JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Tracing the Geographic Origin of Beef in China on the Basis of the Combination of Stable Isotopes and Multielement Analysis

Yan Zhao,*[,†,‡] Bin Zhang,[§] Gang Chen,[†,‡] Ailiang Chen,[†,‡] Shuming Yang,[†,‡] and Zhihua Ye[†,‡]

[†]Institute of Quality Standard and Testing Technology for Agro-Products, Key Laboratory of Agro-product Quality and Safety, Chinese Academy of Agricultural Sciences, Beijing 100081, China

[‡]Key Laboratory of Agro-product Quality and Safety, Ministry of Agriculture, Beijing 100081, China

[§]College of Food and Bioengineering, Henan University of Science and Technology, Luoyang 471023, China

ABSTRACT: The potential for classifying beef samples on the basis of their geographical origin was investigated by stable isotope and multielement analysis using samples from various provinces in China. C and N isotope composition and the concentrations of 23 elements of the defatted beef samples were determined. It was shown that as compared to the Tibet beef fed predominantly on C_3 pasture, maize-fed beef produced in Shandong and Heilongjiang province gave rise to a significant difference in ^{13}C content. Significant differences were also observed in 18 elements among the defatted beef samples. Stable isotope data and multielement concentrations determined in the beef were subjected to multivariate analysis, including principal component analysis (PCA) and discriminant analysis (DA). Eight key variables were identified as providing maximum discrimination among samples. DA gave an overall correct classification rate of 100% and a cross-validation rate of 100%. This research has proved that the geographical origin of beef in China can be identified by a combination of stable isotopes and multielement analysis.

KEYWORDS: *stable isotope, multielement, geographic origin, beef, China*

INTRODUCTION

Verification of the geographic origin of food is of great concern not only to consumers but also to farmers, retailers, and governmental authorities. In China, development of a traceability system for agro-products still lags behind the demands, although the law on quality and safety of agricultural products requiring traceability for agro-products was enacted in October 2006. For meat products, the origin concern has become an important factor strongly influencing the consumer's purchasing decision due to food incidents such as bovine spongiform encephalopathy (BSE), foot-and-mouth disease (FMD), and avian influenza. The traceability system for beef is promoted by the Chinese government to ensure product safety. However, the existing system is currently based on ear tags and life-numbers and ultimately depends on paper records, which can be fraudulently fabricated or mislabeled. Therefore, the establishment of a reliable and scientific system to identify the beef geographic origin is urgently needed in China.

The use of multistable isotope and/or multielement analysis is an increasingly accepted tool for tracing the geographical origin of agro-products, such as cereal,[1−3] wine,[4−10] cheese,[11−17] and honey.[18−20] There have been a number of publications recently relating to the identification of beef geographical origin and production regimen using multiple stable isotope analysis of the bioelements (H, C, N, O, and S) with various degrees of success as well.[21−27] As reported by Guo et al., the percentage of correctly classified samples of cattle from four regions in China, based on the combination of C and N stable isotope ratios, was 78%,[28] indicating that a strategy to improve the correct classification rate is required. The aim of the present study was to demonstrate the potential of the combination of stable isotope (^{13}C and ^{15}N) and multielement analysis for the determination of the geographical origin of beef in China.

MATERIALS AND METHODS

Sources of Samples. A total of 69 cattle raised at four different geographical locations were studied. The cattle were raised at a local farm for at least 12 months before they were slaughtered and consumed. All cattle were at an approximate age of 2 years old. To avoid the effect of seasonal variations, cattle samples were collected almost on the same date. Sixteen cattle samples were collected from Heze city, Shandong province (35° N, 115° E) in July 2011. Heze is located in central eastern China, with a mean annual temperature of 13.5 °C and an average altitude of 50 m. The cattle from Heze are mainly fed maize. Fourteen cattle samples were collected from Harbin city, Heilongjiang province (45° N, 125° E) in August 2011. Harbin is located in northeastern China, with a mean annual temperature of 3.6 °C and an average altitude of 128 m. The cattle from Harbin are mainly fed maize. Fifteen cattle samples were collected from Kunming city, Yunnan province (25° N, 102° E) in October 2011. Kunming is located in southwestern China, with a mean annual temperature of 15.0 °C and an average altitude of 1890 m. The cattle from Kunming are mainly fed C_3 and C_4 pasture. Twenty-four cattle samples were collected from Anduo county, Tibet Autonomous Region (32° N, 90° E) in October 2011. The mean annual temperature there is −2.8 °C, and the average altitude is 4800 m. The cattle from Anduo are mainly fed C_3 pasture. Detailed information for these origins is given in Table 1.

