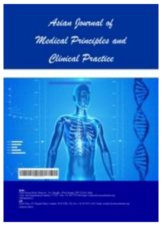

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Origin Identification of Buckwheat from Different Areas in Loess Plateau by Chemical Analysis and Data Mining Techniques

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Authors' contributions

This work was carried out in collaboration among all authors. Author QZ designed the study and performed the statistical analysis. Author JY wrote the protocol and wrote the first draft of the manuscript. Authors QZ and JGX managed the analyses of the study. Author DQW managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

In this study, the quality characteristics of mineral elements, vitamins and amino acids of 39 common buckwheat samples from the eastern, central and western regions of Loess Plateau were analyzed and compared and then effective indicators were selected for discrimination geographical origins. The linear discriminant analysis (LDA) was used to determine the geographical origin of common buckwheat according to three chemical families and their combinations each other. All models showed different correct classification rate and cross-validation rate. Among them, the correct classification rate and cross-validation rate of model based on the relative content of amino acid (the percentage of one amino acid in total amino acids) in combination with mineral elements were both 97.4%. The results demonstrated that the established method using multivariate analysis and data mining techniques was very effective and feasible for identification origin of buckwheat in the Loess Plateau.

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Keywords: Common buckwheat; identification; Loess plateau; data mining; discriminant analysis; chemical analysis.

1. INTRODUCTION

Primary agricultural products as raw food material had the rich nutrition and biological activity components, contributing the high nutritional value and health care function and they were an important guaranty for food quality and safety according to the principle of "from farm to table". However, compositions of agricultural products were largely affected by geographic origins due to differences in growing conditions including climate conditions, soil types, longitude, latitude, and altitude, which influenced the quality and price of produces [1-5]. For these reasons, producers, consumers, traders, the food industry and regulatory authorities are very concerned about the correct labelling of origin, traceability, and quality of agricultural products [2,6]. To accomplish this goal, the European Union (EU) has reinforced national control activities by implementing “protected designation of origin (PDO)”, “protected geographical indication (PGI)” and “traditional speciality guaranteed (TSG)”. In recent decades, numerous efforts and methods based on element, stable isotopic, organic compounds analysis in food with multiple statistical techniques have been used to determine the production origin of food, plant and animal products [7-10].

Common buckwheat (*Fagopyrum esculentum* Moench.) has been used both as a common food source and as a traditional medicine throughout the world because it contained abundant nutritional ingredients and bioactive phyto-chemicals [11-13], which provides basic nutrition and some positive health benefits [14-17]. Buckwheat had modest environmental requirements; it may be grown in poor soils and did not require protection [14,18]. In 2013 the buckwheat world production reached over 2.3 million tons and their cultivation areas were over 2.2 million hectares [18]. Common buckwheat, a major species with agricultural significance originated from Southwest China, has gradually spread around the world. Common buckwheat productions were worldwide concentrated in China, which was the biggest world producer generally. The Loess Plateau of northern China, the largest loess-covering region in the world, was one of the major buckwheat production areas. For example, in 2016, the area planted in the Loess Plateau accounted for more than 60% of the total area of the nation in China [19].

In the present study, the characteristics of some mineral elements, vitamins and amino acids in common buckwheat cultivated from the East (Shanxi Plateau), Central (North Shaanxi Plateau) and West (Hehuang Valley) regions of the Loess Plateau were analyzed and compared to select effective indicators and further determine the geographical origin of common buckwheat based on multivariate analysis and data mining techniques. This study aimed to provide an efficient method for authenticity identification of the geographical origins of common buckwheat in the Loess Plateau.

