Geographical origin traceability of foxtail millet based on the combination of multi-element and chemical composition analysis

Kehong Liang\textsuperscript{a}, Shan Liang\textsuperscript{b,c}, Lingang Lu\textsuperscript{a}, Dazhou Zhu\textsuperscript{a}, and Lei Cheng\textsuperscript{b,c}

\textsuperscript{a}Ministry of Agriculture, Institute of Food and Nutrition Development, Beijing, China; \textsuperscript{b}Beijing Advanced Innovation Center for Food Nutrition and Human Health, Beijing Technology and Business University, Beijing, China; \textsuperscript{c}Beijing Engineering and Technology Research Center of Food Additives, Beijing Technology and Business University, Beijing, China

ABSTRACT
The potential approach of classifying foxtail millet according to geographical origin was investigated using mineral element and chemical composition analysis of samples from various provinces in China. Total 16 mineral elements and five chemical compositions of foxtail millets were analyzed. There were significant differences in 12 elements of millets from different regions. Notable differences were also observed for chemical composition, with Hebei samples showing higher protein content, Henan samples showing higher fat and ash contents and Shandong samples showed higher dietary fiber and amylose contents. Based on the combination of both methods, discriminant analysis provided optimal discrimination among the various geographical origins with a 95.2\% classification rate. Our study provides an effective tool to trace the foxtail millet geographic origin through a combination of multi-element and chemical composition analysis.

ARTICLE HISTORY
Received 24 April 2018
Accepted 26 July 2018

KEYWORDS
Foxtail millet; mineral element; chemical composition; geographic origin

Introduction
Foxtail millet (\textit{Setaria italica}) is one of the most important food and fodder crops in parts of China, India and many African countries.\textsuperscript{[1–3]} It is also grown in Australia and South America.\textsuperscript{[4]} Foxtail millet has gained prominence because of its nutritional significance. It is rich in protein, starch and bran oil,\textsuperscript{[5]} and it is a staple food in China. Moreover, millets also contain a variety of vitamins, minerals and phytochemicals (phenolics and carotenoids),\textsuperscript{[6,7]} so it is considered as potential functional food ingredient to promote better health.

Geographical conditions can influence the chemical composition of millets, including some functional ingredients.\textsuperscript{[8,9]} China has constructed some high quality millet production bases such as \textit{Yuxian} base in Hebei province and \textit{Jinxiang} base in Shandong province. High quality millet usually has a higher price than average millet. However, millet products can be mislabeled and adulterated, which have negative effects on both the consumer and legitimate merchants. In this respect, it is important to establish methods that facilitate recognition of the geographical origin of millet to protect both consumers and producers.

Geographical origin is the most important factor that can affect the chemical composition of cereal grains. Kitta et al.\textsuperscript{[10]} evaluated the relationship between chemical composition and cultivation areas through fatty acid composition. In addition, there have been many reports on using mineral element analysis to identify the origin of food and plants.\textsuperscript{[11–15]} The mineral and trace elemental composition of crops reflects the cultivar and soil conditions where crops grows.\textsuperscript{[16,17]} Stable isotope
analysis has been used to trace the origin of cereal grains, such as rice, wheat, soybean and so on. [18,19] However, it has to be noted that the isotopic fractionation can occur depending on the planting date and variable climatic conditions. [20] No two regions may have identical soil maps, so mineral elements are an effective maker for geographical classification. Recently, chemometric approaches based on the study of mineral elements were used to distinguish wheat of different geographical origins with good results. [21] The element analysis technique has never been proposed for geographical traceability of millets. The objective of this study was to demonstrate the potential of combining mineral elements with chemical composition analysis to determine the geographical origins of foxtail millets in China.

**Materials and methods**

**Sampling**

A total of 21 foxtail millet samples were collected from the 2015 harvests of three major producing regions in China (Hebei, Henan and Shandong provinces). 7 millet samples were collected from Handan city Hebei province (38°N, 114°E) in 2015; 7 millet samples were collected from Zhengzhou city Henan city (34°N, 113°E) in 2015; 7 millet samples were collected from Jinan city Shandong provinces (36°N, 117°E) in 2015. Information on the millet varieties that were employed, sampling locations, and weather conditions in the growing season in the sampling regions are shown in Table 1. These foxtail millet samples were dehulled to remove the inedible husks, then milled into fine powder, sieved through a 60 mesh screen and thoroughly mixed. The prepared samples were stored at −20°C.

