plot of (a) $\delta^2H$ and (b) $\delta^{13}C$ milk samples from 10 dryers across New Zealand. Score labels represent geographical latitudes of processing plants ($n = 46$). The latitude of each milk processing plant is given in the legend.

acids. Compared with the SCFAs, greater hydrogen isotope depletion among the long-chain fatty acids (LCFAs) was observed, except for pentadecanoic acid (C15:0) and α-linolenic acid (C18:3). The extent of depletion of the two latter fatty acids might be attributable to rumen microbial biohydrogenation;[45] however, the source of such variations between the examined fatty acids is difficult to ascertain. The presence of the odd chain length pentadecanoic acid (C15:0) is considered to be indicative of microbial activity in the rumen.[46]

A box plot of the $\delta^{13}C$ variation of milk powder fatty acids is presented in Figure 2b. Differences in the ratio of the carbon isotopes were observed among fatty acids. The largest carbon isotope variation was observed on the SCFAs, specifically C4:0 and C6:0, with C4:0 having the lowest $^{13}C$ among the SCFAs. In contrast, highest $^2H$ was observed for C4:0. Palmitic acid (C16:0) carbon isotope values showed little variation between samples with the average and standard deviation being determined as $-29.5 \pm 0.09‰$; this range reflects a proximate correlation to the carbon isotope ratio of C3 feed plants, for example, grass and grass silage. As C16:0 is one of the predominant fatty acids in plants,[47] this might indicate the

C16:0 as presenting the isotopic identity of the dietary source. The $\delta^{13}C$ content of bulk milk has been shown to be a function of a cow's dietary source.[48] The close agreement of C16:0 to the carbon isotope values of C16:0 and bulk milk powder (Table 2) may support this, although the range of values observed here is small.

Stearic acid (C18:0) is reported to be produced from the biohydrogenation of unsaturated fatty acids (UFA), for example, oleic acid (C18:1) and linoleic acid (C18:2).[49,50] Transformation of UFA into stearic acid is carried out in two steps: hydrolysis of the ester linkages of the corresponding triglyceride catalyzed by rumen bacteria enzymes, followed by biohydrogenation of the UFA.[50] Accordingly, the isotopic composition of stearic acid (C18:0) will be linked to its UFA precursors.

Jahreis and Richter reported the direct synthesis of C18:0 in milk from plant lipids, that is, the biohydrogenation of C18:1 from the feed to give the saturated fatty acid (SFA) of C18:0 in the milk.[51] Because for this study the feed source was not provided, this relationship could not be determined; however, a

significant correlation ($P < 0.001$) between the $\delta^{13}C$ of C18:0 and the $\delta^{13}C$ of C18:1 in milk was observed.

**Multivariate Statistics.** The PCA on the $\delta^2H$ and $\delta^{13}C$ data is presented in Figure 3. The first two PCs were able to explain 91 and 84% of variability of $\delta^2H$ and $\delta^{13}C$ in milk powder, respectively (Figure 3). The exploration of the $\delta^2H$ data was based on values from bulk milk and four fatty acids, which had the highest correlation ($P < 0.01$) to precipitation $\delta^2H$ data as previously reported.[17]

The correlation and significance of the mentioned five variables and their influence on the PCA data structure were examined by variable loadings and are presented in Table 3.

