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Determining the Geographic Origin of Wheat Using Multielement Analysis and Multivariate Statistics

Haiyan Zhao, Boli Guo, Yimin Wei,* Bo Zhang, Shumin Sun, Lei Zhang, and Junhui Yan

Institute of Agro-Food Science & Technology, Chinese Academy of Agricultural Sciences/Key Laboratory of Agricultural Product Processing and Quality Control, Ministry of Agriculture, P.O. Box 5109, Beijing 100193, People's Republic of China

ABSTRACT: The element contents of wheat from four major wheat-producing regions of China were analyzed and used in multivariate statistical analysis to classify wheat according to geographical origin. The concentrations of 15 elements (Be, Na, Mg, Al, K, Ca, V, Mn, Fe, Cu, Zn, Mo, Cd, Ba, and Th) in 240 samples from the 2007/2008 and 2008/2009 harvests were determined by inductively coupled plasma mass spectrometry. The analysis of variance and linear discriminant analysis were applied to classify wheat origin, and the effects of region, variety, and harvest year on the element contents were analyzed in this study. It was concluded that the multielement analysis is a promising method to provide reliable origin information for wheat, although the element profiles and discriminant models were affected by wheat varieties, harvest years, and agricultural practices.

KEYWORDS: Wheat, geographical origin, multielement analysis, ICP-MS, multivariate statistics

INTRODUCTION

Wheat is one of the most important crops in the world, and its distribution, cultivation area, and total trade all occupy the first rank of crops. Wheat production and consumption account for about 25% of the national crop output in China.[1] The chemical composition of wheat is related to varieties, cultivation soil, and climate based on geographical origin, so that quality and commercial value are somewhat different according to their growing conditions.[2−5] Currently, China has constructed some high-quality wheat production bases such as *Gaocheng* base in Hebei province and *Yanjin* base in Henan province. The producers of high-quality wheat usually charge a premium more than average. However, the genuine wheat is usually replaced with inferior or counterfeit products for financial gain, which has a negative effect on both the consumer and the legitimate producer.

Furthermore, with the development of the globalization of food market, many countries have published relevant regulations or laws to ensure the traceability of foods. Regulation (EC) No. 178/2002 requires that food that is placed on the market or is likely to be placed on the market in the community shall be adequately labeled or identified to facilitate its traceability from January 1, 2005.[6] The Food Safety Enhancement Act of 2009 requires that food that is located in the United States or that is intended for import into the United States maintains the full pedigree of the origin.[7] Food Safety of the People's Republic of China requires that the imported prepackaged food needs to state clearly their origins.[8] Wheat (GB 1351-2008, People's Republic of China) stipulates that the origin of wheat should be indicated on the wrappage or in the relevant documentation.[9] The ability to determine the origin of agricultural products is a powerful tool in the enforcement of import laws and requirements.

As a consequence of a number of consumer-driven forces and legislations, it is reasonable to suggest that there should be analytical methods in place that can verify the information provided on origin labels. Verification of the origin of wheat would largely stamp out illegal activities and is important for enforcement options for the food industry and protection of the consumers from deception.

The mineral content of crops is influenced by the soil composition and local environmental factors (rainfall, temperature, sunshine, etc.). Certain regions have specific “fingerprints” of elements, and no two regions are likely to have identical soil maps.[10,11] Consequently, element composition can characterize geographical origin. Furthermore, minerals are significantly more stable in the commodity than organic compounds, so they may be useful markers in terms of geographical classification. Inductively coupled plasma mass spectrometry (ICP-MS) is widely used to determine the elements to classify the origin of food, due to its economical multielement analysis, less consumption time, and less expertise to operate.[12−17] Moreover, to our knowledge, this is the first approach for the only use of multielement analysis for wheat origin identification and the influences of variety and harvest year on wheat authentication.

Here, a method is presented for determining the geographic origin of wheat from four different sites based on two growing years. The objective of this study was to classify accurately the geographical growing regions of wheat based on the mineral fingerprintings combined with multivariate statistics.

