Elucidating the Origin of Milk Products on the Chinese Market Using Hydrogen and Oxygen Stable Isotope Technique

Jingyu Huang, Eyram Norgbey*, Philip Nti Nkrumah, Gloria Appiah-Sefah and Rainizafy Michel

Ministry of Education Key Laboratory of Integrated Regulation and Resource Development on Shallow Lakes, College of Environment, Hohai University, Nanjing, 210098 China.

*Corresponding author
Eyram Norgbey, Ministry of Education Key Laboratory of Integrated Regulation and Resource Development on Shallow Lakes, College of Environment, Hohai University, Nanjing, 210098 China; Tel: +8613813966025; E-mail: eyramnorgbey@outlook.com

Submitted: 27 Feb 2017; Accepted: 08 Mar 2017; Published: 20 Mar 2017

Abstract
Preferre for foreign milk products is the cause of the economically motivated adulteration of milk products on the Chinese market. 42 milk samples from the United States of America, Canada, Southern China, Northern China, Australia and New Zealand were analyzed using δ2H and δ18O stable isotope technique to differentiate the origin of milk products. An isotope ratio mass spectrometer with a combination of a high-temperature conversion elemental analyzer, Thermo-Fisher was used. Statistical analysis was performed using one-way ANOVA. The study revealed δ2H and δ18O had a wide range of mean values: 13.86 to 22.25‰ and -82.86 to -28.5‰, respectively. There was a significant difference in the δ2H (n=7; F=20880, P=7.876E-43) and δ18O (n=7; F=1399.0; P=9.215E-29) composition of the milk samples from the different regions. It was observed that δ2H and δ18O composition is helpful in elucidating milk products from different regions on the Chinese market (P<0.05).

Keywords: Food Safety, Pure Milk, Economically Motivated Adulteration, Stable Isotope Ratios, Origin.

Introduction
Consumers in China are, for many reasons, asking for information about the food they are purchasing: the authenticity of the food and the geographical location of the food. Food adulteration is prominent in China and consumers are skeptical about the origin of food sold on the Chinese market [1]. Food adulteration affects the quality of food, which poses negative effects on consumers’ nutrition and in some cases have led to adverse health effects including death [2]. According to Lu and Wu (2014), criminal cases relating to food safety that include food adulteration grew by 179.8% and 224.6% respectively; in 2011 and 2012 in China [3]. Thus, the need to study food fraud issues in China is necessary and timely. Milk is a major issue for the dairy industry in terms of adulteration. Significant profits on dairy items make them a prime target for adulteration [4]. Because Chinese consumers have a high preference for foreign milk products, they are the most vulnerable when it comes to milk adulteration and mislabeling. As it may not be obvious to detect the authenticity via physical observation, there is the urgent need for robust techniques that could provide unequivocal authentication outcome. Stable isotopes of hydrogen (H) and oxygen (O) in milk water are particularly essential for geographical origin assignment of food connected to regional climatic conditions because they are strongly latitude-dependent [5]. The δ18O content in milk water reflects the isotope composition of the ground water drunk by animals which in turn depends on the altitude, seasonality, total precipitation and distance from the sea [6]. This implies that it may be possible to discriminate among milk products that are obtained from different geographical origin using hydrogen and oxygen stable Isotopic signatures. The main aim of this study is to use the isotopic ratios of H and O within the milk, using mass spectroscopy, to reveal the true geographical origin of milk products on the Chinese market and tackle the mislabeling and deception done by local manufacturers.