Preparation of Samples. Beef samples (∼50 g) were freeze-dried for 24 h and homogenized using ceramic scissors and/or an agate

Received: March 3, 2013
Revised: June 26, 2013
Accepted: June 27, 2013
Published: June 27, 2013

Table 1. Region Information of Beef Samples

region	longitude	latitude	altitude (m)	mean annual temp (°C)	staple feed species	sampling time	no. of samples
Heze city, Shandong province (SD)	115	35	50	13.5	maize	July 2011	16
Harbin city, Heilongjiang province (HLJ)	125	45	128	3.6	maize	Aug 2011	14
Kunming city, Yunnan province (YN)	102	25	1890	15.0	C_3 and C_4 pastures	Oct 2011	15
Anduo county, Tibet Autonomous Region (TB)	90	32	4800	−2.8	C_3 pastures	Oct 2011	24

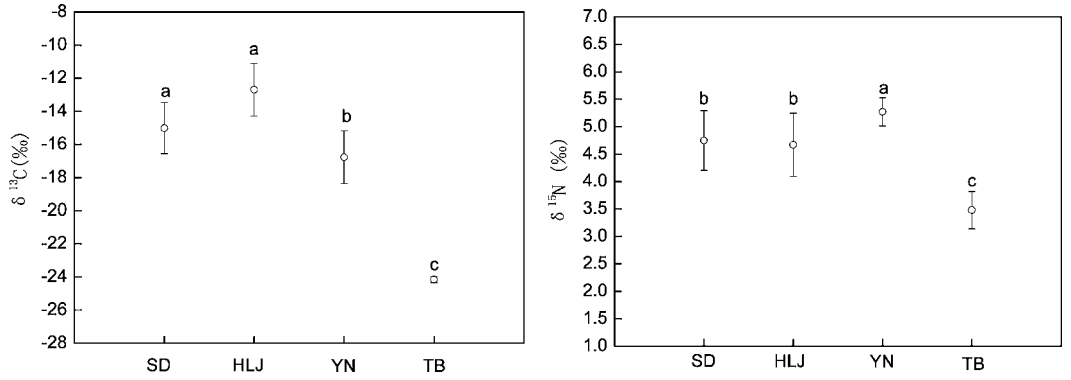

Figure 1. $\delta^{13}C$ and $\delta^{15}N$ values in defatted beef samples. Values are means ± SD. Means with the same letter are not significantly different according to Duncan's multiple-range test; $P < 0.05$.

pestle and mortar. The dry powder obtained was extracted with ether for 6 h in a Soxhlet apparatus. The defatted dry mass (DDM) of beef was rehomogenized using an agate pestle and mortar and stored at −20 °C until analysis.[28,29]

Sample Analysis. *Natural-Abundance (C, N) Stable Isotope Analysis of DDM.* The DDM was introduced into a Sn capsule (C and N isotope analysis).[28] Samples were introduced into the elemental analyzer (Flash EA1112) by an autosampler. The elements of carbon and nitrogen in the samples were converted into CO_2 and NO_x gas by combustion at 1020 °C, and then the NO_x gas generated was reduced over copper wires at 650 °C to N_2. The carrier gas helium ran at a flow rate of 90 mL/min and interfaced through a Conflo III to an isotope ratio mass spectrometer (Delta plus, Thermo Finnigan). The C and N stable isotope composition of each sample was measured in the same run. Nitrogen and carbon isotope data were reported in δ-notation in units of per thousands (‰) relative to the accepted international standards: $\delta^{13}C$‰ relative to Vienna Pee Dee Belimnite (VPDB) and $\delta^{15}N$‰ relative to atmospheric AIR. The delta values were calculated as follows:

$$\delta_X = (R_{samp}/R_{ref} - 1) \times 1000 \quad (1)$$

δ_X is the isotope composition of the sample expressed in delta units (‰, per thousands) relative to the reference material. R_{samp} and R_{ref} are the absolute isotope ratio of the sample and reference material, respectively. The reference working gas CO_2 was calibrated with USGS 24 ($\delta^{13}C_{PDB} = -16.0$‰), and the reference working gas N_2 was calibrated with IAEA N1 ($\delta^{15}N_{air} = 0.4$‰). The analytical precision was 0.2‰ for both C and N.