2. METHODS

2.1 Data Sources

Data of some mineral elements, vitamins and amino acids in common buckwheat were collected from Chinese Crop Germplasm Resources Information System (http://www.cgris.net/) which was an open and shared catalogue of scientific & technological resources in national S&T basic condition platform, providing data for the public. Complete data of 39 common buckwheat samples cultivated in the Loess Plateau were obtained from the database, distributing in the eastern, central and the western regions of the Loess Plateau, respectively. The content of Cu, Mn, Fe, Zn and Ca were determined by atomic absorption method; the content of Se and P were determined by hydride atomic fluorescence spectrometry and spectrophotometric methods, respectively; the content of amino acids were determined using Amino Acid Analyzer; the content of VPP and VE were determined by gas chromatography and photocolorimetric methods, respectively. Details of the samples are shown in Table 1.

2.2 Statistical Analysis

The statistical analysis of the data was performed by Microsoft Excel and the SPSS 17.0 package for windows. One-way analysis of variance was first carried on every single component of all the samples to determine significant differences ($p < 0.05$). Linear discriminant analysis (LDA) using the stepwise method was carried out to evaluate whether samples from different regions could be mathematically distinguished. The statistical significance of each discriminant function was

Table 1. Information of common buckwheat samples

No.	Site	Latitude	Longitude	No.	Site	Latitude	longitude
1-9	Youyu, Shanxi	N112.33	E40.18	20-21	Fugu,Shaanxi	N111.07	E39.05
10	Pinglu, Shanxi	N112.12	E39.53	22-23	Shenmu, Shaanxi	N110.51	E38.83
11	Dingxiang, Shanxi	N112.95	E38.50	24-26	Yulin, Shaanxi	N109.77	E38.30
12	Hongtong, Shanxi	N111.68	E36.25	27	Jingbian, Shaanxi	N108.79	E37.61
13	Fushan, Shanxi	N111.83	E35.97	28	Tianzhu, Gansu	N102.84	E37.24
14,15	Yicheng, Shanxi	N111.68	E35.73	29	Honggu, Gansu	N102.86	E36.33
16	Daning, Shanxi	N110.72	E36.47	30	Minhe, Qinghai	N102.80	E36.30
17	Jixian, Shanxi	N110.65	E36.12	31-34	Huangzhong,Qinghai	N101.57	E36.49
18	Xiangning, Shanxi	N110.80	E35.97	35-39	Ledu, Qinghai	N102.38	E36.49
19	Xiaxian, Shanxi	N111.22	E35.12				

evaluated based on the Wilks' Lambda criterion. Predictive ability of the classification model was evaluated by a cross-validation test, using the 'leave-one-out' procedure.

3. RESULTS

3.1 Elemental Profiles

Characteristics of mineral elements in common buckwheat of the Loess Plateau are shown in Table 2. According to statistics, the average content of P was the highest, while the average content of Se was the lowest among the seven mineral elements in common buckwheat. Mineral element contents of common buckwheat in different regions had their characteristics (Table 2). Compared to other regions, Se content (0.09 μg/g) was the highest, while P and Ca contents (3258.0, 465.0 μg/g, respectively) were the lowest in the eastern region. Common buckwheat samples in the central region had the highest Cu (16.22 μg/g), Fe (130.5 μg/g) contents and lowest Zn (24.29 μg/g), Mn (12.22 μg/g) contents. In the western region, the contents of Zn (34.81 μg/g), Mn (13.01 μg/g), Ca (508.4 μg/g) and P (4524.0 μg/g) were the highest and the contents of Cu (10.50 μg/g) and Se (0.024 μg/g) were the lowest. Comparing the variance degree of seven mineral elements in three regions mentioned above, variation degree of Cu content was found to be the greatest in the central region, followed by the western region, the lowest for the eastern region. Yet interestingly, for other six mineral elements, the variation degree reduced gradually from the eastern to the western regions of the Loess Plateau.

3.2 Vitamin E and Vitamin PP Profiles

As seen in Table 3, the common buckwheat from the eastern and western regions of the Loess Plateau had the highest content of vitamin PP (4.47 mg/100 g) and vitamin E (2.84 mg/100 g), respectively, while both vitamin E (1.38 mg/100 g) and vitamin PP (2.08 mg/100 g) contents were the lowest in the central region. Also, the highest variation degrees of both vitamin E and vitamin PP contents were found in the western region of the Loess Plateau.