Materials and Methods
Sample preparation and Statistical analysis: Forty-two milk samples were collected from different geographical origins, including the United States of America (USA), Canada (CA), Southern part of China (SC), Northern part of China (NC), Australia (AU), New Zealand (NZ). Each δ2H and δ18O composition of the samples (n=7 for each region) represent the mean of 3 replicates. Each liquid milk sample was obtained from genuine sources and was 100% authentic. The data obtained were analyzed using descriptive statistics and one-way analysis of variance (ANOVA) in the Excel Analysis ToolPak. A confidence level of 95% was accepted while...
Stable isotope analysis is a powerful tool for provenance determination of food materials because isotopic compositions of the materials reflect many factors in the natural environment [7]. Renou et al. (2004) indicated stable isotope analysis can be a useful tool in showing a clear disparity between milk products from different regions [8]. Studies indicate that the H and O stable isotope values of animal tissue correlates with the isotopic composition of local precipitation [9]. The δ2H and δ18O composition in water consumed by animals shows a strong correlation with the δ2H and δ18O content present in animal products such as milk [10]. Particularly, Chesson et al. (2010) established that the δ2H and δ18O value of milk water show the isotopic composition of drinking water and water consumed from fresh forage, with minor deviations due to the contribution of food and atmospheric oxygen to the water body. According to Simpkins et al. (1999), the predominant influence on the fractionation of H and O isotopes is evaporation of water, which is reliant on climatic conditions such as humidity, temperature and rainfall. Moreover, the primary driver of the systematic geospatial patterns of H and O isotope ratios in precipitation is the preferential stripping of the heavy isotopes (i.e., 2H and 18O) from water vapor as ocean-saturated air masses move inland and across continents. Hence, at global to regional scales, the spatial variation in the isotopic composition of environmental water resources and human drinking water is consistent with the geographic patterns of precipitation isotopes. Studies indicate that as the δ2H and δ18O content of local water are related to the climatic and geographical features of an area, milk water with δ2H and δ18O can distinguish between milk produced in different areas [11-13].

From the present study, a very high significant difference in the δ2H and δ18O composition of milk water having a probability of 9.678E-6 and 9.215E-29 respectively (P< 0.05) were obtained from the regions under consideration (Table 1). The δ2H and δ18O content present in animal products such as milk water consumed by animals shows a strong correlation with the isotopic composition of local precipitation [9]. The δ2H and δ18O composition in water consumed by animals shows a strong correlation with the δ2H and δ18O content present in animal products such as milk [10]. Particularly, Chesson et al. (2010) established that the δ2H and δ18O value of milk water show the isotopic composition of drinking water and water consumed from fresh forage, with minor deviations due to the contribution of food and atmospheric oxygen to the water body. According to Simpkins et al. (1999), the predominant influence on the fractionation of H and O isotopes is evaporation of water, which is reliant on climatic conditions such as humidity, temperature and rainfall. Moreover, the primary driver of the systematic geospatial patterns of H and O isotope ratios in precipitation is the preferential stripping of the heavy isotopes (i.e., 2H and 18O) from water vapor as ocean-saturated air masses move inland and across continents. Hence, at global to regional scales, the spatial variation in the isotopic composition of environmental water resources and human drinking water is consistent with the geographic patterns of precipitation isotopes. Studies indicate that as the δ2H and δ18O content of local water are related to the climatic and geographical features of an area, milk water with δ2H and δ18O can distinguish between milk produced in different areas [11-13].

From the present study, a very high significant difference in the δ2H and δ18O composition of milk water having a probability of 9.678E-6 and 9.215E-29 respectively (P< 0.05) were obtained from the regions under consideration (Table 1).

Table 1 - The mean δ18O and δ2H isotopic composition of milk samples from all the regions under consideration

| Regions            | δ18O composition (‰) | δ2H composition (‰) |
|--------------------|----------------------|---------------------|
| USA                | 13.86                | -65.93              |
| Canada             | 17.35                | -82.86              |
| Southern part of China | 20.41             | -31.37              |
| Northern part of China | 21.63              | -28.50              |
| Australia          | 22.25                | -39.73              |
| New Zealand        | 21.62                | -63.95              |
| Statistical analysis | n=7; F=1399.0; P= 9.215E-29; 7.876E-43 |

Descriptive statistics; α=0.05, Confidence level=95% (one-way ANOVA). Each δ18O and δ2H composition of the samples represents the mean of 3 replicates.