Multielement Analysis. The defatted dry mass of beef was analyzed after microwave digestion using MARS (CEM Co.) microwave digestion system. Briefly, 0.2 g of defatted beef sample, 10 mL of 65% HNO_3, and 1 mL of hydrogen peroxide solution (31%) were added into a PTFE digestion tube and digested for 40 min by increasing the power to 1600 W and the temperature to 210 °C in a stepwise fashion. The digested solution was diluted to 50 mL with ultrapure water and stored in a plastic flask before analysis.[30]

Twenty-three elements (Be, Na, Mg, K, Ca, Ti, V, Mn, Fe, Co, Ni, Cu, Zn, Ga, Se, Rb, Sr, Zr, Mo, Sn, Sb, Ba, and Bi) were measured by inductively coupled plasma mass spectrometry (ICP-MS, X Series 2, Thermo Fisher, America). The standard matter of chicken (GBW10018) was supplied by the Institute of Geophysical and Geochemical Exploration of China and was used for calculating the recovery and accuracy. After the above-mentioned digestion process and ICP-MS analysis, the recovery and the relative standard deviation (RSD) of each element in the standard matter of chicken (GBW10018) were >90% and <10% (measured in triplicate), respectively, indicating the whole analysis method was validated for elemental analysis. Analysis of each sample was performed in triplicate and quantified using external standards analysis. All of the results were expressed as the average of the triplicate measurements. The internal standards, including Ge, Y, Rh, and Pt, were used to ensure the stability of the instrument. The samples were remeasured whenever the RSD of internal standards was >5%.

Statistical Analysis. Statistical analysis of the data was performed using the SPSS 16.0 package for Windows. ANOVA was carried out for each element. Duncan's multiple comparison was performed to determine the significant difference between the individual regions when the F value was significant in ANOVA. To reduce the dimensionality of the data set and to describe all of the variability of the system using a smaller number of variables, principal component analysis (PCA) was used. The first principal component (PC1) describes the maximum possible variation, and the second PC accounts for the second most and so on. Discriminant analysis (DA) was also performed to evaluate whether cattle from different origins could be distinguished by the analytical parameters. The most significant variables were selected by stepwise analysis of all 69 beef data sets, and then a back substitution test was used to evaluate the prediction capability of the mode for each parameter. Prediction capability is expressed as a percentage of correctly classified samples relative to the entire data set.

RESULTS

Differences in Stable Isotope Concentrations of Defatted Beef among the Regions. The stable carbon and nitrogen isotopic compositions of the DDM of beef are shown in Figure 1. The beef samples from Heilongjiang (HLJ) and Shandong (SD) provinces were characterized by higher $\delta^{13}C$ values (−15.9 to −11.0‰ and −17.0 to −11.2‰, respectively), as compared to those from Tibet (TB) (−23.9 to −24.5‰) and Yunnan (YN) province (−14.18 to −19.9‰).

In comparison to the carbon isotope ratios of these four regions, the nitrogen isotope ratios for all beef samples were rather homogeneous and in a narrow range from 2.8 to 5.7‰.

Table 2. Mean Elemental Concentrations and Standard Deviations for 18 Elements in Defatted Beef Samples[a]

element	SD[b]	HLJ[c]	YN[d]	TB[e]
Na (mg/kg)	2321 ± 407b	3152 ± 31526a	2116 ± 306b	2246 ± 167b
Mg (mg/kg)	881 ± 43b	842 ± 24c	973 ± 34a	992 ± 37a
Ca (mg/kg)	289 ± 31b	366 ± 22a	322 ± 41a	266 ± 27b
K (mg/kg)	14730 ± 1004c	15990 ± 132b	15890 ± 114b	18248 ± 876a
Ti (mg/kg)	127 ± 4b	135 ± 9b	146 ± 11a	140 ± 19a
V (μg/kg)	21.5 ± 4.4a	20.3 ± 3.5a	13.5 ± 2.4c	19.9 ± 5.6b
Mn (μg/kg)	397 ± 92a	185 ± 58d	215 ± 41c	303 ± 48b
Fe (mg/kg)	59.9 ± 6.7c	62.5 ± 3.9b	59.1 ± 3.1c	99.9 ± 7.5a
Co (μg/kg)	5.50 ± 0.5b2	3.99 ± 0.55c	3.49 ± 0.17c	6.14 ± 0.37a
Ni (μg/kg)	503 ± 89c	384 ± 50d	744 ± 120a	610 ± 94b
Cu (μg/kg)	1348.10 ± 766.29b	1272.66 ± 72.34c	1218.59 ± 238.44c	1747.40 ± 433.13a
Zn (mg/kg)	95.56 ± 9.3c	123.8 ± 25.4b	83.8 ± 10.7c	159.9 ± 9.6a
Rb (mg/kg)	31.9 ± 5.2a	22.0 ± 2.7b	29.2 ± 2.7a	12.5 ± 1.9c
Se (μg/kg)	239 ± 24b	257 ± 26a	219 ± 31c	155 ± 15d
Sr (mg/kg)	3.10 ± 0.50a	2.16 ± 0.25c	2.84 ± 0.73b	1.22 ± 0.23d
Zr (μg/kg)	13.4 ± 8.0c	77.6 ± 5.3a	39.3 ± 5.1b	46.4 ± 5.7b
Mo (μg/kg)	51.1 ± 11.2c	79.2 ± 6.0b	78.2 ± 5.6b	96.1 ± 11.9a
Ba (μg/kg)	170 ± 40b	112 ± 25d	144 ± 28c	289 ± 18a

[a]Values represent means ± SD. Values followed by the same letter are not significantly different at $P < 0.05$ according to Duncan's multiple-range test. [b]SD, Shandong province. [c]HLJ, Heilongjiang province. [d]YN, Yunnan province. [e]TB, Tibet Autonomous Region.