**Table 3. Loading and Significance of Selected Variables on the First Two Components**

| $\delta^2H$ | PC1 | *P* value | PC2 | *P* value |
|---|---|---|---|---|
| C4:0 | 0.561 | *0.000* | 0.809 | *0.000* |
| C14:0 | 0.207 | *0.000* | −0.013 | *0.612* |
| C16:0 | 0.454 | *0.000* | −0.411 | *0.000* |
| C18:1 | 0.442 | *0.000* | −0.173 | *0.020* |
| bulk milk | 0.492 | *0.000* | −0.382 | *0.003* |
| $\delta^{13}C$ | PC1 | *P* value | PC2 | *P* value |
| C4:0 | 0.540 | *0.000* | 0.011 | *0.553* |
| C6:0 | 0.527 | *0.000* | −0.186 | *0.028* |
| C8:0 | 0.304 | *0.000* | −0.106 | *0.002* |
| C10:0 | 0.240 | *0.000* | 0.179 | *0.000* |
| C12:0 | 0.273 | *0.000* | 0.037 | *0.030* |
| C14:0 | 0.284 | *0.000* | 0.028 | *0.405* |
| C15:0 | 0.228 | *0.000* | 0.559 | *0.000* |
| C16:0 | 0.151 | *0.000* | −0.026 | *0.387* |
| C18:0 | 0.178 | *0.000* | 0.041 | *0.023* |
| C18:1 | 0.064 | *0.000* | 0.003 | *0.835* |
| C18:3 | −0.102 | *0.002* | 0.777 | *0.000* |
| bulk | 0.064 | *0.000* | −0.039 | *0.222* |

Each variable represents a negative or positive loading on each individual PC. This describes how significantly a variable can explain the data on each component of interest. The proximity and loading similarity of three components, oleic acid (C18:1), palmitic acid (C16:0), and bulk milk, in the positive PC-1 area (Table 3), show that a high degree of correlation exists between the isotopic composition of these compounds and suggests that they might be derived from a common origin, for example, grass or silage. The variable loadings in Table 4 showed that myristic acid (C14:0) has less influence on the separation of

**Table 4. Comparison of the Determined Recognition Abilities (RA; North vs South Island Origin) and Prediction Abilities (PA) Estimated by Two Different Cross-Validation Methods for the LDA Models**[a]

| model | RA | PA (leave one out) | PA (holdout) |
|---|---|---|---|
| a. bulk $\delta^2H + \delta^{13}C + \delta^{15}N$ | 98 | 98 | 97 ± 4 |
| b. FA $\delta^2H$ | 100 | 100 | 98 ± 4 |
| c. FA $\delta^{13}C$ | 100 | 98 | 99 ± 3 |
| d. FA $\delta^2H + \delta^{13}C$ | 100 | 100 | 99 ± 3 |
| e. bulk $\delta^2H$ + FA $\delta^2H$ | 100 | 98 | 98 ± 4 |
| f. bulk $\delta^{13}C$ + FA $\delta^{13}C$ | 100 | 98 | 98 ± 4 |
| g. bulk and FA $\delta^2H + \delta^{13}C$ | 100 | 100 | 97 ± 5 |

[a]All abilities are reported as percentage values with that of the holdout method being the mean value ±1 SD of 1000 random permutations.

south and north regions and no impact on discrimination of subregions within either island on PC2; this is supported by the $P$ value on PC2 ($P < 0.05$). All four fatty acid components are highly correlated (Table 2).

The correlation between and significance of the $\delta^{13}C$ values of the 12 variables of bulk milk and fatty acids on the first two components was examined by variable loadings and is presented in Table 3. From the loading table (Table 3), it can be observed that except for C18:3 ($\alpha$-linoleic acid), the remaining fatty acids have positive loading on component 1, with SCFAs of C6:0 and C4:0 presenting the highest loading. The very small range of C16:0 $\delta^{13}C$ values means it had very little effect on the discrimination of regions compared to other variables. This was surprising as the C16:0 $\delta^2H$ had previously been shown to be highly correlated with precipitation $\delta^2H$.[21]

Thus, it is evident that C10:0, C12:0, and C14:0 fatty acids are providing the means of discriminating the regions from latitudes of −46.31° and −41.38° (Figure 3b). They also have significant ($P \leq 0.05$) positive linear correlation (Table 3), which confirms their similarity in PCA space. Enrichment trends on the $\delta^{13}C$ of SCFA, for example, C4:0 and C6:0, toward northern regions were observed (data not shown), and this seems to increase their correlation to the bulk milk powder carbon isotope value. Part of this correlation may be due to the average temperatures in the northern regions being higher and thereby promoting the fermentation of LCFA to SCFAs in feeds such as silage. This has been observed on the positive region of PC1 loading plots (Table 3), which are predominantly occupied by samples from warmer regions, that is, higher latitudes (Figure 3b).