**Multi-element analysis**

The analysis of multi-element data from millet was according to the method by Zhao et al. [21] The millet samples were analyzed after microwave digestion using MARS (CEM Company) microwave digestion system. 0.2 g of sample, 10 mL of 65% HNO₃, 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 ultra pure water and stored in a plastic flask before analysis.

Multi-elements were measured using Inductively Coupled Plasma Mass Spectrometry (ICP-MS, X Series 2, Thermo Fisher, America). Standard solutions for Ca, K, Mg, P, Fe, Mn, Cu, Zn, Se, Mo, Co, Ni, Rb, Sr, Cs, and Ba were prepared using multi-element calibration standard solutions, and all determination coefficients of the standard curves were higher than 0.99. Because there is no matching certified reference material (CRM) for millets, the analytical quality control was routinely validated using a rice reference material, GSB-1 (GWB 10010). The recovery and the relative

**Table 1.** Information on the foxtail millet varieties employed in this study, and weather conditions in the growing seasons in different regions.

| Region                  | Varieties                                                                 | Latitude (°) | Longitude (°) | Accumulative temperature (°C) | Sunshine duration (h) | Precipitation (mm) |
|-------------------------|---------------------------------------------------------------------------|--------------|---------------|-------------------------------|----------------------|-------------------|
| Hebei province (HB)     | Jigu 19, Heng 2015–3, 14–628, RS5358, 14–697, Heng 2015–2, G239           | 38N          | 114E          | 3 032                         | 572.3                | 169.4             |
| Henan province (HN)     | Jigu 19, Heng 2015–3, 14–628, RS5358, 14–697, Heng 2015–2, G239           | 34N          | 113E          | 3 091                         | 641.9                | 117.7             |
| Shandong province (SN)  | Jigu 19, Heng 2015–3, 14–628, RS5358, 14–697, Heng 2015–2, G239           | 36N          | 117E          | 3 144                         | 802.7                | 245.3             |
standard deviation (RSD) of these 16 elements in millet samples were found to be higher than 90% and lower than 10%, respectively.

**Proximate composition**

Crude protein, crude fat and ash contents were determined using standard methods. Dietary fiber was estimated using the methods of Englyst and Hudson. Amylose content was estimated using the methods of Sowbhagya and Bhattacharya. Potato amylase was used as a standard.

**Statistical analysis**

All statistical analyses of the data (16 mineral elements, protein, fat, dietary, amylose, ash contents) were performed in the SPSS 19.0 package for Windows. One-way ANOVA was completed for each of the parameters. Duncan’s multiple comparison test was performed to determine significant differences between the different regions, with a significance level of \( P < 0.05 \). Principal component analysis (PCA) was used to describe sample clusters from different origins. Discriminant analysis (DA) was also performed to assess the ability to discriminate the geographic provenance of millets. The most significant variables were selected by stepwise analysis. Variable selection was based on Wilks’ lambda. A cross-validation procedure was applied to assess the model.

**Results and discussion**

**Mineral elements in millet from different regions**

The 16 elemental concentrations (Ca, K, Mg, P, Fe, Mn, Cu, Zn, Se, Mo, Co, Ni, Rb, Sr, Cs, and Ba) of 21 millet samples from three different regions are reported in Table 2. The most relatively abundant mineral elements in millet were P and K, which were both greater than 1 g/kg in content. Ca, Mg, Fe, Mn and Zn were each between 10 mg/kg and 1 g/kg. Cu, Rb, Sr, Mo, Ni and Ba were each between 0.1 mg/kg and 10 mg/kg. Co, Cs and Se were each between 10 μg/kg and 100 μg/kg. The contents of K, Mg, Ca, Fe, Zn, Mn, Cu and Mo were similar to that reported by Zhang et al. Se was lower than the values reported in Liu et al. Our samples were generally rich in P compared to the millet reported by Verma et al. To the best of our knowledge, no literature is available concerning Co, Mo, Ni, Rb, Sr, Cs, and Ba concentrations in millet.