As shown in Figure 1, significant differences existed between samples from cultivated lands and pasture locations. The nitrogen isotope values ranged from 3.6 to 5.7‰ for SD beef, from 3.0 to 4.9‰ for HLJ beef, from 4.9 to 5.6‰ for YN beef, and from 2.8 to 4.1‰ for TB.

Differences in Multielement Concentrations of Defatted Beef among the Regions. It has been shown by the ANOVA test that 18 of 23 elements (Na, Mg, K, Ca, Ti, V, Mn, Fe, Co, Ni, Cu, Zn, Se, Rb, Sr, Zr, Mo, and Ba) in the defatted beef were significantly different among the four regions (Table 2). As indicated by Duncan's multiple comparison, each region had a characteristic element content fingerprint. For SD samples, the contents of Mn and Sr were the highest among the four regions. HLJ samples were generally characterized by the highest contents of Na, Se, and Zr. YN samples had the highest content of Ni. TB samples could be separated from others on the basis of their higher contents of K, Fe, Cu, Co, Zn, Ba, and Mo.

Principal Component Analysis and Discriminant Analysis. Two stable isotopes and 18 elements in the defatted beef samples from these four regions were analyzed using PCA (Figure 2). The first four factors explained 77% of the total variability. The values of δ^{13}C, Fe, Cu, Zn, Mo, and δ^{15}N had the highest weight on the first PC (explaining 31.5% of the variability), and TB samples could be distinguished. Na, Ca, and Se dominated the second PC (explaining 25.1% of variability), and HLJ samples could thus be separated from the others. Ni and Ti contents showed the highest weight on the third PC (explaining 14.9% of variability), and YN samples can be recognized. It was more difficult to explain for the grouping of elements in the remaining PCs, where the dominant elements were Cu and Co in PC4, Rb and Sr in PC5, and Zr in PC6.

For a better understanding of the discriminating efficiency of each element, linear discriminant analysis was carried out on the basis of 2 stable isotopes and 18 elements composition of defatted beef samples. Eight elements (δ^{13}C‰, δ^{15}N‰, Mg, K, Mn, Zn, Se, and Zr) were selected to establish a classification model using a stepwise discriminant procedure, which was then evaluated by a cross-validation procedure. A satisfactory classification was obtained with an overall correct classification

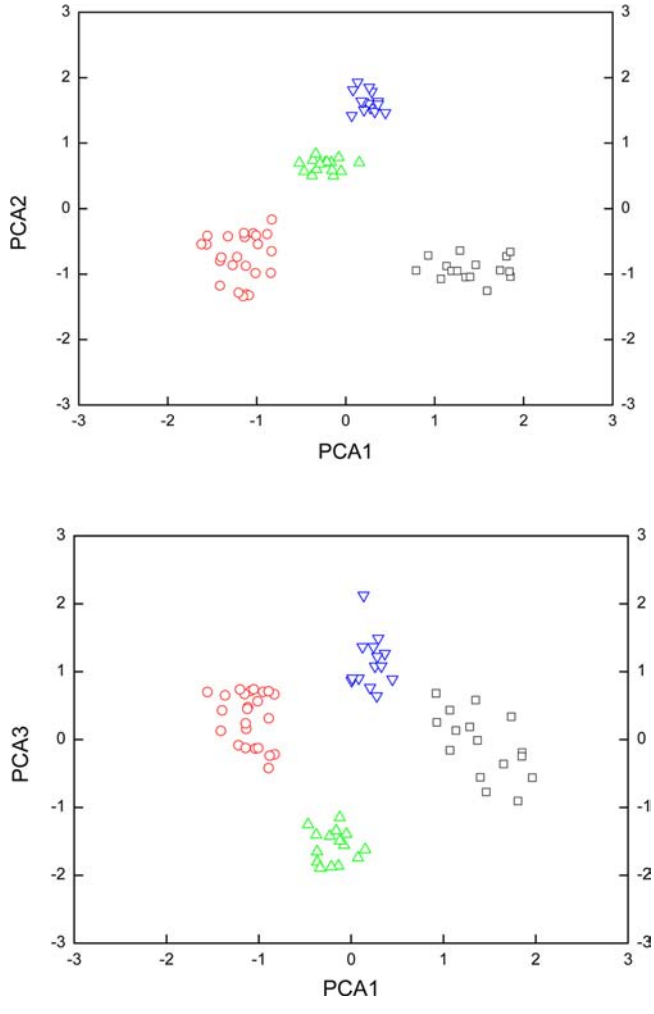

Figure 2. Principal component analysis of defatted beef from four regions: (□) SD; (○) TB; (△) YN; (▽) HLJ.

