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Elucidating the Origin of Milk Products on the Chinese Market Using Multi Element Stable Isotope Technique

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Abstract

Preference for foreign milk products is the cause of the economically motivated adulteration of milk products on the Chinese market. The present study was done to ascertain the feasibility of utilizing δ^2H, $\delta^{18}O$ and $\delta^{15}N$ stable isotope techniques in elucidating the geographical origin 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. 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 that both δ^2H and $\delta^{18}O$ 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 δ^2H (n=7; F=20880, P=7.876E-43) and $\delta^{18}O$ (n=7; F=1399.0; P=9.215E-29) composition of the milk samples from the different regions. It was observed that δ^2H and $\delta^{18}O$ composition is helpful in elucidating milk products from different regions on the Chinese market (P<0.05). The range of the mean $\delta^{15}N$ values of the milk samples was very close, 3.06 to 5.61‰, indicating a possible limitation in employing $\delta^{15}N$ isotopic technique. The nitrogen stable isotope could not provide a clear distinction for the milk products because $\delta^{15}N$ of an animal reflects that of the diet. Hence in cases of similar diet, it may not be possible to provide a distinction between the animals using this technique.

Keywords: mislabeled milk; economically motivated adulteration; stable isotope ratios, food safety;

Introduction

Food adulteration is a global problem and more prominent in China [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. This implies that 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 and mislabeling [3]. Qiao and others (2010) bemoaned that because of the melamine adulterated dairy products in China, confidence in China's dairy industry has reduced and consumption of dairy products in China has dropped significantly. Also, because dairy products manufactured in particular regions may have some added value [4], there is the tendency that milk products manufactured in a region (for instance China) may be labeled as products of different geographical origin. In view of these challenges, there is the need to develop a robust technique to improve upon existing techniques that seek to address these crucial problems.

Stable isotopes of hydrogen (H) and oxygen (O) are particularly essential for geographical origin assignment of food connected to regional climatic conditions because they are strongly latitude-dependent [5]. H and O isotopic signatures are also affected by altitude, seasonality, total precipitation and distance from the sea [6]. The application of the nitrogen (N) stable isotope technique in authenticating milk products is based on its ability to give an indication of the dietary background of the animal sources from which the milk was obtained [7]. This study tends to apply H, O and N stable isotope to reveal the authenticity and geographical locations of commercially distributed milk products on the Chinese market.

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 δ^2H, $\delta^{18}O$ and $\delta^{15}N$ 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 an alpha value of 0.05 was used in the -one-way ANOVA.

Milk water: Moderate rennet (activity ≥105 U/g) obtained from the USA was added to pure milk and the samples were left overnight at room temperature. After, the milk water was collected by filtration and then frozen at −20 °C until analysis.

Standards: Stable isotope ratios are expressed in delta (δ) notation, was used to describe the isotopic difference between the sample and an international standard, which is defined by the equation.

$$\delta(‰)=[(R\_s/R\_std)-1]*1000 \qquad (1)$$

Where R_s was the isotope ratio (i.e., $^2H/^1H$, and $^{18}O/^{16}O$) of the sample, and R_{std} was that of the reference materials. Variations in stable isotope ratios were reported as parts per thousand (‰) deviations from internationally accepted standards: Vienna Standard Mean Ocean Water (VSMOW) for oxygen and hydrogen.

Measurements: The liquid milk samples were analyzed using an isotope ratio mass spectrometer (IRMS) operating in the continuous flow mode (Integra CN, Sercon, Cershire, UK) with a combination of a high temperature conversion elemental analyzer (TC/EA) attached Flash Element Analyzer 1112 HT-Delta V advantage, Thermo-Fisher for oxygen isotope analysis. For hydrogen isotopic analysis, the IRMS was equipped with an energy filter to suppress ^{+4}He ions on the attached Flash EA 1112 HT-Delta V advantage, Thermo-Fisher. The reactor consists of a glassy carbon tube with glassy carbon filling, ensuring that neither sample nor reaction gasses can get into contact with oxygen-containing surfaces. The reaction gasses are separated in an isothermal gas chromatograph, which is also part of the TC/EA. The gasses were admitted to the IRMS for isotope analysis. Measurements were made using calibration of the system with reference hydrogen and oxygen gas and reference standard material of VSMOW in regular repetitions.

Results and Discussion

Results

Hydrogen and Oxygen Stable Isotopes

The mean δ^2H and $\delta^{18}O$ composition of milk samples are shown in Table 1 respectively. The range of the mean values for δ^2H and $\delta^{18}O$ are -82.86 to -28.50‰ and 13.86 to 22.25‰ respectively. The difference in the δ^2H (F=20880; P= 7.876E-43; n=7) and $\delta^{18}O$ (F=1399.0; P= 9.215E-29; n=7) composition of the samples were highly significant. Table 2 shows discrimination between the regions-of-origins of milk using oxygen and hydrogen stable isotope technique.

Table 1: The mean $\delta^{18}O$, δ^2H and $\delta^{15}N$ isotopic composition of milk samples from all the regions under consideration

Regions	$\delta^{18}O$ composition (‰)	δ^2H composition (‰)	$\delta^{15}N$ composition (‰)
USA	13.86	-65.93	3.32
Canada	17.35	-82.86	4.30
Southern part of China	20.41	-31.37	3.76
Northern part of China	21.63	-28.50	5.15
Australia	22.25	-39.73	5.61
New Zealand	21.62	-63.95	3.06
Statistical analysis	n=7; F=1399.0; P= 9.215E-29;	n=7; F=20880; P= 7.876E-43	n=7 F=15.46; P= 7.832E-7;

Descriptive statistics; α=0.05, Confidence level=95% (one-way ANOVA). Each $\delta^{18}0$, δ2H and $\delta^{15}N$ composition of the samples represents the mean of 3 replicates.

