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Research Article

Using Mineral Elements to Authenticate the Geographical Origin of Yak Meat

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Abstract

Labeling systems for niche market food products is becoming increasingly important to address consumers' expectations. Yak meat is 'green' product from natural extensive rangeland on Qinghai-Tibetan plateau. A trace technique is essential for consumers to know the origin of yak meat. In the current study, mineral fingerprints were investigated for their potential to classify yak meat according to geographical origin. The concentration of more than 50 mineral contents in 24 yak meat samples from three regions on Qinghai-Tibetan plateau were analyzed by ICP-MS. Multivariate statistical analyses were used to identify the most relevant indicators of origin. Seven elements (Na, As, Ni, Se, Rb, Cd and Ti) were selected for further routine analyses based on the significant origin differences ($P<0.05$). The three minerals (Se, Rb, Ti) were selected by statistics analysis and established discriminant model for yak meat traceability. Linear discriminate analysis gave an overall correct classification rate of 91.7% and cross-validation rate of 87.5%. These results demonstrate the usefulness of multi-element fingerprints as indicators for authenticating the geographical origin of yak meat.

Keywords: *Yak meat, Mineral elements, Geographical origin, Authentication*

Yak Etinin Coğrafi Kökenini Doğrulamak Amacıyla Mineral Elementlerin Kullanılması

Öz

Pazardaki gıda ürünlerinin kökenini belirtmek için etiketleme sistemleri, tüketicilerin beklentilerini karşılamak için giderek daha önemli bir hal almaktadır. Yak eti, Qinghai-Tibet platosu üzerindeki doğal geniş meralardan elde edilen 'yeşil' bir üründür. Tüketicilerin yak etinin kaynağını bilmeleri için bir takip tekniği gereklidir. Bu çalışmada, yak etinin coğrafi kökenine göre sınıflandırılma potansiyeli açısından mineral parmak izleri araştırıldı. Qinghai-Tibet platosundaki üç bölgeden gelen 24 yak eti örneğinde 50'den fazla mineral içeriğin konsantrasyonu ICP-MS ile analiz edildi. En önemli kaynak göstergelerini tanımlamak için çok değişkenli istatistiksel analizler kullanıldı. Önemli orijin farklılıklarına bağlı olarak, rutin analiz için yedi element (Na, As, Ni, Se, Rb, Cd ve Ti) seçildi ($P<0.05$). Üç mineral (Se, Rb, Ti), yak eti izlenebilirliği amacıyla istatistik analiz sonucu seçildi ve ayırıcı model olarak belirlendi. Doğrusal ayırıcı analiz, %91.7 doğru sınıflandırma oranı ve %87.5 çapraz doğrulama oranı vermiştir. Bu sonuçlar çoklu element parmak izlerinin, yak etinin coğrafi kökenini doğrulamada bir gösterge olarak yararlı olduğunu göstermektedir.

Anahtar sözcükler: *Yak eti, Mineral maddeler, Coğrafi orijin, Doğrulama*

INTRODUCTION

The yak *(Bos grunniens)* is a ruminant which lives in harsh conditions in the mountains of Central Asia. More than 90% of the world's total yak population are currently herded in Chinese territories, mainly on the Qinghai-Tibetan plateau in area of altitude ranging from 2500 m to 6000 m [1,2]. Through thousands of years of evolution, the yak has

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adapted to survive in a cold and anoxic environment [3]. Products from yaks (meat, milk) and functions of yak farming (workload, energy, etc) are numerous and closely related to the daily life of the local human population. The yak meat is the main source of protein and the most important economic income for farmers [4]. It is of good quality with a fine texture, high protein and low fat content, and rich in amino acids compared with that of Chinese beef cattle [5,6].

Recently, the demand for yak meat is increasing in most areas of China since it is extensively produced in this scenic area far from the pollution and its reputation for good quality, high protein and low fat content, favorable amino acid and fatty acid profile compared with *Bos Taurus* cattle [2]. Moreover, face to the rapid development of the intensive beef cattle industry in the grain production areas in China, the yak meat production needs to be financially supported by the differentiated product label [7]. The labeling system for differentiated meat products has been developed in Europe [8], many of these labels have defined the geographic restriction of the production location, and some of them has even been developed exclusively for geographic origin such as Protected Geographical Indication. Now, it is difficult to clarify the food origin in Chinese market except organic food which is labeled. Therefore, it is necessary to develop routine analytical methods to authenticate the geographic origin of yak meat with the ultimate goal to set up a similar label for yak meat product.