dx.doi.org/10.1021/jf400947y | J. Agric. Food Chem. 2013, 61, 7055−7060

rate of 100% and a cross-validation rate of 100% (Table 3). This classification model could clearly discriminate samples among four different regions.

Table 3. Classification of Beef Samples in Different Regions and Percentage of Observations Correctly Classified

			predicted group membership[a]				
			SD[b]	HLJ[c]	YN[d]	TB[e]	total
original	count	SD	16				16
		HLJ		14			14
		YN			15		15
		TB				24	24
	%		100	100	100	100	100[f]
cross-validated	count	SD	16				16
		HLJ		14			14
		YN			15		15
		TB				24	24
	%		100	100	100	100	100[g]

[a]The number of correctly classified observations are tabulated diagonally. [b]SD, Shandong province. [c]HLJ, Heilongjiang province. [d]YN, Yunnan province. [e]TB, Tibet Autonomous Region. [f]100% of empirical grouped observations correctly classified. [g]100% of cross-validated grouped observations correctly classified.

DISCUSSION

The $\delta^{13}C$ value in beef has been found to be highly dependent on the diet composition, particularly with regard to the proportion of C_3 and C_4 plant material.[28−32] The feeds of cattle from these four regions mentioned are significantly different from each other. For example, Shandong and Heilongjiang provinces are located in eastern China, where maize is one of the primary crops. The cattle from Shandong and Heilongjiang provinces are mainly fed C_4 plant maize, whereas rice straw, soybean, and wheat account for only a small proportion of feed. Yunnan is located in southwestern China, where cattle are fed both C_3 and C_4 grasses, whereas for Tibet, the climate there is very special and its altitude is as high as 4800 m. Cattle from Tibet are mainly fed C_3 grass. Such different diet compositions may result in higher $\delta^{13}C$ values in the beef samples from Shandong and Heilongjiang, as compared to other two provinces, in which the $\delta^{13}C$ value in the beef from Tibet is the lowest. These data are in good agreement with the findings by Guo et al.,[28] who observed that the $\delta^{13}C$ values in beef DDM samples from Jilin are in the range from −15.6 to −10.9‰, because Heilongjiang is adjacent to Jilin, and both provinces possess similar crops and climates. In a previous study, Schmidt et al. investigated the discrimination of organic beef from conventional beef by lower carbon isotopic composition, because fodder for the former is abundant in C_3 plants. It has also been believed that the $\delta^{13}C$‰ value of beef above −20‰ was not in accordance with organic farming for central Europe.[21] In our work, lower $\delta^{13}C$‰ in the beef from TB was detected, as compared to that of samples from other locations, because the cattle living in TB were predominantly fed C_3 grass rather than C_4 plant maize.

Both diet and region are the main factors influencing the value of $\delta^{15}N$ in cattle tissue.[28,29] Cattle from Shandong and Heilongjiang mainly fed on cereal feed, whereas those from Yunnan mainly fed on pasture. The cereal crop used as the feed was grown with the help of synthetic nitrogen-based fertilizers, which may lead to lower $\delta^{15}N$ values in the cereals that will then be transformed to the cattle through ingestion.[33,34] This is why the $\delta^{15}N$ value in the beef from Yunnan was significantly higher than that in the cattle fed on cereal. The $\delta^{15}N$ value of beef from Tibet was the lowest among the four regions. It is indicated that leguminous grass directly utilizing atmospheric nitrogen (such as clover) is the main pasture in Anduo county, Tibet Autonomous Region,[29] and the high elevation could be another key factor influencing the $\delta^{15}N$ value. As reported by Bontempo et al, $\delta^{15}N$ was confirmed to be <4‰ for alpine products of milk and cheese, which is comparable with the present study.[35]

It has been shown in the present study that the beef in each region had a typical profile of element composition. Multiple elements were also analyzed as one promising group to determine the origin of beef because their retention from the local environment can provide a site-specific geographic profile. The multielement profiles of soil are different with regard to several environmental and geographical factors.[36] Heze city is located in the central eastern plain of a section of the Yellow River. Certain element contents (Mn and Sr) in the soil there were higher than in the other three locations.[37] At another site, Harbin city, certain element contents (Na and Zr) in the soil were higher than at the other three locations.[37] In addition, because this area is the main crop production region, certain essential elements such as Ca and Se may easily accumulate in the soil. Certain element contents (Ti and Ni) in the soil of Yunnan province were higher than their average levels in China, resulting in the accumulation of such elements in the beef from Yunnan. Tibet is special in both location and climate. Specifically, for Anduo county, abundance mineral resources such as Fe, Zn, Cu, and Mo have been discovered.[38] Migration of certain elements through the food chain has already been found and quantified.[22,29] According to these differences, multielement analysis was used for the classification of beef samples based on their original regions in China.