The lack of correlation ($P > 0.05$) between $\alpha$-linolenic acid (C18:3) and bulk milk powder $\delta^{13}C$ was unexpected (Table 2) as this is the predominant fatty acid in forage.[52] A lack of correlation is also observed between $\delta^2H$ of $\alpha$-linolenic acid (C18:3) and $\delta^2H$ of bulk milk powder (Table 2). However, it has been reported that a large portion of this fatty acid in feed is converted to stearic acid (C18:0) in the rumen and contributed as such in milk.[53] Molkentin demonstrated the potential of $\delta^{13}C$ of $\alpha$-linolenic acid (C18:3) as a discriminant factor to determine conventional and organic fresh milk despite an overlapping interference between the two mentioned dietary regimens.[54] However, monitoring $\alpha$-linolenic acid (C18:3) $\delta^{13}C$ values is not practical for milk powder, as the drying process and storage conditions will likely affect its concentration in milk powder.

*Regional Discrimination.* Seven LDA models, a−g (Table 4), were determined for different combinations of variables made up of bulk and/or fatty acid isotopic compositions. The fatty acid data used were from butyric acid (C4:0), caproic acid (C6:0), myristic acid (C14:0), and oleic acid (C18:1). The models developed were (a) C, N, and H isotope ratios for bulk milk powder, (b) H isotope ratios for milk powder fatty acids, (c) C isotope ratios for milk powder fatty acids, (d) C and H isotope ratios for milk powder fatty acids, (e) H isotope ratios for bulk milk powder and fatty acids, (f) C isotope ratios for bulk milk powder and fatty acids, and (g) C and H isotope ratios for bulk milk powder and fatty acids.

The efficacy of the models for discrimination of the milk powders originating from either the North or South Island of New Zealand was assessed by calculation of the recognition ability (RA) and prediction ability (PA) of the different models. The RA of a discriminant model was determined from the confusion matrix of predicted class against the actual authentic
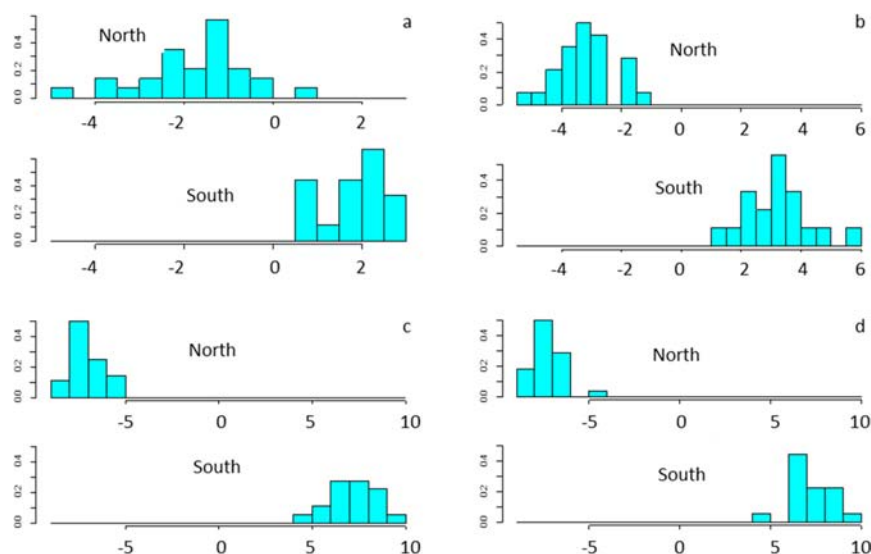
**Figure 4.** Histograms showing the frequencies of prediction (North Island or South Island origin) scores for four LDA models developed using (a) bulk $\delta^2H + \delta^{13}C + \delta^{15}N$, (b) bulk $\delta^2H$ + FA $\delta^2H$, (c) FA $\delta^2H + \delta^{13}C$, and (d) bulk and FA $\delta^2H + \delta^{13}C$.