Efforts have been made to develop analytical tools to quantify specific compounds in the product or the animal tissues that can act as tracers of the animal's geographic origin. Methods involving the measurement of quantifiable components at both an elemental and molecular level have been applied, including stable isotopes [9,10]; and trace elements [11]. Since specific feed stuffs are associated with a particular geographical region [12], the "markers" related to feeding background such as fatty acids [13], volatile compounds [14] and carotenoids [15,16] could also be useful for assignment of geographical origin [17]. However, most of yaks were fed exclusively by grass from natural grassland on the Qinghai-Tibetan plateau, the "markers" related to grass-feeding could not be applied to authenticate the geographical origin of yak. Successful authentication of geographical origin of beef has been achieved by analyzing mineral elements [11]. Since the production system and the chemical composition of yak meat differ from that of beef cattle, it is necessary to verify the reliability of this method in yak meat. The objective of this study was to primary analyze the mineral composition of yak meat from three major yak-producing regions on Qinghai-Tibetan plateau, and to investigate the feasibility labeling yak meat origin by its mineral elements concentrations of yak meat to authenticate its geographical origin.

MATERIAL and METHODS

Sample Origin

All animals were used for according to the Guide for the Care and Use of Laboratory Animals of Qinghai Province [18]. Twenty-four, 3-4 years old, male yaks were randomly selected from Da-tong (DT, n=10), He-nan (HN, n=8) and Lu-qu (LQ, n=6) County on Qinghai-Tibetan plateau. Da-tong County is located in the eastern part of Qinghai Province, the soil type includes alpine frozen soil, gray-drab soil and alpine meadow soil [19]. The grassland type in DT is mainly alpine meadow, and mountain meadow [19]. He-nan County is located in southeast part of Qinghai Province where the soil is constituted by alpine desert soil, alpine meadow soil, chernozem, mountain meadow soil, gray-drab soil and bog soil. Alpine meadow is the main grassland vegetation in HN [20]. Lu-qu County is located in the Gannan Tibetan Autonomous Prefecture of Gansu Province, in southeastern of Qinghai-Tibetan Plateau is constituted by alpine meadow soil, alpine shrub meadow soil, swamping meadow soil and mountain steppe soil [21].

Sample Preparation

The yaks were slaughtered in September 2013. Meat samples of 500 g were collected from the *longissimus* muscle of yak carcasses, and stored at -20°C prior to processing. Each sample was cut into small pieces for freeze-drying over 48 h before being pulverized in a ball mill. The crude fat of yak muscle powder was extracted with petroleum ether in a soxhlet apparatus, and the residue, mostly de-fatted proteins, was used for further analysis [10,22].

Mineral Analysis

The detailed procedures for biochemical methods are described by references [10,22]. Briefly, the samples were analyzed after microwave digestion using MARS microwave digestion system (CEM Corp. North Carolina, America). A 0.2 g of de-fatted yak sample, with 6 mL of 65% HNO_3 and 2 mL of 37% HCl were added into a Teflon PTFE digestion tube and digested for 40 min by increasing the power to 1600W and the temperature to 180°C in a stepwise fashion. The digested solution was diluted to 50 mL with ultra-pure water (Resistivity, in MW × cm at 25°C:18.2) and stored in plastic tube before analysis. Contents of more than 50 mineral elements (Na, Mg, Al, K, Ca, Sc, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Rb, Sr, Y, Mo, Ru, Rh, Pd, Ag, Cd, Sn, Sb, Te, Cs, Ba, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Lu, Yb, Hf, Ir, Pt, Au, Tl, Pb, Th, U) were measured by Inductively Coupled Plasma Mass Spectrometry (ICP-MS, 7500a, Agilent, America). Optimisation of the method using this instrument was done for higher sensitivity and lower detection limits. The optimised operation conditions for analysis of the diluted samples were as follows: radio frequency power of 1600 W, plasma gas flow rate of 1.12

L/min, auxiliary gas flow rate of 0.5 L/min, nebulisation chamber temperature at 2°C, the oxide indice of 0.45%, and dual current indice of 1.01%.

Analysis of each sample was quantified by using external standard. The Environmental Calibration Standard (a series of ICP-MS Standards) supplied by Agilent was used as a standard solution and the determination coefficient of standard curve was higher than 0.99. The internal standards Ge, In, Bi and other elements were used to ensure the stability of the instrument. The samples were analysed twice in each run when the Relative Standard Deviation (RSD) of internal standards was higher than 3%.