In a previous study, Franke et al. has demonstrated the suitability of element signature for authentication of poultry meat and dried beef samples from different origins, including Switzerland, Austria, Australia, the United States, and Canada. The results showed that the element compositions were significantly different among samples from various locations, and thus they could be discriminated on such basis.[39] The element contents were also found to be varied in beef samples from four regions of China, in which Se, Sr, Fe, Ni, and Zn were regarded as good tracers for beef origin identification, which could result in 98.4% correct classification for all samples.[40] In our study, it was shown that the beef samples from different regions in China possessed their typical multielement profile mainly based on the elemental compositions of the local soil. The beef from different regions was also analyzed using IRMS and ICP-MS by Heaton et al. After multivariant analysis, the beef from identified geographic origins was correctly classified.[29] In the present study, the combination of stable isotope and multielement data can be successfully adopted for the classification of the geographic origin of beef after PCA and DA.

AUTHOR INFORMATION

Corresponding Author

*(Y.Z.) Phone: +86-010-8210-6558. Fax: +86-010-8210-6560. E-mail: yzhao2010@yahoo.cn.

Funding

This work was funded by the Special Fund for Agro-scientific Research in the Public Interest (No. 201203046), the Fundamental Research Funds for Chinese Central Public-interest Institution (No.2012ZL015), and the Chinese Program of International S&T Cooperation (No. S2012ZR0202).

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We greatly thank Dr. Sun B. Z. (Institute of Animal Sciences (IAS), Chinese Academy of Agricultural Sciences (CAAS), for his assistance in collecting the beef samples.

■ REFERENCES

(1) Asfaha, D. G.; Quetel, C. R.; Thomas, F.; Horacek, M.; Wimmer, B.; Heiss, G.; Dekant, C.; Deters-Itzelsberger, P.; Hoelzl, S.; Rummel, S.; Brach-Papa, C.; Van Bocxstaele, M.; Jamin, E.; Baxter, M.; Heinrich, K.; Kelly, S.; Bertoldi, D.; Bontempo, L.; Camin, F.; Larcher, R.; Perini, M.; Rossmann, A.; Schellenberg, A.; Schlicht, C.; Froeschl, H.; Hoogewerff, J.; Ueckermann, H. Combining isotopic signatures of $^{87}Sr/^{86}Sr$ and light stable elements (C, N, O, S) with multi-elemental profiling for the authentication of provenance of European cereal samples. *J. Cereal Sci.* **2011**, *53*, 170−177.

(2) Suzuki, Y.; Chikaraishi, Y.; Ogawa, N. O.; Ohkouchi, N.; Korenaga, T. Geographical origin of polished rice based on multiple element and stable isotope analyses. *Food Chem.* **2008**, *109*, 470−475.

(3) Kelly, S. D.; Baxter, M.; Chapman, S.; Rhodes, C.; Dennis, J.; Brereton, P. The application of isotopic and elemental analysis to determine the geographical origin of premium long grain rice. *Eur. Food Res. Technol.* **2002**, *214*, 72−78.

(4) Day, M.; Zhang, B. L.; Asselin, G. J.; Morlat, R. Characterization of the region and year of production of wines by stable isotopes and elemental analyses. *J. Int. Sci. Vigne Vin* **1995**, *29*, 75−87.

(5) Rossmann, A.; Schmidt, H. L.; Reniero, F.; Versini, G.; Moussa, I.; Merle, M. H.; Lebensm, Z. Stable carbon isotope content in ethanol of EC data bank wines from Italy, France and Germany. *Z. Lebensm. Unters. Forsch.* **1996**, *203*, 293−301.

(6) Tardaguila, J.; Bertamini, M.; Reniero, F.; Versini, G.; Pardo, J. Composition in oxygen-18 of must water in the wine harvest: I. Influence of the water regime. *Alimentaria* **1996**, *34*, 95−99.

(7) Tardaguila, J.; Pardo, J. E.; Gomez, R.; Blanch, A. I. Authentication of wine with oxygen-18 and deuterium analysis. *Alimentaria* **1997**, *35*, 93−96.