| Mineral element | Hebei | Henan | Shandong | Mean | SEM   | P-value |
|-----------------|-------|-------|----------|------|-------|---------|
| Ca (mg/kg)      | 100.22| 108.87| 112.23   | 107.11| 6.20  | 0.307   |
| K (g/kg)        | 1.91b | 2.13a | 2.18     | 2.07 | 0.14  | 0.010   |
| Mg (mg/kg)      | 866.01b | 885.45b | 887.17b | 879.54| 11.75 | 0.009   |
| P (g/kg)        | 2.73b | 3.10a | 3.12     | 2.98 | 0.22  | 0.003   |
| Fe (mg/kg)      | 20.39a | 27.61a | 20.55    | 22.85| 4.12  | 0.111   |
| Mn (mg/kg)      | 12.42a | 13.28a | 12.23    | 12.64| 0.56  | 0.195   |
| Cu (mg/kg)      | 5.85ab | 6.14ab | 5.41b    | 5.80 | 0.37  | 0.309   |
| Zn (mg/kg)      | 26.08b | 30.33a | 29.89    | 28.77| 2.34  | 0.017   |
| Se (μg/kg)      | 36.55b | 36.84a | 38.99a   | 37.46| 1.33  | 0.033   |
| Mo (μg/kg)      | 291.29b | 363.90a | 302.94ab | 329.89| 36.52| 0.018   |
| Co (μg/kg)      | 23.20a | 9.10b | 7.83b    | 13.38| 8.53  | 0.000   |
| Ni (μg/kg)      | 714.32a | 484.80b | 480.91b  | 560.01| 133.65| 0.000   |
| Rb (mg/kg)      | 4.14b | 12.02a | 10.60a   | 8.92 | 4.20  | 0.000   |
| Sr (mg/kg)      | 0.82b | 1.64a | 1.58a    | 1.35 | 0.46  | 0.000   |
| Cs (μg/kg)      | 14.18b | 55.80a | 47.79a   | 39.26| 22.08 | 0.000   |
| Ba (μg/kg)      | 424.66a | 321.21b | 303.54a  | 349.80| 65.43 | 0.002   |

Note: Means in the same row with different superscripts indicate a significant difference (\( P < 0.05 \)).
ANOVA test showed that 12 of 16 elements (K, Mg, P, Zn, Se, Mo, Co, Ni, Rb, Sr, Cs, and Ba) in the millet were significantly different among the three regions. The results indicated that Hebei samples had the highest contents of Co, Ni and Ba. Henan and Shandong samples had a higher contents of K, P, Zn, Se, Rb, Sr, and Cs. Furthermore, Henan samples could be distinguished from Shandong samples based on high levels of Cu. The content of Cu in Henan soil samples was higher than that in Shandong soil samples. All of the above element profiles provide valuable information about the geographical origin of millet samples.

Chemical composition in millet samples from different regions

The chemical composition of millet samples is shown in Table 3. The protein and fat contents in the millet samples were 10.28 g/100 g and 3.40 g/100 g, respectively, which was similar to the values reported by Verma et al. [27] Dietary fiber content of foxtail millet was 2.37 g/100 g, which was lower than the value of 3.6 g/100 g found by Devisetti et al. [29] This difference may be attributed to the differences in foxtail millet varieties. The amylose content was 21.47 g/100 g. Fujita et al. [30] reported the amylose content of millet was 11.4 to 27.1%. Thus, the results of the present study were within the reported range. The ash content of millet was observed to be 1.39 g/100 g, which was close to the results provided by Devisetti et al. [29] Protein, fat, dietary fiber, amylose and ash content were significantly different \((P < 0.05)\) among the samples from different regions. The protein content in the Hebei samples was 10.87 g/100 g, higher than Henan and Shandong samples. The amylose and ash contents were higher in millet from Henan and Shandong, respectively, than in Hebei. The fat content was the highest in the Henan samples and the lowest in the Shandong samples, whereas the dietary fiber content was the highest in samples obtained from Shandong and the lowest in samples from Hebei. Several studies reported higher fat content and dietary fiber for millet than other cereals such as maize, rice, and sorghum. [31–33]