As shown in Table 2, the variation in δ2H values by geographical origin showed there were high significant differences in following regions when compared: USA vs. CA, AU vs. NZ, SC vs. NC, USA vs. AU, USA vs. NZ, USA vs. NC, USA vs. SC, SC vs. AU, SC vs. NZ, SC vs. CA, NC vs. AU, NC vs. NZ, NC vs. CA, AU vs. CA, NZ vs. CA. Also from (Table 2).
The variation in δ18O values by geographical origin also showed there were high significant differences in following regions when compared: USA vs. CA, AU vs. NZ, SC vs. NC, USA vs. AU, USA vs. NZ, USA vs. NC, USA vs. SC, SC vs. AU, SC vs. NZ, SC vs. CA, NC vs. AU, NC vs. CA, AU vs. CA, NZ vs. CA. Showing a P value less than 0.05 which indicate that δ2H and δ18O values in the milk sample can differentiate between the regions under study when compared, showing the true geographical origin of milk. So in an instance when a fraudster insists his milk product is from a particular region, it is possible to authenticate the geographical region of the milk product using hydrogen and oxygen stable isotope technique. The extremely high significant difference observed in the samples obtained from different regions-of-origins is explained by the geographic variation in water isotope ratios.

However, O stable isotope technique failed to provide a unique distinction between the milk samples obtained from the Northern part of China and New Zealand (P= 0.8615). This may be due to the fact that animals may have a similar diet which supports works by Renou et al [8], Ritz et al. (2005) also explained that difference in the type of breed and difference in time elapsed since last pregnancy of the milk producing animal can also affect the δ18O composition [14]. Also, similar climatic conditions within the production regions may be the cause. This is because the content of δ18O depends on climatic conditions [5]. In a past study, stable isotope values were utilized to develop a new analytical approach enabling the identification of milk samples from different geographical origins [6]. The results they found were quite consistent with our results, which is that milk samples from the six different regions, the United States of America, Canada, Southern China, Northern China, Australia and New Zealand, could be easily discriminated and classified by δ2H and δ18O.

As a result of the extreme significant difference in the δ2H and δ18O composition of the milk samples obtained from the different geographical regions, fraudulent manufacturers cannot label milk products obtained from China as a milk obtained from a different geographical region. From our study, H and O stable isotope techniques may be useful in providing a unique discrimination in the origin of production of commercially distributed milk on the Chinese market. And thus could easily prevent the cheat of domestic pure milk to foreign pure milk or mislabeling pure milk.

### Conclusions

China exports food to most parts of the world and it is not uncommon to find substandard products or mislabeled products in the Chinese market. Thus it is important that food exported to other countries from China are genuine and consumers are given what they have paid for. In this study, stable hydrogen and oxygen isotopic compositions (δ2H, δ18O) of 42 pure milk from various cultivated areas (the United States of America, Canada, Southern China, Northern China, Australia and New Zealand), were applied to discriminate the geographical origin of pure milk. It is clear from our discussions above that it is possible with varying degrees of certainty to determine the geographical origin of milk using δ2H, and δ18O.

### References

1. Huang J, Nkrumah PN, Appiah-Sefah G, Tang S (2013) Authentication of pure L-Leucine products manufactured in china by discriminating between plant and animal sources using nitrogen stable isotope technique. J Food Sci 78: 490-494.
2. Gossner CM-E, Schlundt J, Ben Embarek P, Hird S, Lo-Foodong D, et al. (2009) The melamine incident: implications for international food and feed safety. Environ Health Perspect 117: 1803-1808.
3. Lu F, Wu X (2014) China food safety hits the “gutter.” Food Control 41: 134-138.
4. Xue J, Zhang W (2013) Understanding China’s food safety problem: An analysis of 2387 incidents of acute foodborne illness. Food Control 30: 311-317.
5. Kelly SD (2003) Using stable isotope ratio mass spectrometry (IRMS) in food authentication and traceability. Food Authent traceability 156-183.
6. Luo D, Dong H, Luo H, Xian Y, Guo X, et al. (2015) Multi-Element (C, N, H, O) Stable Isotope Ratio Analysis for Determining the Geographical Origin of Pure Milk from