Nitrogen Stable Isotopes

The mean $\delta^{15}N$ composition of milk samples is depicted in Table 1 having a mean range value between 3.064 and 5.150‰. The difference in the δ15Ncomposition of the samples had an F value of 15.46 and a P value of 7.832E-7. Table 2 shows discrimination between the regions-of-origins of milk using nitrogen stable isotope technique

Table 2: Discriminating between the regions-of-origins of milk using Oxygen, Hydrogen and Nitrogen stable isotope technique

Regions	Oxygen Isotope Analysis		Hydrogen Isotope Analysis		Nitrogen Isotope Analysis	
	P	Feasibility	P	Feasibility	P	Feasibility
USA vs. Canada	2.27E-9	Yes	3.176E-13	Yes	0.06744	No
Australia vs. New Zealand	0.0004262	Yes	6.068E-14	Yes	0.0003266	Yes
Southern part of China vs. Northern part of China	2.2408E-5	Yes	1.427E-6	Yes	0.01455	Yes
USA vs. Australia	2.146E-12	Yes	1.904E-14	Yes	7.270E-5	Yes
USA vs. New Zealand	9.146E-13	Yes	9.678E-6	Yes	0.4948	No
USA vs. Northern part of China	2.0566E-12	Yes	5.9104E-16	Yes	0.001936	Yes
USA vs. Southern part of China	4.732E-11	Yes	2.901E-15	Yes	0.1437	No
Southern part of China vs. Australia	1.558E-6	Yes	5.074E-10	Yes	0.0009106	Yes
Southern part of China vs. New Zealand	1.5531E-5	Yes	7.596E-15	Yes	0.1204	No
Southern part of China vs. Canada	2.871E-8	Yes	1.511E-16	Yes	0.5805	No
Northern part of China vs. Australia	0.0009054	Yes	2.236E-11	Yes	0.3529	No
Northern part of China vs. New Zealand	0.8615	No	1.686E-15	Yes	0.003113	Yes
Northern part of China vs. Canada	4.2271E-10	Yes	4.2271E-10	Yes	0.01208	Yes
Australia vs. Canada	2.558E-10	Yes	4.557E-16	Yes	0.0003088	Yes
New Zealand vs. Canada	2.161E-10	Yes	2.442E-13	Yes	0.0355	Yes

Discussion

Elucidating the regions-of-origins of milk products on the Chinese market using hydrogen, oxygen and nitrogen stable isotope technique

Stable isotope analysis of 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 [9,10]. Theδ^2H andδ^{18}O composition in water consumed by animals shows a strong correlation with the δ^2H and δ^{18}O content present in animal products such as milk [11]. Particularly, Chesson et al. (2010) established that theδ^2H and δ^{18}O 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 [12].

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., ^{2}H and ^{18}O) 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 δ^2H and δ^{18}O content of local water are related to the climatic and geographical features of an area, milk water with δ^2H and δ^{18}O can distinguish between milk produced in different areas [11,13]. From the present study, a very high significant difference in the δ^2H and δ^{18}O 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). As shown in Table 2, the variation in δ^2H 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 δ^{18}O 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 δ^2H and δ^{18}O 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 [14]. 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. 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 δ^{18}O composition [8,15]. Also, similar climatic conditions within the production regions may be the cause. This is because the content of δ^{18}O 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 [16]. 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 δ^2H and δ^{18}O. As a result of the extreme significant difference in the δ2H and δ^{18}O 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.

The application of the nitrogen stable isotope technique in authenticating milk products is based on its ability to give an indication of the dietary background of the animal sources from which the milk was obtained [7]. From Table 1, the milk samples of USA origin had lower δ^{15}N values relative to the samples produced in Canada. It must be noted that the difference in the δ^{15}N composition of the milk samples from the respective origins was statistically insignificant ($F=4.469$; $P= 0.06744$). Hence, it can be asserted that the δ^{15}N composition of the milk samples from USA and Canada may have been obtained from animal sources with similar dietary background. Actually, the mean± S.E. δ^{15}N values of the milk samples from the respective origins (Table 1) confirm this assertion. The relatively lower δ^{15}N composition of the milk samples from both sources give an indication that the dietary source is grass. This is because it has been established that grasses tend to be relatively depleted in δ^{15}N compared with maize and rape [17]. It may also be as a result of the presence of nitrogen fixing plants in the diet [18]. Also, the relatively lower δ^{15}N composition may mean that the dietary sources might have been grown using synthetic fertilizer [19]. This gives an indication of the kind of agricultural practices these animals are subjected to [20]. These diverse reasons confirm the assertion that because there exists different biosynthesis pathway of different plant species, habitat influences, or possible fertilizer application, δ^{15}N values have to be interpreted very carefully. Furthermore, because the difference in the δ^{15}N composition of the milk samples from the respective origins was not statistically significant, nitrogen stable isotope technique may not be appropriate to distinguish between milk samples from North America (USA and Canada) on the basis of using the technique as a proxy for geographical origin determination. The same can be said for the following countries or regions; the USA and NZ, USA and SC, SC and NZ, SC and CA, as shown in Table 2 where the difference in the δ^{15}N composition of the milk samples from the respective origins was statistically insignificant.

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, oxygen and nitrogen isotopic compositions (δ^2H, $\delta^{18}O$, $\delta^{15}N$) 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 δ^2H and $\delta^{18}O$. The nitrogen stable isotope could not provide a clear distinction for the milk products because $\delta^{15}N$ of an animal reflects that of the diet. Hence in cases of similar diet, it may not be possible to provide a distinction between the animals using this technique.

Disclosure of conflicts of interest

All authors do not have any conflict of interest.

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