Statistical Analysis

The statistical analysis of the data was performed using the SPSS 19.0 package for windows. Data were first analyzed for the homogeneity of their variance using the test of Shapiro. Differences between means were analyzed using the Kruskal-Wallis H test since most of the data did not follow the normal distribution.

The mineral elements, in the de-fatted yak meat, which differed according to the regions on the northeastern of Qinghai-Tibetan plateau were analyzed using principle components analysis (PCA). In order to better visualize the relative distribution of the defatted yak meat samples according to their geographical origin, cluster analysis (CA) was performed using the first three principal component normalization scores. The clustering analysis was performed using the Euclidean distance and clustering methods using the sum of squares. To ascertain the discriminating efficiency of each element in yak meat, stepwise discriminant analysis was carried out on the basis of the mineral element compositions found to be significantly different among the regions.

RESULTS

The means of seven (Na, As, Ni, Se, Rb, Cd, Ti) of the 50 elements analysed in de-fatted yak meat, were significantly different among the regions ($P<0.05$) *(Table 1)*. The Na, As and Ti concentrations were significantly higher in DT County than HN and LQ County. And no differences were shown between HN and LQ County. The Ni and Cd concentrations in HN County were lower than DT County, but similar with LQ County. The Se and Rb concentrations in HN and DT County were higher than in LQ County, but no differences between HN and DT County.

Following PCA, the first three factors explained 77.40% of the total variability *(Table 2)*. The contents of Cd and Ti had the highest weight on the first PC (explaining 46.80% of the variability). Na and Se were the most important variables explaining variation in the second PC (16.20% of variability). Rb content showed the highest weight on the third PC (explaining 14.40% of variability).

Scatter plots of scores on the first two principal components presented in *Fig. 1*. The three regions reflecting differences in patterns of mineral composition were easily distinguished. Compared with the other two regions, samples from DT is more dispersed. The largely horizontal distribution of DT and LQ samples reflect their classification with regards to differences in Na and Se in PC2. On the other hand, the vertical distribution of samples from HN represents variation due to differences in Cd and Tl on PC1.

Table 2. *Correlations of the first 3 principal components with the original variables and variance explained by these principal components*

Mineral Element of De-Fatted Muscle	PC1	PC2	PC3
Na	0.175	0.859	-0.119
As	0.668	0.309	0.093
Ni	0.706	0.291	-0.374
Se	0. 216	0.811	0.250
Rb	0.114	0.093	0.943
Cd	0.869	-0.055	0.179
Ti	0.817	0.454	0.138
Variance contribution, %	46.8	16.20	14.40
Cumulative variance contribution, %	46.8	63.00	77.40

Table 1. *Mineral elements compositions of de-fatted muscle of yak sample from different regions (mg/kg, DM)*

Element	HN[1]		DT[2]		LQ[3]		P-value
	Mean±SD	CV	Mean±SD	CV	Mean±SD	CV	
Na	1533±161.7[b]	0.105	2104±704.2[a]	0.335	1540±199.40[b]	0.130	0.032
As	0.032±0.012[b]	0.375	0.055±0.017[a]	0.309	0.030±0.010[b]	0.333	0.036
Ni	0.050±0.011[b]	0.220	0.079±0.032[a]	0.405	0.063±0.012[ab]	0.190	0.002
Se	0.040±0.027[a]	0.675	0.056±0.016[a]	0.286	0.008±0.004[b]	0.5	0.000
Rb	5.602±1.517[a]	0.271	4.826±3.316[a]	0.687	2.153±0.770[b]	0.358	0.037
Cd	0.002±0.000[b]	0	0.005±0.002[a]	0.4	0.003±0.001[ab]	0.333	0.012
Ti	0.001±0.000[b]	0	0.003±0.001[a]	0.333	0.001±0.001[b]	1	0.000

[1] HN, He-nan County; [2] DT, Da-tong County; [3] LQ, Lu-qu County; [a,b] Superscripts within rows indicate significant difference at 0.05 level