| Table 2: Discriminating between the regions-of-origins of milk using Oxygen and Hydrogen stable isotope technique |
|---|---|---|---|
| Regions | Oxygen Isotope Analysis | Hydrogen Isotope Analysis |
| | P | Feasibility | P | Feasibility |
| USA vs. Canada | 2.27E-9 | Yes | 3.176E-13 | Yes |
| Australia vs. New Zealand | 0.0004262 | Yes | 6.068E-14 | Yes |
| Southern part of China vs. Northern part of China | 2.2408E-5 | Yes | 1.427E-6 | Yes |
| USA vs. Australia | 2.146E-12 | Yes | 1.904E-14 | Yes |
| USA vs. New Zealand | 9.146E-13 | Yes | 9.678E-6 | Yes |
| USA vs. Northern part of China | 2.0566E-12 | Yes | 5.9104E-16 | Yes |
| USA vs. Southern part of China | 4.732E-11 | Yes | 2.901E-15 | Yes |
| Southern part of China vs. Australia | 1.558E-6 | Yes | 5.074E-10 | Yes |
| Southern part of China vs. New Zealand | 1.5531E-5 | Yes | 7.596E-15 | Yes |
| Southern part of China vs. Canada | 2.871E-8 | Yes | 1.511E-16 | Yes |
| Northern part of China vs. Australia | 0.0009054 | Yes | 2.236E-11 | Yes |
| Northern part of China vs. New Zealand | 0.8615 | No | 1.686E-15 | Yes |
| Northern part of China vs. Canada | 4.2271E-10 | Yes | 4.2271E-10 | Yes |
| Australia vs. Canada | 2.558E-10 | Yes | 4.557E-16 | Yes |
| New Zealand vs. Canada | 2.161E-10 | Yes | 2.442E-13 | Yes |
Different Regions. Food Anal Methods 9: 437.

7. Nakashita R, Suzuki Y, Akamatsu F, Iizumi Y, Korenaga T, et al. (2008) Stable carbon, nitrogen, and oxygen isotope analysis as a potential tool for verifying geographical origin of beef. Anal Chim Acta 617: 148-152.

8. Renou J, Deponge C, Gachon P, Bonnefoy J, Ritz P (2004) Characterization of animal products according to geographic origin and feeding diet using nuclear magnetic resonance and isotope ratio mass spectrometry. Anal Chim Acta 517: 63-66.

9. Ehleringer JR, Bowen GJ, Chesson LA, West AG, Podlesak DW, Cerling TE, et al. (2008) Hydrogen and oxygen isotope ratios in human hair are related to geography. Proc Natl Acad Sci U S A 105: 2788-2793.

10. Kelly S, Heaton K, Hoogewerff J (2005) Tracing the geographical origin of food: The application of multi-element and multi-isotope analysis. Trends Food Sci Technol 16: 555-567.

11. Chesson LA, Valenzuela LO, O’Grady SP, Cerling TE, Ehleringer JR (2010) Hydrogen and Oxygen Stable Isotope Ratios of Milk in the United States. J Agric Food Chem 58: 2358-2363.

12. Simpkins WA, Patel G, Collins P, Harrison M, Goldberg D (1999) Oxygen isotope ratios of juice water in Australian oranges and concentrates. J Agric Food Chem 47: 2606-2612.

13. Bontempo L, Lombardi G, Paolotti R, Ziller L, Camin F (2012) H, C, N and O stable isotope characteristics of alpine forage, milk and cheese. Int Dairy J 23: 99-104.

14. Ritz P, Gachon P, Garel J, Bonnefoy J, Coulon J, et al. (2005) Food Chemistry Milk characterization: effect of the breed 91: 521-523.