Principle component analysis and discriminant analysis

The millet samples from three regions can be partially differentiated by PCA of the mineral elements (Figure 1a), in which the Hebei samples can be distinctly separated from the samples from the other two regions. However, it is difficult to distinguish the samples from the Henan and Shandong regions by mineral signature. Present results showed that the element contents in foxtail millet samples from the three regions had distinct characteristics. The different characteristics could be linked to the soil system. Among these three locations, K content is lower in Hebei soil, [34] and the levels of Zn and Mn are higher in Henan soil. [35] Higher P levels were found in Shandong soil. [36] The corresponding contents of these elements were detected in foxtail millet samples from the corresponding locations.

Five chemical compositions and 12 elements in the foxtail millet samples from three regions were analyzed using PCA (Figure 1b). Approximately 84.45% of the total variability can be explained by the first five components. Dietary fiber, Sr, Rb and Cs showed the largest PC1, which explained 41.67% of the variability. K, Mg and P had the highest weight in PC2. The score plot of PC1 and PC2 showed an initial distinction between samples from the three regions. Compared to the individual

| Components                  | Hebei       | Henan       | Shandong    | Mean   | SEM  | P-value |
|-----------------------------|-------------|-------------|-------------|--------|------|---------|
| Protein content (g/100g)    | 10.87<sup>a</sup> | 9.88<sup>b</sup> | 10.08<sup>b</sup> | 10.28  | 0.52 | 0.006   |
| Fat content (g/100g)       | 2.70<sup>b</sup>  | 3.99<sup>a</sup>  | 3.52<sup>c</sup>  | 3.40   | 0.65 | 0.000   |
| Dietary fiber content (g/100g) | 1.68<sup>c</sup> | 2.59<sup>a</sup> | 2.83<sup>b</sup> | 2.37   | 0.61 | 0.000   |
| Amylose content (g/100g)   | 20.35<sup>b</sup> | 21.61<sup>a</sup> | 22.46<sup>a</sup> | 21.47  | 1.06 | 0.003   |
| Ash content (g/100g)       | 1.31<sup>b</sup>  | 1.45<sup>a</sup>  | 1.41<sup>a</sup>  | 1.39   | 0.07 | 0.004   |

Note: Means in the same row with different superscripts indicate a significant difference \((P < 0.05)\).
mineral elements data, the combination of both methods significantly improved the classification of foxtail millet samples of various geographical origins.

To develop a mathematical model to classify millet samples from the three regions, discriminant analysis was carried out using SPSS software based on five chemical compositions and 12 elements (Figure 2). Six factors (including protein, fat, dietary fiber, amylose, Se and Rb) were selected to establish a classification procedure, which was then evaluated in a cross-validation procedure. A satisfactory classification was obtained with a recognition ability of 95.2% of the original grouped samples and a cross-validation rate of 95.2% (Table 4).

There has been little investigation into the traceability of millet. Agronomic traits such as panicle exsertion and longest finger length have been reported to be linked to geographical provenance. However, agronomic traits are usually not available when millet is used as food. Protein, fat and amylose contents were significantly different among samples from diverse geographic regions of China. Climate was the main factor influencing the chemical composition of millet. Different climate characteristics such as temperature, sunshine duration and precipitation have led to the accumulation of different traits (Table 1). Liu et al. found that the protein and fat contents in millet as well as accumulative

Figure 1. Principal component analysis of millets from different regions. (a) PCA of mineral elements; (b) PCA of the combination of mineral elements and chemical compositions.
temperature, sunshine duration and precipitation were strongly correlated. Analyses of proteins could be used to determine of geographical origin, due to the fact that the presence of these analysis is usually associated with environment. Jiang et al.\cite{40} reported that mineral element contents of rice accessions correlations between most of the 17 amino acid contents and Mg, Ca, and Zn, between protein content and Na, Mg, Zn, Cu, or Mn content. Zhou et al.\cite{41} revealed positive correlations between protein content and Cu or Zn content. The findings in this study demonstrate that the combination of mineral elements and chemical composition can improve the differentiation of foxtail millets with various origins and be an effective tool for geographic regional determination of foxtail millet.