3.3 Amino Acids Profiles

Table 4 showed the characteristics of amino acids in common buckwheat from different regions. The content of each of amino acids and total amino acids from the western region samples was the highest. And there was a decreasing trend for the variation degree of the total amino acids content from the east to the western regions.

3.4 Linear Discriminant Analysis (LDA)

For achieving better classification and identification of the common buckwheat samples, LDA was carried out based on variables with significant differences ($p < 0.05$). The LDA classification results based on different chemical families and their combinations each other are summarized in Table 5. The correct classification rate of model 1, model 2 and model 3 were 51.3%, 61.5%, 82.1%, and their cross-validation rate respectively reached to 43.6%, 61.5%, 76.9%, indicating that the distribution of amino acids and vitamins compositions of buckwheat samples from different origins were similar, which made it difficult to determine the origins. Also, based on the relative content of amino acids was carried out. As compared to model 1, the correct classification rate and cross-validation rate of model 4 increased from 51.3% to 97.4% and from 43.6% to 87.2% respectively, which was consistent with the previous study [20]. Among all models, the correct classification rate and cross-validation rate of model 10 based on the relative content of amino acid in combination with mineral elements were both 97.4%. In model 11, the combination of relative content of amino acids, mineral elements and vitamins content was taken as the variable and the correct classification rate increased to 100%. However, the cross-validation rate dropped to 94.9%. Also, we found that the content of amino acid seemed to be no obvious effect to distinguish the origin of the buckwheat combining the results of all models.

3.5 Discriminant Analysis Results

Generally, a high percentage of correct classification represented the stability and the strong relationship between the variables and the origins, while a high predictive percentage of the validated set indicated the high ability to classify the origin of an unknown sample based on the relative profiles of selected variables [8-10]. The result of cross-validation was more real, objective and reliable. Additionally, considering the efficiency, economics, operation convenience of discrimination model in practical applications, therefore model 10 based on the relative content of amino acid in combination with mineral elements was selected to further discriminant analysis.

Table 2. Descriptive statistics of mineral elements contents (μg/g) of common buckwheat from different regions

	East region (n=19)			Central region (n=8)			West region (n=12)		
	Mean±SD	**Range**	**CV (%)**	**Mean±SD**	**Range**	**CV (%)**	**Mean±SD**	**Range**	**CV (%)**
Cu	11.63±1.96	7.62-15.28	16.9	16.22±13.45	4.88-45.18	82.9	10.50±3.24	7.99-19.04	30.3
Zn	24.44±8.85	15.23-41.04	36.2	24.29±8.44	17.68-37.90	34.7	34.81±6.04	24.96-45.59	15.2
Fe	118.2±79.1	47.7-353.8	67.0	130.5±80.5	50.4-306.6	61.7	122.8±50.8	61.0-235.1	38.4
Mn	12.96±3.14	8.50-20.00	24.2	12.22±2.59	8.04-16.07	21.2	13.01±2.62	9.55-17.04	19.5
Ca	465.0±252.9	218.6-1202.0	54.4	504.2±237.3	224.3-962.6	47.1	508.4±131.2	291.1-728.2	24.4
P	3258±1207	2061-5557	37.1	3953±788	2761-5196	19.9	4524±602	3316-5398	12.9
Se	0.090±0.074	0.016-0.236	82.0	0.057±0.026	0.024-0.087	45.4	0.024±0.010	0.012-0.040	40.9

Table 3. Descriptive statistics for vitamin content (mg/100 g) in common buckwheat from different regions