MATERIALS AND METHODS

Sampling. A total of 240 wheat samples were collected from the 2007/2008 and 2008/2009 harvests from four major wheat-producing regions in China (Hebei, Henan, Shandong, and Shaanxi provinces). To obtain a representative sampling, the main wheat-producing counties and towns and the most common varieties were chosen in each province. Also, the samples were collected from the same locations each year. The number of samples per province per year was 30. The information on wheat varieties employed, sampling locations, and weather conditions in

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the growing seasons in the sampling regions is shown in Table 1. Also, the locations of these production areas are marked on the map in Figure 1. At each farm, approximately 5 kg of samples was harvested and then labeled. For each sample, 100 g was chosen from 5 kg samples as an analytical sample.

Sample Preparation. The samples were rinsed with deionized water obtained from Milli-Q Millipore system (Bedford, MA) repeatedly after picking out stones, weeds, etc. and then dried in an oven (DHG-9140A, Yiheng, Shanghai, China) at 38 °C for 10 h, so that the moisture was consistent as much as possible. The dried samples were finally ground to the whole wheat flour with a Cyclotec 1093 lab mill (Foss Tecator, Denmark).

The samples were digested using a MARS (CEM Co., Matthews, NC) microwave digestion system. About 0.1 g of the whole wheat flour, 8 mL of 65% MOS grade HNO_3 (Beijing Institute of Chemical Reagents, Beijing, China), and 3 mL of 37% MOS grade HCl (Beijing Institute of Chemical Reagent) were added into a Teflon digestion vessel and digested for 40 min by increasing the power to 1600 W and the temperature to 240 °C in a stepwise fashion. The digested solution was quantitatively transferred into a 50 mL Teflon volumetric flask and made up to volume with 18.2 MΩ cm water obtained from Milli-Q Millipore system.

Element Measurement. The contents of 16 isotopes (^{9}Be, ^{23}Na, ^{24}Mg, ^{27}Al, ^{39}K, ^{44}Ca, ^{51}V, ^{55}Mn, ^{57}Fe, ^{65}Cu, ^{66}Zn, ^{97}Mo, ^{111}Cd, ^{113}Cd, ^{137}Ba, and ^{232}Th) were measured using an ICP-MS (7500a, Agilent Technologies, Santa Clara, CA) in the standard mode. Optimization was done for maximum sensitivity while maintaining the oxide ratio as low as possible. The optimized operating conditions for analysis of the diluted samples were as follows: The radio frequency power was 1200 W, while the carrier gas and peristaltic pump flow rates were 1.12 L/min and 0.5 mL/min, respectively. The nebulization chamber temperature was 2 °C, and the oxide and double charge indices were 0.45 and 1.01%, respectively. The limits of detection (LODs) of all of the elements evaluated from three times the standard deviation of 10 replicate blank measurements are shown in Table 2.

Each sample was analyzed in triplicate and quantified using external standards analysis to monitor possible shift of initial calibration. All of the results were expressed as the average of triplicate measurements. The Environmental Calibration Standard (Part #5183-4688) supplied by Agilent Co. was used for standard solution, and all of the determination coefficients of standard curves were higher than 0.99. The internal standards ^{7}Li, ^{72}Ge, ^{89}Y, ^{115}In, ^{159}Tb, and ^{209}Bi (1 ng L^{-1}, Part #5183-4680, Agilent) were used to correct for matrix effects and compensate for possible variations in instrument performance during the determination. Each internal standard was used to monitor the target isotopes with the similar atomic weights. The sample was remeasured whenever the relative standard deviation value of internal standard concentration was higher than 3%.

The certified reference material (CRM) of wheat flour (GBW10011) from China Procurement Center of Certified Reference Materials (Beijing, China) was analyzed in triplicate by the proposed methodology for validating the elemental concentrations used as data. The results obtained are shown in Table 3. The recovery of each element was higher than 90%.

Chemometric Analysis. Analysis of variance (ANOVA) and linear discriminant analysis (LDA) were applied, respectively, to the two-year data sets with SPSS for Windows version 16.0 (SPSS Inc., Chicago, IL) in an effort to classify wheat samples based on the multielement profiles of each sample. Each wheat sample was considered as an assembly of 15 variables (i.e., the contents of the analyzed elements), which constituted their chemical descriptors.