**Conclusion**

This study reports on the content of major nutritional components (protein, fat, dietary fiber, amylase, ash) and 16 mineral elements of millet samples from three provinces in China. The results show that millet classification can be improved by combining both nutritional data and mineral element data Our study provides an efficient method to determine the geographical origin of millets, which has great importance for the millet industry.

**Table 4.** Classification of foxtail millet samples in different regions and the percentage of observations that were correctly classified.

| Predicted group membership\(^a\) | HB | HN | SD | Total |
|----------------------------------|----|----|----|-------|
| Original                         |    |    |    |       |
| Count                            | 7  | 7  | 1  | 7     |
| %                                | 100| 100| 85.7| 95.2\(^b\) |
| Cross-validated                  |    |    |    |       |
| Count                            | 7  | 7  | 1  | 7     |
| %                                | 100| 100| 85.7| 95.2\(^c\) |

Note: \(^a\) The numbers of correctly classified observations are tabulated diagonally; \(^b\) 95.2% of empirically grouped observations were correctly classified; \(^c\) 95.2% of cross-validated grouped observations were correctly classified.

Figure 2. Score plot of discriminant functions 1 and 2 to discriminate millet from different regions.
Acknowledgments

This research was supported by National Agricultural Products Quality and Safety Risk Assessment of Major Projects (GJFP201801501); National Agricultural Products Quality and Safety Risk Assessment of Major Projects (GJFP201801504); The fund of the Beijing Engineering and Technology Research Center of Food Additives, Beijing Technology & Business University (BTBU); Projects of Beijing Technology and Business University Youth Fund (No. QNJ2017-06); Projects of Beijing Municipal Science and Technology Project (Grant No. D17110500190000); The Agricultural Science and Technology Innovation Program (Y2018PT34).

Funding

This work was supported by the National Agricultural Products Quality and Safety Risk Assessment of Major Projects [GJFP201801501,GJFP201801504]; Projects of Beijing Technology and Business University Youth Fund [No. QNJ2017-06]; Projects of Beijing Municipal Science and Technology Project [Grant No. D17110500190000]; The fund of the Beijing Engineering and Technology Research Center of Food Additives, Beijing Technology & Business University (BTBU); The Agricultural Science and Technology Innovation Program [Y2018PT34].