	East region (n=19)			Central region (n=8)			West region (n=12)		
	Mean±SD	**Range**	**CV (%)**	**Mean±SD**	**Range**	**CV (%)**	**Mean±SD**	**Range**	**CV (%)**
VE	1.64±1.04	0.23-4.28	63.5	1.38±0.51	0.37-2.04	36.7	2.84±2.02	1.52-8.51	69.9
VPP	4.47±1.24	2.73-7.75	27.7	2.08±0.99	1.18-3.45	47.7	2.24±1.36	1.09-6.36	63.1

Table 4. Descriptive statistics for amino acids content (g/100 g) in common buckwheat of different regions

	East region (n=19)			Central region (n=8)			West region (n=12)		
	mean±SD	**range**	**CV (%)**	**mean±SD**	**range**	**CV (%)**	**mean±SD**	**range**	**CV (%)**
Asp	1.15±0.30	0.80-1.72	25.8	1.16±0.16	0.87-1.31	14.1	1.29±0.17	0.99-1.55	13.2
Thr	0.45±0.10	0.30-0.65	23.3	0.45±0.05	0.37-0.52	11.1	0.51±0.07	0.36-0.60	13.9
Ser	0.59±0.15	0.36-0.86	24.7	0.60±0.09	0.45-0.69	14.7	0.70±0.10	0.50-0.80	14.0
Glu	2.28±0.66	1.41-3.49	29.1	2.39±0.35	1.82-2.69	14.7	2.56±0.35	1.99-3.18	13.6
Gly	0.64±0.14	0.47-0.92	22.7	0.71±0.06	0.61-0.78	8.5	0.76±0.10	0.57-0.94	13.1
Ala	0.51±0.10	0.39-0.70	19.7	0.54±0.06	0.44-0.61	11.5	0.62±0.09	0.48-0.75	14.1
Cys	0.16±0.07	0.08-0.28	42.5	0.18±0.03	0.14-0.21	13.9	0.19±0.07	0.09-0.31	38.0
Val	0.56±0.12	0.41-0.78	22.3	0.61±0.08	0.46-0.68	13.4	0.68±0.09	0.51-0.80	13.9
Met	0.19±0.07	0.10-0.31	34.0	0.19±0.02	0.16-0.21	10.3	0.21±0.04	0.15-0.28	17.7
Ile	0.46±0.15	0.32-0.74	32.5	0.49±0.08	0.35-0.63	17.3	0.52±0.10	0.37-0.75	19.0
Leu	0.78±0.18	0.58-1.13	23.5	0.80±0.11	0.61-0.91	13.7	0.87±0.11	0.68-1.03	12.7
Tyr	0.30±0.09	0.20-0.54	31.5	0.32±0.03	0.26-0.36	11.0	0.37±0.06	0.30-0.48	14.7

	East region (n=19)			Central region (n=8)			West region (n=12)		
	mean±SD	**range**	**CV (%)**	**mean±SD**	**range**	**CV (%)**	**mean±SD**	**range**	**CV (%)**
Phe	0.54±0.11	0.42-0.77	19.6	0.58±0.09	0.42-0.67	14.7	0.63±0.09	0.53-0.78	14.5
Lys	0.65±0.13	0.49-0.88	19.5	0.71±0.08	0.58-0.80	11.2	0.77±0.09	0.60-0.93	12.2
His	0.26±0.06	0.18-0.37	23.9	0.28±0.04	0.22-0.32	14.3	0.31±0.04	0.24-0.39	14.4
Arg	1.07±0.26	0.75-1.63	24.1	1.15±0.21	0.78-1.41	18.7	1.31±0.18	0.93-1.56	14.2
Pro	0.45±0.10	0.31-0.66	22.2	0.46±0.09	0.30-0.61	19.7	0.60±0.17	0.37-0.84	27.8
Trp	0.12±0.04	0.07-0.18	32.8	0.11±0.03	0.07-0.14	26.2	0.14±0.02	0.08-0.17	17.9
Total	11.15±2.68	8.03-16.51	24.0	11.70±1.53	9.02-13.16	13.1	13.01±1.64	9.91-15.45	12.6