origin of the milk powders. The RA is the percentage of correct predicted assignments of the training data achieved by the discriminant model. The prediction ability of the model was determined by cross-validation. Cross-validation was undertaken by two methods; the first was cross-validation by leave-one-out classification. The second method of cross-validation was to randomly determine two-thirds of the data set to be utilized in determining the LDA model, and the other third that was held out was used to test the model assignments. This method was repeated 1000 times to give a robust estimation of the mean prediction ability. In both cases the percentage of correct predicted assignments represents the prediction ability of the cross-validated model. The recognition abilities and prediction abilities of the seven models investigated are shown in Table 4.

The model using bulk isotope measurements ($\delta^2H + \delta^{13}C + \delta^{15}N$) provides good discrimination, although the frequency histogram (Figure 4a) shows a wide spread in values, particularly in the north, and, hence, some overlap. The FA $\delta^2H$ + bulk $\delta^2H$ model shows better discrimination and no overlap in the frequency histogram (Figure 4b). The best performing model is the one developed using FA $\delta^2H + \delta^{13}C$ (Figure 4c); the prediction scores cluster tightly, and their groups are at their most distant in terms of prediction score. Similar results were found for the model using all available data (bulk and FA $\delta^2H + \delta^{13}C$, Figure 4d).

The stable isotope (H, C, and N) composition of bulk milk powder sourced from across New Zealand shows geographic variation, although the relationship with latitude is not strong and there is considerable overlap in values between regions (i.e., different locations within one island). An LDA model developed using these bulk isotope parameters was effective in distinguishing samples from the North and South Islands.

The $\delta^2H$ values of CSIA of butyric (C4:0), myristic (C14:0), palmitic (C16:0), and oleic acid (C18:1) plus the $\delta^2H$ of bulk milk powder enabled regional discrimination. The LDA model prepared using only the $\delta^2H + \delta^{13}C$ values of the above FAs provided the best separation of the North and South Island samples. The LDA model built using only FA isotope data performed as well as one prepared using all bulk and FA isotopic data. Therefore, it can be concluded that these fatty

acids can be utilized as biomarkers in milk powder to convey reliable isotopic information that can be used to track milk powders back to their origin. The technique relies upon appropriate authentic-region reference samples being available, so extension of this application to other countries requires these background data be collected.

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■ **REFERENCES**

(1) Romdhane, K. Determination of identity and quality of dairy products. In *Sensory Analysis of Foods of Animal Origin*; CRC Press: Boca Raton, FL, 2010; 456 pp.

(2) Jia, X.; Huang, J.; Luan, H.; Rozelle, S.; Swinnen, J. China's milk scandal, government policy and production decisions of dairy farmers: the case of Greater Beijing. *Food Policy* **2012**, *37*, 390−400.

(3) Lyu, J. C. A comparative study of crisis communication strategies between Mainland China and Taiwan: the melamine-tainted milk powder crisis in the Chinese context. *Public Relat. Rev.* **2012**, *38*, 779−791.

(4) Baldwin, A.; Pearce, D. Milk powder. In *Encapsulated and Powdered Foods*; CRC Press: Boca Raton, FL, 2005; 528 pp.

(5) New Zealand Government, Ministry of Primary Industries, Facts and figures; http://www.mpi.govt.nz/agriculture/pastoral/dairy (accessed May 4, 2013).

(6) Kornexl, B. E.; 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. *Z. Lebensm. Unters. Forsch* **1997**, *205*, 19−24.

(7) Camin, F.; Perini, M.; Colombari, G.; Bontempo, L.; Versini, G. Influence of dietary composition on the carbon, nitrogen, oxygen and

hydrogen stable isotope ratios of milk. *Rapid Commun. Mass Spectrom.* **2008**, *22*, 1690−1696.