(8) Martin, G. J.; Mazure, M.; Jouitteau, C.; Martin, Y. L.; Aguile, L.; Allain, P. Characterization of the geographic origin of Bordeaux wines by a combined use of isotopic and trace element measurements. *Am. J. Enol. Vitic.* **1999**, *50*, 409−417.

(9) Dutra, S. V.; Adami, L.; Marcon, A. R.; Carnieli, G. J.; Roani, C. A.; Spinelli, F. R.; Leonardelli, S.; Ducatti, C.; Moreira, M. Z.; Vanderlinde, R. Determination of the geographical origin of Brazilian wines by isotope and mineral analysis. *Anal. Bioanal. Chem.* **2011**, *401*, 1571−1576.

(10) Fauhl-Hassek, C. Trends in wine authentication. *Bull. O.I.V.* **2009**, *82*, 93−100.

(11) Manca, G.; Camin, F.; Coloru, G. C.; Del Caro, A.; Depentori, D.; Franco, M. A.; Versini, G. Characterization of the geographical origin of pecorino sardo cheese by casein stable isotope (C^{13}/C^{12} and N^{15}/N^{14}) ratios and free amino acid ratios. *J. Agric. Food Chem.* **2001**, *49*, 1404−1409.

(12) Chiacchierini, E.; Bogoni, P.; Franco, M.; Giaccio, M.; Versini, G. Characterisation of the regional origin of sheep and cow cheeses by casein stable isotope ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) ratios. *J. Commodity Sci.* **2002**, *41*, 303−315.

(13) Giaccio, M.; Signore, A.d.; Giacomo, F.d.; Bogoni, P.; Versini, G.; del Signore, A.; di Giacomo, F. Characterization of cow and sheep cheeses in a regional scale by stable isotope ratios of casein ($^{13}C/^{12}C$, $^{15}N/^{14}N$) and glycerol ($^{18}O/^{16}O$). *J. Commodity Sci.* **2003**, *42*, 193−204.

(14) Pillonel, L.; Badertscher, R.; Froidevaux, P.; Haberhauer, G.; Holzl, S.; Horn, P.; Jakob, A.; Pfammatter, E.; Piantini, U.; Rossmann, A.; Tabacchi, R.; Bosset, J. O. Stable isotope ratios, major, trace and radioactive elements in emmental cheeses of different origins. *Lebensm.-Wiss. Technol.* **2003**, *36*, 615−623.

(15) Pillonel, L.; Tabacchi, R.; Bosset, J. Analytical methods for the determination of the geographic origin of Emmental cheese. Summary of a screening study. *Mitt. Geb. Lebensmittelunters. Hyg.* **2003**, *94*, 60−69.

(16) 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.

(17) Camin, F.; Wehrens, R.; Bertoldi, D.; Bontempo, L.; Ziller, L.; Perini, M.; Nicolini, G.; Nocetti, M.; Larcher, R. H, C, N and S stable isotopes and mineral profiles to objectively guarantee the authenticity of grated hard cheeses. *Anal. Chim. Acta* **2012**, *711*, 54−59.

(18) Kropf, U.; Golob, T.; Necemer, M.; Kump, P.; Korosec, M.; Bertoncelj, J.; Ogrinc, N. Carbon and nitrogen natural stable isotopes in Slovene honey: adulteration and botanical and geographical aspects. *J. Agric. Food Chem.* **2010**, *58*, 12794−12803.

(19) Kropf, U.; Korosec, M.; Bertoncelj, J.; Ogrinc, N.; Necemer, M.; Kump, P.; Golob, T. Determination of the geographical origin of Slovenian black locust, lime and chestnut honey. *Food Chem.* **2010**, *121*, 839−846.

(20) Schellenberg, A.; Chmielus, S.; Schlicht, C.; Camin, F.; Perini, M.; Bontempo, L.; Heinrich, K.; Kelly, S. D.; Rossmann, A.; Thomas, F.; Jamin, E.; Horacek, M. Multielement stable isotope ratios (H, C, N, S) of honey from different European regions. *Food Chem.* **2010**, *121*, 770−777.

(21) Molkentin, J.; Giesemann, A. Differentiation of organically and conventionally produced milk by stable isotope and fatty acid analysis. *Anal. Bioanal. Chem.* **2007**, *388*, 297−305.

(22) Franke, B. M.; Koslitz, S.; Micaux, F.; Piantini, U.; Maury, V.; Pfammatter, E.; Wunderli, S.; Gremaud, G.; Bosset, J. O.; Hadorn, R.; Kreuzer, M. Tracing the geographic origin of poultry meat and dried beef with oxygen and strontium isotope ratios. *Eur. Food Res. Technol.* **2008**, *226*, 761−769.