Table 5. Summary of models and accuracy

Model no.	Variable types	Effective indicators	Correct classification rate	Cross-validation rate
1	Amino acid content	Pro	51.3%	43.6%
2	Vitamin	VPP	61.5%	61.5%
3	Mineral element	Cu、Zn、P、Se	82.1%	76.9%
4	Relative content of amino acid	Asp、Leu、Glu、Val、Lys、His、Ser	97.4%	87.2%
5	Amino acid content＋vitamin	Ala、VPP	79.5%	74.4%
6	Amino acid content＋mineral element	Cu、Zn、P、Se	82.1%	76.9%
7	Mineral element＋vitamin	Cu、Zn、P、Se、VPP	89.7%	82.1%
8	Amino acid content＋mineral element＋vitamin	Cu、Zn、P、Se、VPP	89.7%	82.1%
9	Relative content of amino acid＋vitamin	VPP、Asp、Glu、Val、Ser	87.2%	84.6%
10	Relative content of amino acid＋mineral element	Asp、Glu、Val、Leu、Lys、Cu、Zn、Mn、Se	97.4%	97.4%
11	Relative content of amino acid＋mineral element＋vitamin	Asp、Glu、Val、Thr、Cu、Zn、Mn、Se、VPP	100%	94.9%

Table 6. Classification of common buckwheat in different regions and percentage of observations correctly classified by LDA

			Predicted group membership			
			ER[a]	CR[b]	WR[c]	Total
Original	Count	ER	19	0	0	19
	%	CR	0	8	0	8
		WR	0	1	11	12
			100	100	91.7	97.4
Cross-validated	Count	ER	19	0	0	19
	%	CR	0	8	0	8
		WR	0	1	11	12
			100	100	91.7	97.4

[a] ER, East region. [b] CR, Central region. [c] WR, West region

In model 10, nine variables (Zn, Mn, Cu, Se and relative content of Asp, Glu, Val, Leu and Lys) were thought to contribute significantly for distinguishing the origin (Table 5). The two functions based on Wilks' lambda values explained the 100% of the variance (Function 1 explained 88.3% of the total variance and function 2 explained 11.7%).

Function1=29.045+391.213Lys–571.738Leu+ 651.597Val+345.106Glu–1126.252Asp+ 0.114Cu+0.082Zn–0.430Mn+2.064Se

Function2=–14.122+142.590Lys–94.743Leu+ 51.847Val+116.417Glu–117.715Asp+0.115Cu– 0.145Zn–0.014Mn+13.268Se

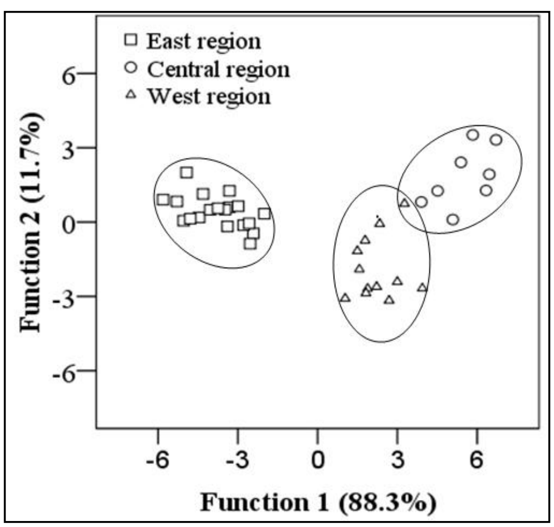

Fig. 1. Scatter plot of common buckwheat from different regions based on the two discriminant functions

The separation of buckwheat from different regions of Loess Plateau was checked by plotting the two functions scores (Fig. 1). It was found that buckwheat from different regions was well classified to each other. The LDA results of model 10 are summarized in Table 6. Based on selected 9 indicators, this method classified effectively the buckwheat grown in the eastern, central and western of the Loess Plateau and both the correct classification rate and cross-validation rate were 97.4%, exhibiting a satisfactory performance of this model for classifying common buckwheats of different origins of Loess Plateau. The accuracy of that in the eastern and central region was 100% and the accuracy rate was 91.7% (11/12) in the western region because one case in the western region was misclassified in the central region. The results showed that the common buckwheat from various origins of Loess Plateau can be classified based on the combination of multivariate analysis and data mining techniques.