ANOVA. ANOVA was performed with each single variable on 2007/2008 and 2008/2009 samples, separately, and used to examine whether there were significant differences between different sets of data from

Table 1. Information on Wheat Varieties Employed, Locations, and Weather Conditions in the Growing Seasons in the Sampling Regions

region	varieties (no. of samples)		latitude	longitude	average temperature (°C)		total precipitation (mm)	
	2007/2008	2008/2009			2007/2008	2008/2009	2007/2008	2008/2009
Hebei Province	Hengguan 35 (3), Shixin 828 (3), Han 7086 (3), Shimai 15 (3), Shimai 14 (3), Shimai 12 (2), Shijiazhuang 8 (2), Shixin 733 (1), Jimai 22 (1), Le 639 (1), Kenong 199 (2), Liangxing 99 (1), 78-1 (1), Shimai 10 (1), Jishi 02-1 (1), Shi 4185 (1), Gao 9415 (1)	Hengguan 35 (8), Shixin 828 (2), Han 7086 (5), Shimai 15 (3), Shimai 14 (5), Shimai 12 (2), Shijiazhuang 8 (1), Shixin 733 (1), Jimai 22 (1), Heng 5229 (1), Han 6172 (1)	36°N[a]−42°N	113°E[b]−119°E	9.8	10.5	26.8	21.3
Henan Province	Aikang 58 (5), Zhoumai 18 (5), Zhoumai 16 (4), Xinmai 19 (3), Xinmai 18 (2), Xinong 979 (1), Yumai 44 (1), Wenmai 49-198 (1), Pingan 1 (1), Yunong 015 (1), Zhengmai 9023 (1), Zhaoke 88 (1), Han 3475 (1), Han 4589 (1), Yumai 18 (1), Lankao 18 (1)	Aikang 58 (6), Zhoumai 18 (1), Zhoumai 16 (6), Xinmai 19 (1), Xinmai 18 (2), Xinong 979 (4), Yumai 44 (1), Zhumai 4 (1), Hengguan 35 (1), Zhengmai 366 (1), Zhoumai 22 (1), Jimai 4 (1), Pingan 6 (1), Lankaoaizao 8 (1), Fengwu 981 (2)	31°N−36°N	110°E−116°E	10.9	11.8	20.6	18.2
Shandong Province	Jining 16 (6), Jimai 22 (5), Liangxing 99 (1), Taishan 9818 (3), Taimai 1 (2), Jimai 21 (1), Weimai 8 (6), Zibo 12 (4), Wennong 6 (2)	Jining 16 (6), Jimai 22 (11), Liangxing 99 (5), Taishan 9818 (4), Taimai 1 (1), Taishan 23 (2), Linmai 4 (1)	34°N−38°N	114°E−122°E	10.1	10.7	23.9	22.5
Shaanxi Province	Xiaoyan 22 (16), Xinong 889 (4), Xinong 979 (2), Xiaoyan 22-3 (3), Xinong 88 (3), Jinmai 47 (1), Wunong 148 (1)	Xiaoyan 22 (24), Xinong 889 (2), Xinong 979 (1), Xiaoyan 22-3 (2), 757 (1)	31°N−39°N	105°E−111°E	10.2	10.9	25.3	30.4

[a] N, Northern. [b] E, East.

different regions at the 95% confidence level. Then, Duncan's multiple comparison was applied to compare means when there were statistically significant differences among the element concentrations.

LDA. LDA, used as classification procedures, was carried out on the 2007/2008 samples (LDA1), the 2008/2009 samples (LDA2), and the two-year samples (LDA1 + 2), respectively. The stepwise method was used to select those elements having sufficient discriminatory power to distinguish one province mineral data from another. On the base of selected variables, this procedure rendered a number of orthogonal linear discriminant functions that provided the best discrimination between the groups equal to the number of categories minus 1.[18,19] The statistical significance of each discriminant function was evaluated on the basis of the Wilks' Lambda factor after the function was removed. This parameter ranges from 1.0 (no discriminatory power) to 0.0 (perfect discriminatory power). The separation among groups in the discriminant space was checked by plotting the first three function scores. Finally, to verify the power and the stability of the model, "leave-one-out" cross-validation discriminant analysis was performed.

Figure 1. Regions of samples.