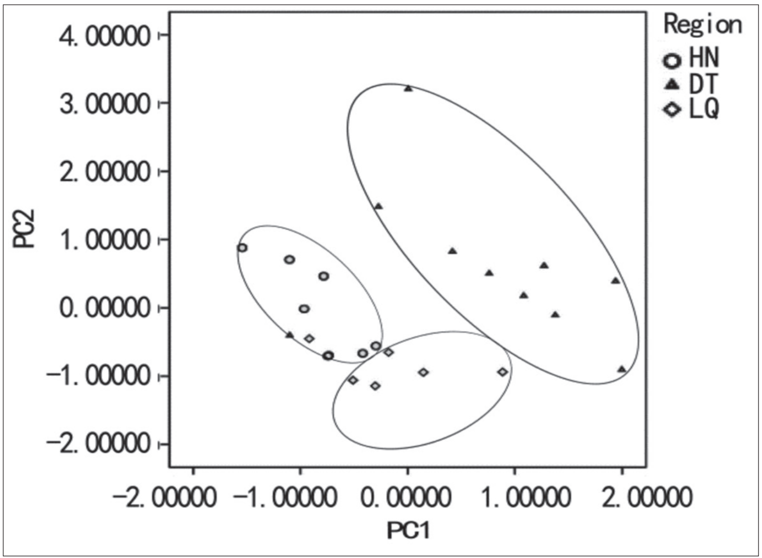

Fig 1. Scatter plots of de-fatted muscle of yak samples scores on the first three principle components. HN, He-nan County; DT, Da-tong County; LQ, Lu-qu County

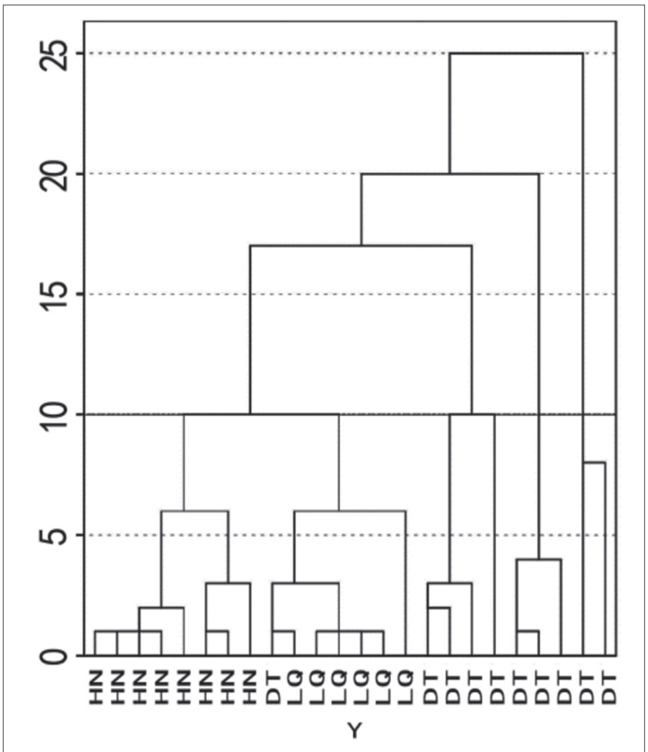

Fig 2. Dendrogram of Cluster analysis. HN, He-nan County; DT, Da-tong County; LQ, Lu-qu County

Yak samples from different regions were separated into six clusters based on the dendrogram cut at a distance of 10 *(Fig. 2)*. The first cluster was mainly composed of samples from He-nan County (n=8). The second clusters were composed of samples from Lu-qu County (n=8). Finally, the rest of cluster mainly comprised the samples from Da-tong County (n=9). Overall, the cluster results were generally in agreement with the actual origin of samples, which implied that multi-element information could be suitably utilised to classify yak samples from the different regions.

By statistics analysis, three elements (Se, Rb, Ti) were selected to establish a classification model using a stepwise discriminant procedure. The model establish as follows:

$Y_{HN} = -7.316+151.354Se+1.347Rb-1433.200Ti$
$Y_{DT} = -9.619+133.994Se+0.545Rb+2312.683Ti$
$Y_{LQ} = -2.272+10.447Se+0.203Rb+1071.610Ti$

Samples from LQ had an overall correct classification rate and cross-validation rate of 100%; HN and DT samples had an overall correct classification rate of 87.5% and 90%, respectively *(Table 3)*. A satisfactory classification was obtained with an overall correct classification rate of 91.7% and a cross-validation rate of 87.5%. These results were consistent with those obtained by PCA and CA, and reconfirmed the feasibility of multi-element analysis for yak geographical origin traceability.