4. DISCUSSION

The element analysis has been applied to determine geographical origin of some farm products including coffee [8], tea [10], garlic [21], apple [22], maize [23], Chinese cabbage [24,25], wheat [26,27] and other crops [6,28] and showed good results of the method. In the present study, four selected elements (Cu, Zn, P and Se) were found to contribute the ability to distinguish the producing area when only mineral elements were analyzed independently, and the overall correct classification rate and cross-validation rate were 82.1% and 76.9%, respectively. Besides, some organic compounds were also utilized to study the producing area of some products [29,30]. Amino acids, a class of important food components, contributed directly to the taste of foods and colouring substances formed [31,32]. Some studies reported that distinguishing methods based on amino acids analysis can effectively determine the geographic origin of some products [32,33]. In the present study, the correct classification rate (51.3%) and cross-validation rate (43.6%) based on the amino acids content alone were far lower than that based on the content of mineral elements. Some studies reported that element compositions are considered to be a very effective means for studying the geographic origin of products because mineral contents were more stable and less variable than organic compounds in food [26,34], which confirmed the results mentioned above. Nevertheless, the results of discrimination based on the relative content of amino acids has been greatly improved with more accurate even than that of mineral elements in this study, which might be because using the relative content of amino acids as variables could reduce the impact of fluctuations of total amino acid content on one amino acid content [20]. In the last several years, multiple discriminate analyses based on combinations of different types of ingredients have been used to discriminate the origin of agricultural product to avoid the one-sidedness of variation of a kind of constituent [29,30,35]. In the present study, the multivariate analysis results based on the combination of relative content of different amino acids and mineral elements was found to have nine variables (Zn, Mn, Cu, Se, and relative content of Asp, Glu, Val, Leu, Lys) played a very important role to distinguish the geographical origin, and perform best with 97.4% accuracy rate for discriminating the producing area of common buckwheat grown in various areas of the Loess Plateau.

The east region of the Loess Plateau had complex topography with loessal soils and brown soils. Also, the distance of Shanxi Plateau was longer from north to south, therefore differences in temperature and climate conditions of Shanxi Plateau were very obvious. These factors may be one of the main reasons leading to the large variation coefficients in the contents of amino acids and Mn, Zn, P, Fe, Ca, Se of buckwheat in Shanxi Plateau. The central region was mainly in the continental middle temperate zone and the soil mainly made of loessal soils. The west region located in the transition zone from the Loess Plateau to the Qinghai-Tibet Plateau was a type plateau continental climate with an arid climate, low rainfall, evaporation, high altitude, and the temperature difference between day and night. Common buckwheat in the west region had the highest contents of Zn, Mn, Ca, P, Vitamin E and amino acids, which may be related to the special geographical environment.

5. CONCLUSION

In summary, the multivariate discriminate analysis combining the relative content of amino acids and mineral elements contents was a simple, accurate and effective method to distinguish the geographical origin of common buckwheat from different areas of the Loess Plateau. Some variations in certain variables (Zn, Mn, Cu, Se and relative content of Asp, Glu, Val, Leu, Lys) could act as a classifier for discriminating geographical origin. A correct classification rate of 97.4% for common buckwheat of different regions of the Loess Plateau in China was got by LDA. The present study provided a good method for the quality, safety and food authenticity of common buckwheat, which was very important for both consumers and producers.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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