Table 2. LODs of Elements (ng g^{-1})

element	LOD	element	LOD	element	LOD
Be	0.039	Ca	0.069	Zn	0.017
Na	0.005	V	0.023	Mo	0.016
Mg	0.016	Mn	0.013	Cd	0.013
Al	0.043	Fe	0.017	Ba	0.011
K	0.012	Cu	0.012	Th	0.008

RESULTS

ANOVA. As a result of ANOVA, the concentration means of Be, K, Cd, and Th were not significantly different among the 2007/2008 samples; therefore, they were rejected for further statistical analysis. While the concentration means of all of the elements for the 2008/2009 samples were found to be significantly different. The mean values and the standard deviations of the concentrations of samples are listed in Tables 4 and 5, respectively.

Most of the element average concentrations in the samples from 2007/2008 were higher than those from 2008/2009, but the characteristics on the contents of some elements from a particular region were similar for two-year samples. The samples from Hebei had the lowest Ca and K contents, while they had the highest V and Mo contents. Henan samples had the lowest Na, Mg, and Mn contents, while they had the highest Cd contents. The concentration of Zn in Shandong samples was lowest, but Ba was the highest. The samples from Shaanxi showed the highest levels of Al, K, Ca, Mn, and Fe. All of the above element profiles gave valuable information about the geographical origin of wheat samples.

LDA. The canonical discriminant functions of the 2007/2008 samples were derived based on the following elements: Ba, Mn, Ca, Mg, and V. These three functions explained the 100% of the variance (function 1 explained 64.8% of the total variance, function 2 explained 31.0%, and function 3 explained 4.2%). Wilks' Lambda values were 0.069, 0.307, and 0.818 for functions 1, 2, and 3, respectively ($p = 0.000$). To investigate the impact of

Table 4. Element Concentrations (μg g^{-1}) in the Wheat Samples from the 2007/2008 Harvest from Different Provinces[a]

element	Hebei	Henan	Shandong	Shaanxi
Be	0.002 ± 0.003 a	0.001 ± 0.002 a	0.003 ± 0.007 a	0.003 ± 0.003 a
Na	32 ± 12 a	29.3 ± 8.6 a	34 ± 11 a	48 ± 17 b
Mg	1629 ± 120 bc	1496 ± 118 a	1557 ± 145 ab	1692 ± 225 c
Al	9.2 ± 4.3 a	11.2 ± 8.4 a	10.1 ± 7.9 a	17.2 ± 8.8 b
K	4233 ± 327 a	4238 ± 404 a	4260 ± 359 a	4311 ± 601 a
Ca	482 ± 68 a	541 ± 89 b	646 ± 88 c	676 ± 107 c
V	2.7 ± 1.2 c	1.95 ± 0.88 b	2.1 ± 1.2 b	1.1 ± 1.4 a
Mn	41.2 ± 5.3 a	37.4 ± 6.6 a	45.9 ± 8.9 b	61 ± 11 c
Fe	52 ± 11 a	48.7 ± 9.6 a	54 ± 22 a	79 ± 29 b
Cu	5.51 ± 0.86 b	4.77 ± 0.61 a	4.85 ± 0.87 a	6.32 ± 0.99 c
Zn	22.1 ± 5.7 b	19.6 ± 3.7 a	17.6 ± 2.4 a	23.0 ± 6.2 b
Mo	0.87 ± 0.30 b	0.53 ± 0.20 a	0.45 ± 0.25 a	0.75 ± 0.50 b
Cd	0.038 ± 0.016 a	0.055 ± 0.067 a	0.040 ± 0.041 a	0.035 ± 0.026 a
Ba	3.2 ± 1.1 a	3.4 ± 1.4 a	7.7 ± 1.9 b	3.06 ± 0.86 a
Th	0.021 ± 0.079 a	0.007 ± 0.012 a	0.018 ± 0.058 a	0.03 ± 0.10 a

[a] Data in this table are shown by means ± standard deviations, and the different letters mean significant differences ($p < 0.05$).