(8) Chikaraishi, Y.; Suzuki, Y.; Naraoka, H. Hydrogen isotopic fractionations during desaturation and elongation associated with polyunsaturated fatty acid biosynthesis in marine macroalgae. *Phytochemistry* **2004**, *65*, 2293−2300.

(9) Crittenden, R. G.; Andrew, A. S.; LeFournour, M.; Young, M. D.; Middleton, H.; Stockmann, R. Determining the geographic origin of milk in Australasia using multi-element stable isotope ratio analysis. *Int. Dairy J.* **2007**, *17*, 421−428.

(10) Ritz, P.; Gachon, P.; Garel, J. P.; Bonnefoy, J. C.; Coulon, J. B.; Renou, J. P. Milk characterization: effect of the breed. *Food Chem.* **2005**, *91*, 521−523.

(11) Rossmann, A. Determination of stable isotope ratios in food analysis. *Food Rev. Int.* **2001**, *17*, 347−381.

(12) Ariyama, K.; Shinozaki, M.; Kawasaki, A. Determination of the geographic origin of rice by chemometrics with strontium and lead isotope ratios and multielement concentrations. *J. Agric. Food Chem.* **2012**, *60*, 1628−1634.

(13) Lee, K.; Lee, A.; Gautam, M.; Kim, J.; Shin, W.; Choi, M.; Bong, Y.; Hwang, G. A multi-analytical approach for determining the geographical origin of ginseng using strontium isotopes, multiele-ments, and ^{1}H NMR analysis. *J. Agric. Food Chem.* **2011**, *59*, 8560−8567.

(14) Monahan, F.; Osorio, M.; Moloney, A.; Schmidt, O. Multi-element isotope analysis of bovine muscle for determination of international geographical origin of meat. *J. Agric. Food Chem.* **2011**, *59*, 3285−3294.

(15) Scampicchio, M.; Mimmo, T.; Capici, C.; Huck, C.; Innocente, N.; Drusch, S.; Cesco, S. Identification of the origin and process induced changes in milk by stable isotope ratio mass spectrometry. *J. Agric. Food Chem.* **2012**, *60*, 11268−11273.

(16) Manca, G.; Franco, M. A.; Versini, G.; Camin, F.; Rossmann, A.; Tola, A. Correlation between multielement stable isotope ratio and geographical origin in Peretta cows' milk cheese. *J. Dairy Sci.* **2006**, *89*, 831−839.

(17) Brescia, M. A.; Monfreda, M.; Buccolieri, A.; Carrino, C. Characterisation of the geographical origin of buffalo milk and mozzarella cheese by means of analytical and spectroscopic determinations. *Food Chem.* **2005**, *89*, 139−147.

(18) Camin, F.; Wietzerbin, K.; Cortes, A. B.; Haberhauer, G.; Lees, M.; Versini, G. Application of multielement stable isotope ratio analysis to the characterization of French, Italian, and Spanish cheeses. *J. Agric. Food Chem.* **2004**, *52*, 6592−6601.

(19) Rossmann, A.; Kornexl, B.; Versini, G.; Pichlmayer, F.; Lamprecht, G. Origin assignment of milk from alpine regions by multielement stable isotope ratio analysis (SIRA). *Riv. Sci. Aliment.* **1998**, *27* (1), 9−12.

(20) Richter, E.; Spangenberg, J.; Klevenhusen, F.; Soliva, C.; Kreuzer, M.; Leiber, F. Stable carbon isotope composition of c9,t11-conjugated linoleic acid in cow's milk as related to dietary fatty acids. *Lipids* **2012**, *47*, 161−169.

(21) Ehtesham, E.; Baisden, W. T.; Keller, E. D.; Hayman, A. R.; Van Hale, R.; Frew, R. D. Correlation between precipitation and geographical location of the $\delta^2$H values of the fatty acids in milk and bulk milk powder. *Geochim. Cosmochim. Acta* **2013**, *111*, 105−116.

(22) Scrimgeour, C. Chemistry of fatty acids. In *Bailey's Industrial Oil and Fat Products*, part 1, 6th ed.; Wiley: London, UK, 2005; 749 pp.