References

[1] Jonese, M. K.; Liu, X. Origins of Agricultural in East Asia. Science 2009, 324, 730–731. DOI: 10.1126/science.1172082.
[2] Devi, P. B.; Vijayabharathi, R.; Sathyabama, S.; Malleshi, N. G.; Priyadarisini, V. B. Health Benefits of Finger Millet (Eleusine Coracana L.) Polyphenols and Dietary Fibre: A Review. Journal of Food Science and Technology 2014, 51, 1021–1040. DOI: 10.1007/s13197-011-0584-9.
[3] Zhang, G. Y.; Liu, X.; Quan, Z. W.; Cheng, S. F.; Xu, X.; Pan, S. K.; Xie, M.; Zeng, P.; Yue, Z.; Wang, W. L. Genome Sequence of Foxtail Millet (Setaria Italica) Provides Insights into Grass Evolution and Biofuel Potential. Nature Biotechnology 2012, 30, 549–556. DOI: 10.1038/nbt.2185.
[4] Sreenivasulu, N.; Miranda, M.; Prakash, H. S.; Wobus, U.; Weschke, W. Transcriptome Changes in Foxtail Millet Genotypes at High Salinity: Identification and Characterization of a PHGPX Gene Specifically Upregulated by NaCl in a Salt-Tolerant Line. Journal of Plant Physiology 2004, 161, 467–477. DOI: 10.1016/j.jplph.2004.0176-1617-0112.
[5] Pawar, V. S.; Pawar, V. D. Melting Characteristics and Biochemical Changes of Foxtail Millet. Journal of Food Science and Technology-Mysore 1997, 34, 416–418.
[6] Liu, R. H. Whole Grain Phytochemicals and Health. Journal of Cereal Science 2004, 46, 207–219. DOI: 10.1016/j.jcsc.2007.06.010.
[7] Kumar, K. V. P.; Dharmaraj, U.; Sakhare, S. D.; Inamdar, A. A. Flour Functionality and Nutritional Characteristics of Different Roller Milled Streams of Foxtail Millet (Setaria Italica). Lwt-Food Science and Technology 2016, 73, 274–279. DOI: 10.1016/j.lwt.2016.06.028.
[8] Kalinova, J.; Moudry, J. Content and Quality of Protein in Proso Millet (Panicum Miliaceum L.) Varieties. Plant Food for Human Nutrition 2006, 61, 45–49. DOI: 10.1007/s11130-006-0013-9.
[9] Wen, Y.; Liu, J.; Meng, X.; Zhang, D.; Zhao, G. Characterization of Proso Millet Starches from Different Geographical Origins of China. Food Science and Biotechnology 2014, 23, 1371–1377. DOI: 10.1007/s10068-014-0188-z.
[10] Kitta, K.; Ebihara, M.; Iizuka, T.; Yoshikawa, R.; Ishihiki, K.; Kawamoto, S. Variations in Lipid Content and Fatty Acid Composition of Major Non-Glutinous Rice Cultivars in Japan. Journal of Food Composition and Analysis 2005, 18, 269–278. DOI: 10.1016/j.jfca.2004.10.001.
[11] Dutra, S. V.; Adam, L.; Marcon, A. R.; Carnieli, G. J.; Roani, C. A.; Spinelli, F. R.; Vanderlinde, R. Determination of the Geographical Origin of Brazilian Wines by Isotope and Mineral Analysis. Analytical and Bioanalytical Chemistry 2011, 401, 1571–1576. DOI: 10.1007/s00216-011-5181-2.
[12] Gonzalez-Martín, M. I.; Moncada, G. W.; Gonzalez-Perez, C.; San Martin, N. Z.; Lopez-Gonzalez, F.; Ortega, I. L.; Hernandez-Hierro, J. M. Chilean Flour and Wheat Grain: Tracing Their Origin Using near Infrared Spectroscopy and Chemometrics. Food Chemistry 2014, 145, 802–806. DOI: 10.1016/j.foodchem.2013.08.103.
[13] Maione, C.; Batista, B. L.; Campiglia, A. D.; Barbosa, F.; Barbosa, R. M. Classification of Geographic Origin of Rice by Data Mining and Inductively Coupled Plasma Mass Spectrometry. Computers and Electronics in Agriculture 2016, 121, 101–107. DOI: 10.1016/j.compag.2015.11.009.
[14] Karabagias, I. K.; Halatsi, E. Z.; Karabournioti, S.; Kontakos, S.; Kontominas, M. G. Impact of Physicochemical Parameters, Pollen Grains, and Phenolic Compounds on the Correct Geographical Differentiation of Fir Honeyes Produced in Greece as Assessed by Multivariate Analyses. International Journal of Food Properties 2017, 20, S520–S533. DOI: 10.1080/10942912.2017.1300811.
[38] Liu, W. H.; Lu, B.; Zhou, N. J. Studies on the Effects of Various Ecological Factors on the Contents of Protein and Fat in Millet. Journal of Shanxi Agricultural University 1995, 3, 244–247. In Chinese.

[39] Wang, J.; Kliks, M. M.; Qu, W. Y.; Jun, S. J.; Shi, G. Y.; Li, Q. X. Rapid Determination of the Geographical Origin of Honey Based on Protein Fingerprinting and Barcoding Using MALDI TOF MS. Journal of Agricultural and Food Chemistry 2009, 57, 10081–10088. DOI: 10.1021/jf902286p.

[40] Jiang, S. L.; Wu, J. G.; Feng, Y.; Yang, X. E.; Shi, C. H. Correlation Analysis of Mineral Element Contents and Quality Traits in Milled Rice (Oryza Stavia L.). Journal of Agricultural & Food Chemistry 2007, 55, 9608–9813. DOI: 10.1021/jf071785w.

[41] Zhou, C. S.; Liu, W. H.; Fan, B. W.; Luo, L. Study on Correlation between Trace Elements and Quality and Output of Rice in Sichuan. Guangdong Trace Elements Sciences 2003, 10, 56–59. in Chinese.