DISCUSSION

The elemental analysis in this study demonstrated that mineral element content of yak meat from three regions on Qinghai-Tibetan plateau were different due to their geographic origin *(Table 1)*. A number of techniques have been used to determine the origin of feed source and these techniques are included in a review [23]. Perhaps the most common technique is the measurement of stable isotopes in meat using isotope ratio mass spectrometry (IRMS). Authenticating meat origin using IRMS has been endorsed because the environmental and management factors that affect the stable isotope ratios of bioelements that end up in animal tissue directly reflect where that meat was derived. The stable isotopes of C and N was used to successfully classify beef from different regions in China [10]. However, routine use of IRMS has been limited as there are large costs associated with setting up reference databanks [24]. Others have combined multi-element and stable isotope analysis to improve classification, but indicated that using multi-mineral analysis was as good as stable isotopes for identifying meat origin [11].

The use of mineral elements to classify meat from different origins has previously been demonstrated for beef [11], lamb [22], pork [25] and poultry [11] meat. Those studies used mineral composition to trace meat back to different countries. The feasibility of discriminating meat source over vast geographical boundaries, ie different countries, is achieved because of large differences in geological profiles, soil formation and climate. The results of this study demonstrated that yak meat source can be discriminated at a much smaller scale, ie between regions within

Table 3. *Classification of yak samples in different regions and percentage of observations correctly classified*

Predicted Group Membership [a]						
Original Group			**Predict Group Membership**			**Total**
			HN[1]	**DT[2]**	**LQ[3]**	
Original	Number	HN	7	0	1	8
		DT	0	9	1	10
		LQ	0	0	6	6
	Original count %		87.5	90	100	91.7[b]
Cross-validated	Number	HN	7	0	1	8
		DT	1	8	1	10
		LQ	0	0	6	6
	Cross-validated count %		87.5	80	100	87.5[c]

[1] HN, He-nan County; [2] DT, Da-tong County; [3] LQ, Lu-qu County; [a] The number of correctly classified observations are tabulated diagonally; [b] 91.7% of empirical grouped observations correctly classified; [c] 87.5% of cross-validated grouped observations correctly classified

a province. This is made possible by the unique farming system in these areas where the farm practice is essentially organic. Because there is virtually no importation of fertilizer or supplemental feed, which would otherwise introduce external sources on dietary minerals, it is possible to discriminate yak meat from different areas in Qinghai province.

The mineral composition of meat is influenced by the environment [22] and the geological processes which influence the soil profile. There was a research demonstrated the environmental influence and geographical variation in mineral content of pastures grown in extensive grassland regions of southern Patagonia [26]. In this research the soil characteristics differed markedly among the three Counties [27]. For example, Da-tong County is characterized by its saline, chestnut, alpine meadow soils and high arsenic content [28]. Two minerals which characterized yak meat to this area [29]. The soil in Lu-qu County is poor salinization [21], which was reflected by the lack of variation in Na in the PCA analysis *(Fig. 1)*. The content and availability of minerals in soils and plants influence the intake and utilisation by grazing animals.

The classification of the different yak meat samples to their origin had a combined cross-validation rate of 87.5%. While we acknowledge the small sample size of the present study, the ability of PCA and LDA to clearly identify yak samples from different origins, provides some confidence in using minerals to authenticate yak meat origin. In agreement with our research, beef geographical origin based on multi-element analysis was also able to discriminate beef from four regions in China, with 98.4% correct classification [30]. In previous studies, there were investigated the suitability of element signatures for authenticating poultry meat and dried beef samples from different origins [11,31]. Those authors concluded that element composition in meat samples could be used to discriminate samples from different countries. However, they noted that classification confidence declined when a large proportion of the diet was imported and the deposition of mineral elements in lean tissue no longer reflected the environment. Similarly, it is recognized that the way in which the meat is processed, i.e. curing, deboning, seasoning, can lead to changes of mineral contents. Because of the unique environment in which yak meat is farmed and the organic practices used, the risk of misclassification is relatively small. But we would highlight the potential impact of factory processing of yak meat beyond the farm gate which could reduce confidence in both origin and perceived 'purity' of marketed products.

In summary, this study provides evidence that yak meat can be authenticated according to its geographical origin using mineral contents. Se, Rb, Ti were selected from more than 50 minerals by statistics analysis and established discriminant model for yak meat traceability. Multivariate statistical analysis gave an overall correct classification rate of 91.7% and cross-validation rate of 87.5%.

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