(23) Wassenaar, L.; Hobson, K. Comparative equilibration and online technique for determination of non-exchangeable hydrogen of keratins for use in animal migration studies. *Isot. Environ. Health Stud.* **2003**, *39*, 211−217.

(24) Hobson, K. A.; Wunder, M. B.; Van Wilgenburg, S. L.; Clark, R. G.; Wassenaar, L. I. A method for investigating population declines of migratory birds using stable isotopes: origins of harvested lesser scaup in North America. *PLoS ONE* **2009**, *4*, e7915.

(25) Qi, H.; Coplen, T. B. Investigation of preparation techniques for $\delta^2$H analysis of keratin materials and a proposed analytical protocol. *Rapid Commun. Mass Spectrom.* **2011**, *25*, 2209−2222.

(26) Meier-Augustein, W.; Hobson, K. A.; Wassenaar, L. I. Critique: Measuring hydrogen stable isotope abundance of proteins to infer origins of wildlife, food and people. *Bioanalysis* **2013**, *5*, 751−767.

(27) Jensen, R. G.; Newburg, D. S. Chapter 6, Section B. Bovine milk lipids. In *Handbook of Milk Composition*; Robert, G. J., Ed.; Academic Press: San Diego, CA, 1995; 919 pp.

(28) Bligh, E. G.; Dyer, W. J. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **1959**, *37*, 911−917.

(29) Kramer, J.; Fellner, V.; Dugan, M.; Sauer, F.; Mossoba, M.; Yurawecz, M. Evaluating acid and base catalysts in the methylation of milk and rumen fatty acids with special emphasis on conjugated dienes and total trans fatty acids. *Lipids* **1997**, *32*, 1219−1228.

(30) Morrison, D. J.; Cooper, K.; Preston, T. Reconstructing bulk isotope ratios from compound-specific isotope ratios. *Rapid Commun. Mass Spectrom.* **2010**, *24*, 1799−1804.

(31) Wang, Y.; Sessions, A. L. Memory effects in compound-specific D/H analysis by gas chromatography/pyrolysis/isotope-ratio mass spectrometry. *Anal. Chem.* **2008**, *80*, 9162−9170.

(32) Wong, W. W.; Hachey, D. L.; Zhang, S.; Clarke, L. L. Accuracy and precision of gas chromatography/combustion isotope ratio mass spectrometry for stable carbon isotope ratio measurements. *Rapid Commun. Mass Spectrom.* **1995**, *11*, 1007−1011.

(33) R Development Core Team. R: A language and environment for statistical computing; R Foundation for Statistical Computing, Vienna, Austria, 2013.

(34) DeNiro, M. J.; Epstein, S. Influence of diet on the distribution of carbon isotopes in animals. *Geochim. Cosmochim. Acta* **1978**, *42*, 495−506.

(35) Peterson, B. J.; Fry, B. Stable isotopes in ecosystem studies. *Annu. Rev. Ecol. Syst.* **1987**, *18*, 293−320.

(36) Tieszen, L. L. Natural variations in the carbon isotope values of plants: implications for archaeology, ecology, and paleoecology. *J. Archaeol. Sci.* **1991**, *18*, 227−248.

(37) Kalac, P. The required characteristics of ensiled crops used as a feedstock for biogas production: a review. *J. Agrobiol.* **2011**, *28*, 85−96.

(38) Bahar, B. Alteration of the carbon and nitrogen stable isotope composition of beef by substitution of grass silage with maize silage. *Rapid Commun. Mass Spectrom.* **2005**, *19*, 1937−1942.

(39) Pillonel, L.; Badertscher, R.; Casey, M.; Meyer, J.; Rossmann, A.; Schlichtherle-Cerny, H.; Tabacchi, R.; Bosset, J. O. Geographic origin of European Emmental cheese: characterisation and descriptive statistics. *Int. Dairy J.* **2005**, *15*, 547−556.