Table 3. Results Obtained through the Analysis of the CRM[a]

element	CRM (μg g^{-1})	experimental value (μg g^{-1})	recovery (%)	element	CRM (μg g^{-1})	experimental value (μg g^{-1})	recovery (%)
Be	0.00085	0.00084 ± 0.0	98 ± 1	Fe	18.5 ± 3.1	17.9 ± 0.5	97 ± 2
Na	17 ± 5	16.3 ± 1.3	96 ± 8	Cu	2.7 ± 0.2	2.5 ± 0.1	93 ± 4
Mg	450 ± 70	439 ± 8	98 ± 2	Zn	11.6 ± 0.7	11.2 ± 0.4	97 ± 3
Al	104 ± 10	100 ± 4	96 ± 3	Mo	0.48 ± 0.05	0.45 ± 0.02	94 ± 3
K	1400 ± 60	1369 ± 38	98 ± 3	Cd	0.018 ± 0.004	0.017 ± 0.002	93 ± 8
Ca	340 ± 20	334 ± 5	98 ± 1	Ba	2.4 ± 0.3	2.3 ± 0.2	94 ± 6
V	0.034 ± 0.012	0.032 ± 0.001	94 ± 3	Th	0.002	0.002 ± 0.0	92 ± 6
Mn	5.4 ± 0.3	5.2 ± 0.2	96 ± 3				

[a] The experimental values in this table are shown by means ± standard deviations.

Table 5. Element Concentrations ($\mu g\ g^{-1}$) in the Wheat Samples from the 2008/2009 Harvest from Different Provinces[a]

element	Hebei	Henan	Shandong	Shaanxi
Be	0.125 ± 0.034 b	0.138 ± 0.039 b	0.086 ± 0.023 a	0.135 ± 0.057 b
Na	19.7 ± 8.5 b	14.6 ± 2.5 a	15.2 ± 2.3 a	16.2 ± 3.1 a
Mg	1103 ± 105 b	1006 ± 109 a	1053 ± 59 ab	1067 ± 93 b
Al	1.7 ± 1.5 a	4.3 ± 4.5 b	0.9 ± 1.1 a	7.6 ± 7.0 c
K	2920 ± 323 a	3156 ± 274 b	3102 ± 207 b	3380 ± 333 c
Ca	312 ± 36 a	324 ± 39 ab	319 ± 55 ab	341 ± 41 b
V	1.31 ± 0.40 b	1.01 ± 0.35 a	1.06 ± 0.58 ab	1.24 ± 0.64 ab
Mn	46.5 ± 6.7 b	40.7 ± 5.6 a	43.0 ± 7.5 ab	53.2 ± 7.9 c
Fe	32.2 ± 5.3 b	32 ± 11 b	27.7 ± 4.3 a	38.5 ± 6.7 c
Cu	5.6 ± 1.0 b	4.9 ± 1.0 a	4.79 ± 0.54 a	5.40 ± 0.66 b
Zn	27.7 ± 4.5 b	29.6 ± 6.4 b	23.9 ± 2.8 a	28.7 ± 4.9 b
Mo	0.94 ± 0.45 b	0.52 ± 0.19 a	0.62 ± 0.22 a	0.54 ± 0.19 a
Cd	0.026 ± 0.010 a	0.038 ± 0.037 b	0.017 ± 0.009 a	0.019 ± 0.006 a
Ba	3.4 ± 1.3 a	3.1 ± 1.2 a	4.6 ± 1.4 b	3.43 ± 0.97 a
Th	0.005 ± 0.002 c	0.006 ± 0.003 c	0.001 ± 0.001 a	0.003 ± 0.002 b

[a] Data in this table are shown by means ± standard deviations, and the different letters mean significant differences ($p < 0.05$).

Table 6. Classification of Wheat Samples from the 2007/2008 Harvest from Different Regions

			predicted group membership				
			Hebei	Henan	Shandong	Shaanxi	total
original	count	Hebei	23	5	1	1	30
		Henan	5	23	2	0	30
		Shandong	1	1	27	1	30
		Shaanxi	1	1	0	28	30
%			76.7	76.7	90.0	93.3	84.2
cross-validated	count	Hebei	21	7	1	1	30
		Henan	6	22	2	0	30
		Shandong	1	3	25	1	30
		Shaanxi	1	2	0	27	30
%			70.0	73.3	83.3	90.0	79.2

the experimental variables on the classification results, the coefficients of the discriminant functions were examined. The most contributions to classification were Ba (−0.990) and Mn (+0.866) in discriminant function 1. The highest coefficient in the discriminant function 2 was Ca (+0.555). Discriminant function 3 was formed mainly by Mg (+0.675) and V (+0.565). The average correct classifications of 84.2 and 79.2% of the samples for original and cross-validation were obtained, respectively (Table 6). Between Hebei and Henan samples, there were some misclassifications in both directions, which led to the lower classifications. There were other misclassifications in other groups, too. Fisher's linear discrimination functions for each province were as follows:

Group 1 (Hebei) = 0.063 Mg + 0.042 Ca + 3.600 V + 0.167 Mn − 0.142 Ba − 70.998

Group 2 (Henan) = 0.057 Mg + 0.051 Ca + 2.845 V + 0.137 Mn − 0.027 Ba − 62.800

Group 3 (Shandong) = 0.058 Mg + 0.055 Ca + 2.875 V + 0.110 Mn + 2.228 Ba − 77.926

Group 4 (Shaanxi) = 0.053 Mg + 0.074 Ca + 2.537 V + 0.611 Mn − 1.671 Ba − 89.009

The separation between the geographical origins of wheat in the discriminant space was checked by plotting the first three function scores, shown in Figure 2a, and strong visual regional clustering was observed, but samples from Hebei and Henan had some overlaps.

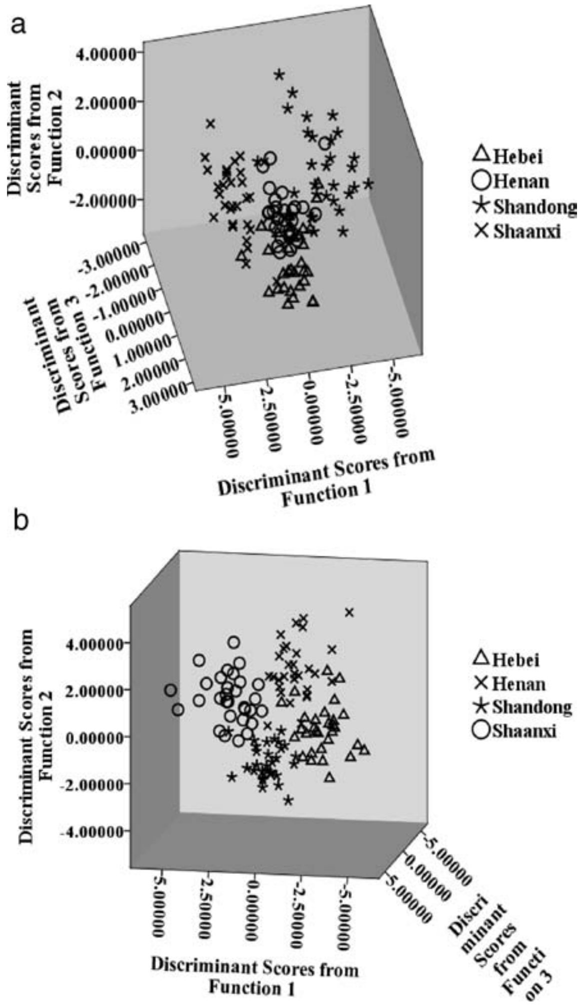

Figure 2. (a) Scatter plot of discriminant scores from functions 1−3 of 2007/2008 samples. (b) Scatter plot of discriminant scores from functions 1−3 of 2008/2009 samples.

For the 2008/2009 samples, the discriminant elements were Th, Al, Mn, Mo, Be, Ba, K, Na, Mg, Zn, and Cd. New three canonical discriminant functions that explained the 100% of the variance (function 1 explained 53.3% of the total variance, function 2 explained 33.1%, and function 3 explained 13.6%) were obtained. Wilks' Lambda values were 0.036, 0.163, and 0.523 for functions 1, 2, and 3, respectively ($p = 0.000$). Th (−0.351), K (+0.281), and Mo (−0.244) were the variables with the highest weights in the discriminant function 1. Discriminant function 2 was formed mainly by Th (+0.433), Be (+0.335), Zn (+0.302), Al (+0.299), and Ba (−0.289). The highest coefficients in the discriminant function 3 were Mn (+0.615), Fe (+0.368), Mo (+0.354), Na (+0.349), and Mg (+0.340). These three discriminant functions had a recognition ability of 95.8% of the original grouped samples, and the mean classification rate in the cross-validation classification matrix was 92.5%, reflecting that the recognition of the four classes was satisfactory (Table 7). Fisher's linear discrimination functions for each province were as follows:

Group 1 (Hebei) = 138.353 Be + 0.723 Zn + 7.688 Mo − 118.925 Cd − 1.224 Ba + 2044.663 Th + 0.807 Mn − 0.959 Al + 0.028 K + 0.061 Mg − 0.678 Na − 111.194

Table 7. Classification of Wheat Samples from the 2008/2009 Harvest from Different Regions

			predicted group membership				
			Hebei	Henan	Shandong	Shaanxi	total
original	count	Hebei	30	0	0	0	30
		Henan	2	27	1	0	30
		Shandong	0	0	29	1	30
		Shaanxi	0	0	1	29	30
%			100.0	90.0	96.7	96.7	95.8
cross-validated	count	Hebei	27	2	1	0	30
		Henan	3	26	1	0	30
		Shandong	0	0	29	1	30
		Shaanxi	0	0	1	29	30
%			90.0	86.7	96.7	96.7	92.5

Table 8. Classification of Wheat Samples from the 2007/2008 and 2008/2009 Harvests from Different Regions

			predicted group membership				
			Hebei	Henan	Shandong	Shaanxi	total
original	count	Hebei	38	6	9	7	60
		Henan	4	49	5	2	60
		Shandong	5	10	42	3	60
		Shaanxi	5	7	0	48	60
%			63.3	81.7	70.0	80.0	73.8
cross-validated	count	Hebei	37	6	9	8	60
		Henan	6	47	5	2	60
		Shandong	5	10	42	3	60
		Shaanxi	6	8	0	46	60
%			61.7	78.3	70.0	76.7	71.7

Group 2 (Henan) = $157.690\ \mathrm{Be} + 1.004\ \mathrm{Zn} + 3.305\ \mathrm{Mo} - 34.973\ \mathrm{Cd} - 1.356\ \mathrm{Ba} + 1517.858\ \mathrm{Th} + 0.704\ \mathrm{Mn} - 0.568\ \mathrm{Al} + 0.039\ \mathrm{K} + 0.032\ \mathrm{Mg} - 0.972\ \mathrm{Na} - 113.243$

Group 3 (Shandong) = $88.988\ \mathrm{Be} + 0.782\ \mathrm{Zn} + 1.533\ \mathrm{Mo} - 70.207\ \mathrm{Cd} + 0.267\ \mathrm{Ba} - 743.449\ \mathrm{Th} + 0.699\ \mathrm{Mn} - 0.091\ \mathrm{Al} + 0.031\ \mathrm{K} + 0.056\ \mathrm{Mg} - 0.581\ \mathrm{Na} - 103.008$

Group 4 (Shaanxi) = $149.679\ \mathrm{Be} + 1.069\ \mathrm{Zn} + 1.639\ \mathrm{Mo} - 69.889\ \mathrm{Cd} - 1.316\ \mathrm{Ba} - 986.963\ \mathrm{Th} + 1.053\ \mathrm{Mn} + 0.355\ \mathrm{Al} + 0.040\ \mathrm{K} + 0.033\ \mathrm{Mg} - 0.743\ \mathrm{Na} - 131.912$

Figure 2b presents a clear separation of the four regions. However, results from LDA1 + 2 (Al, V, Mn, Mo, Cd, and Ba as discriminant variables) showed a poor classification of two-year samples, giving only 73.8 and 71.1% correct assignation for original and cross-validation, respectively (Table 8).

Furthermore, the selected discriminant elements for LDA1, LDA2, and LDA1 + 2 were different, but Mn and Ba were contained in three LDA models, and they were the principle discriminating elements in wheat origin analysis for Shaanxi and Shandong provinces, respectively.