(23) Bahar, B.; Schmidt, O.; Moloney, A. P.; Scrimgeour, C. M.; Begley, I. S.; Monahan, F. J. Seasonal variation in the C, N and S stable isotope composition of retail organic and conventional Irish beef. *Food Chem.* **2008**, *106*, 1299−1305.

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

(25) Osorio, M. T.; Moloney, A. P.; Schmidt, O.; Monahan, F. J. Beef authentication and retrospective dietary verification using stable isotope ratio analysis of bovine muscle and tail hair. *J. Agric. Food Chem.* **2011**, *59*, 3295−3305.

(26) Nakashita, R.; Suzuki, Y.; Korenaga, T.; Watanabe, N.; Tanaka, K. Stable isotope analysis for tracing the geographical origin of beef. *Bunseki Kagaku* **2009**, *58*, 1023−1028.

(27) Bong, Y.-S.; Shin, W.-J.; Lee, A. R.; Kim, Y.-S.; Kim, K.; Lee, K.-S. Tracing the geographical origin of beefs being circulated in Korean markets based on stable isotopes. *Rapid Commun. Mass Spectrom.* **2010**, *24*, 155−159.

(28) Guo, B. L.; Wei, Y. M.; Pan, J. R.; Li, Y. Stable C and N isotope ratio analysis for regional geographical traceability of cattle in China. *Food Chem.* **2010**, *118*, 915−920.

(29) Heaton, K.; Kelly, S. D.; Hoogewerff, J.; Woolfe, M. Verifying the geographical origin of beef: the application of multi-element isotope and trace element analysis. *Food Chem.* **2008**, *107*, 506−515.

(30) Sun, S. M.; Guo, B. L.; Wei, Y. M.; Fan, M. T. Analysis of stable carbon and nitrogen isotope compositions and geographical origins of sheep tissues. *Sci. Agric. Sin.* **2010**, *43*, 1670−1676.

(31) Nakashita, R.; Suzuki, Y.; Akamatsu, F.; Iizumi, Y.; Korenaga, T.; Chikaraishi, Y. Stable carbon, nitrogen, and oxygen isotope analysis as a potential tool for verifying geographical origin of beef. *Anal. Chim. Acta* **2008**, *617*, 148−152.
(32) Bong, Y. S.; Shin, W. J.; Lee, A. R.; Kim, Y. S.; Kim, K.; Lee, K. S. Tracing the geographical origin of beefs being circulated in Korean markets based on stable isotopes. *Rapid Commun. Mass Spectrom.* **2010**, *24*, 155−159.
(33) Kelly, S.; Heaton, K.; Hoogewerff, J. Tracing the geographical origin of food: the application of multi-element and multi-isotope analysis. *Trends Food Sci. Technol.* **2005**, *16*, 555−567.
(34) Bahar, B.; Moloney, A. P.; Monahan, F. J.; Harrison, S. M.; Zazzo, A.; Scrimgeour, C. M.; Begley, I. S.; Schmidt, O. Turnover of carbon, nitrogen, and sulfur in bovine longissimus dorsi and psoas major muscles: Implications for isotopic authentication of meat. *J. Anim. Sci.* **2009**, *87*, 905−913.
(35) Bontempo, L.; Lombardi, G.; Paoletti, R.; Ziller, L.; Camin, F. H, C, N and O stable isotope characteristics of alpine forage, milk and cheese. *Int. Dairy J.* **2012**, *23*, 99−104.
(36) Perez, A. L.; Smith, B. W.; Anderson, K. A. Stable isotope and trace element profiling combined with classification models to differentiate geographic growing origin for three fruits: effects of subregion and variety. *J. Agric. Food Chem.* **2006**, *54*, 4506−4516.
(37) Wei, F. S.; Zhang, C.; Chen, J. S.; Wu, Y. Y. Study on the background contents on 61 elements of soil in China. *J. Environ. Sci.−China* **1991**, *12*, 12−19.
(38) Sun, L. X.; Bai, Z. D.; Xun, D. B.; Li, H. K.; Sun, B. Geological characteristics and zircon U-Pb SHRIMP dating of the plagiogranite in Amduo ophiolites, Tibet (in Chinese). *Geol. Surv. Res.* **2011**, *34*, 10−15.
(39) Franke, B. M.; Haldimann, M.; Reimann, J.; Baumer, B.; Gremaud, G.; Hadorn, R. Indications for the applicability of element signature analysis for the determination of the geographic origin of dried beef and poultry meat. *Eur. Food Res. Technol.* **2007**, *225*, 501−509.
(40) Guo, B. L.; Wei, Y. M.; Pan, J. R.; Li, Y. Determination of beef geographical origin basedon multid-element analysis (in Chinese). *Sci. Agric. Sin.* **2007**, *40*, 2842−2847.