(40) Knobbe, N.; Vogl, J.; Pritzkow, W.; Panne, U.; Fry, H.; Lochotzke, H.; Preiss-Weigert, A. C and N stable isotope variation in urine and milk of cattle depending on the diet. *Anal. Bioanal. Chem.* **2006**, *386*, 104−108.

(41) Steele, K. W.; Daniel, R. M. Fractionation of nitrogen isotopes by animals: a further complication to the use of variations in the natural abundance of ^{15}N for tracer studies. *J. Agric. Sci.* **1978**, *90*, 7−9.

(42) Cheng, L.; Kim, E. J.; Merry, R. J.; Dewhurst, R. J. Nitrogen partitioning and isotopic fractionation in dairy cows consuming diets based on a range of contrasting forages. *J. Dairy Sci.* **2011**, *94*, 2031−2041.

(43) Ouellet, D. R.; Demers, M.; Zuur, G.; Lobley, G. E.; Seoane, J. R.; Nolan, J. V.; Lapierre, H. Effect of dietary fiber on endogenous nitrogen flows in lactating dairy cows. *J. Dairy Sci.* **2002**, *85*, 3013−3025.

(44) Gat, J. R. *Isotope Hydrology; A Study of the Water Cycle*; Series on Environmental Science and Management; Imperial College Press: London, UK, 2010; Vol. 6, 189 pp.

(45) Bauman, D. E. Biosynthesis of conjugated linoleic acid and its incorporation into meat and milk in ruminants. In *Advances in Conjugated Linoleic Acid Research*; Yurawecz, M. P., Mossoba, M. M., Kramer, J. K. G., Pariza, M. W., Nelson, G. J., Eds.; AOCS Publishing: Champaign, IL, 1999; Vol. 1, 480 pp.

(46) Bauchart, D.; Doreau, M.; Kindler, A. Effect of fat and lactose supplementation on digestion in dairy cows. 2. Long-chain fatty acids. *J. Dairy Sci.* **1987**, *70*, 71−80.

(47) Jahreis, G.; Fritsche, J.; Steinhart, H. Monthly variations of milk composition with special regard to fatty acids depending on season and farm management systems − conventional versus ecological. *Lipid/Fett* **1996**, *98*, 356−359.

(48) Minson, D. J.; Ludlow, M. M.; Troughton, J. H. Differences in natural carbon isotope ratios of milk and hair from cattle grazing tropical and temperate pastures. *Nature* **1975**, *256*, 602−602.

(49) Song, M. K.; Kennelly, J. J. Biosynthesis of conjugated linoleic acid and its incorporation into ruminant's products. *Asian−Aust. J. Anim. Sci.* **2003**, *16*, 306−314.

(50) Thomas, J.; Beverly, T. Conjugated linoleic acid synthesis within the gut microbial ecosystem of ruminants. In *Advances in Conjugated Linoleic Acid Research*; Yurawecz, M. P., Kramer, J. K. G., Gudmundsen, O., Pariza, M. W., Banni, S., Eds.; AOCS Publishing: Champaign, IL, 2006; Vol. 3, 280 pp.

(51) Jahreis, G.; Richter, G. H. The effect of feeding rapeseed on the fatty-acid composition of milk lipids and on the concentration of metabolites and hormones in the serum of dairy cows. *J. Anim. Physiol. Anim. Nutr.* **1994**, *72*, 71−79.

(52) Clapham, W. M.; Foster, J. G.; Neel, J. P. S.; Fedders, J. M. Fatty acid composition of traditional and novel forages. *J. Agric. Food Chem.* **2005**, *53*, 10068−10073.

(53) Wyss, U.; Collomb, M. Influence of the botanical composition of grass, hay or silage on the fatty acid composition of milk. *Rencon. Autour. Rech. Rum.* **2006**, *13*, 325.

(54) Molkentin, J. Authentication of organic milk using $\delta^{13}$C and the $\alpha$-linolenic acid content of milk fat. *J. Agric. Food Chem.* **2009**, *57*, 785−790.