DISCUSSION

The results showed that the element contents in wheat samples from four provinces had their own characteristics. The different characteristics could be linked to the soil system. The main soil type in Hebei and Henan is fluvo-aquic soil, while brown earth and dark loessial soil are the main types in Shandong and Shaanxi sampling regions, respectively.[20] The content of K is lower in Hebei soil.[21] The levels of Na and Mn in Henan soil are lower than in other regions, while the content of Cd is higher.[22,23] Higher Ba levels and lower Zn levels are in Shandong soil.[24,25] The Shaanxi soil is enriched in minerals such as Ca, Mn, Al, and Fe.[26−29] The results showed that the influence of natural geochemistry on the element concentrations of the studied wheats seemed to be evident. The satisfactory classification results further confirmed that the discrimination between wheats coming from different regions can be afforded by creating unique signatures with element chemical profiling.

However, the element profiles of the samples are affected not only by soil but by other factors, such as varieties, climates, and agricultural practices.[30−35] The climates of four provinces have their own characteristics. Hebei province belongs to the temperate continental monsoon climate. Henan province is the north subtropical to warm temperate transition zone. Shandong is the warm temperate monsoon climate type. The sampling regions in Shaanxi belong to the warm semiarid or subhumid climate. Different climatic characteristics lead to different annual average temperature and precipitation (Table 1). Because of the adaptability of wheat to climates, main varieties are different in different regions, and there are many main varieties in each province. The two-year samples in this study were the main varieties and from the same location in each province, so the varieties were numerous, and many of them were regional or yearly, not found in any other location or year. It could be observed from Table 1, for Hebei, Henan, Shandong, and Shaanxi samples, that the number of 2007/2008 varieties was 17, 16, 9, and 7, separately, while 2008/2009 was 11, 15, 7, and 5, separately. The number of the same varieties for two-year samples was 9, 7, 5, and 4, separately, from Hebei, Henan, Shandong, and Shaanxi. Also, the number of samples with the same varieties was 19, 15, 15, and 21, separately. Therefore, varietal differences could be influential. Furthermore, all of the samples in our database were grown for the purpose of sale without regard to the origin. Therefore, wheat farmers applied fertilizers as needed. There probably was a great deal of variability in the fertilizer application from field to field as well as province to province, which could also contribute to the variation of element concentrations such as K and could not be evaluated. The classification of LDA1 + 2 was lower than LDA1 and LDA2, the possible reason of which is most likely to be the interannual variation compromising the model's classification success.

The contributions of region, variety, harvest year, and their interactions to the element contents in 240 samples were examined by means of a multivariate analysis of variance (MANOVA) test. As a result, there were significant effects on the element contents for region ($p < 0.01$), variety ($p < 0.01$), harvest year ($p < 0.01$), and the interation between variety and harvest year ($p < 0.05$). There was no significant interaction effect between the region and the wheat variety or harvest year. This result was consistent with the above results and may explain why some discriminant elements appeared in LDA2 but no in LDA1 (i.e., Th, Al, Mo, Be, K, Na, Zn, and Cd), as well as some discriminant elements appearing in LDA1 but no in LDA2 (i.e., Ca and V). The significant effects on the element contents for variety, harvest year, and the interation between variety and harvest year may be the reason why the differences of many element concentrations were large between two-year samples grown at the same locations. However, the characteristics of the element contents for each province changed little. For the single element, the contributions of these factors were different. For the element Mn, there were significant effects only for region ($p < 0.01$) and the interaction between variety and harvest year ($p < 0.05$). However, the influence of region was stronger. For the element Ba, the influence order of each factor was as follows: harvest year > region > the interaction between variety and harvest year > variety. Therefore, more elements should be determined so that those more related to soil composition such as Mn could be selected for identification. Furthermore, extending the database with more representative samples is also necessary to improve the statistical representation of the origins, thus providing more reliable classification functions and probably higher classified rates for the authentication of wheat.

The findings in this paper demonstrate that there are indicative elements for each sampling region within China, such as Ba for Shandong province and Mn for Shaanxi province. It is promising to classify wheats according to their geographic origins using multielement analysis in combination with statistical processing, although there are impacts of wheat varieties, harvest years, and agricultural practices on the element concentrations in wheats. The ease and efficiency of multielement analysis make it an optimal choice for geographic regional determination of wheat.

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