Tracing the geographical origin of rice by stable isotopic analyses combined with chemometrics

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ABSTRACT

Multivariate stable isotope analysis combined with chemometrics was used to investigate and discriminate rice samples from six rice producing provinces in China (Heilongjiang, Jilin, Jiangsu, Zhejiang, Hunan and Guizhou) and four other Asian rice producing countries (Thailand, Malaysia, Philippines, and Pakistan). The stable isotope characteristics were analyzed for rice of different species cultivated with varied farming methods at different altitudes and latitudes/longitudes. The index groups of δ^{13}C, δ^{15}N, δ^{18}O, $^{207/206}$Pb and $^{208/207}$Pb were screened and established for the selected samples with different geographical features by means of principal component analysis (PCA) and discriminant analysis (DA), which would provide a sound technical solution for rice traceability and serve as a template for further research on the traceability of other agricultural products, especially plant-derived products.

1. Introduction

Rice is one of the most important agricultural products and provides nutrition to more than half of the world's population (Pinson et al., 2015). According to the Food and Agriculture Organization (FAO), as the world's leading rice producing region, Asia is home to many top rice exporters of the world (FAOSTAT, 2012). In line with improved living standards, the geographical origin of rice has become one of the major considerations for consumers. Rice with a geographical origin label, for example Jasmine rice from Thailand, Koshihikari from Japan and Basmati from Pakistan, is considered high-quality rice and is usually charged a premium. With the consumer market of high-quality rice appearing, there emerges a risk that unscrupulous producers sell inferior or counterfeit products as premium rice for additional economic benefits (Suzuki, Chikaraishi, Ogawa, Ohkouchi, & Korenaga, 2008). Hence, it is highly essential to accurately determine rice authenticity, preventing fake and inferior products in order to protect consumers' rights and improve the credibility of producers and traders in the light of the increase in global trade and free markets (Gonzalvez, Armenta, & de la Guardia, 2009).

In recent years, analytical techniques used to determine the geographical origins of agricultural products have made great progress (Karoui & Debaerdemaeker, 2007; Luykx & van Ruth, 2008). In particular, the method for determining the geographical origins of various foods has been developed and validated based on multi-isotope analysis in parallel with multi-elemental chemometric methods, involving rice (Chen et al., 2016; Chung et al., 2017; Yuan et al., 2018), animal-derived food (Camin, Bontempo, Perini, & Piasentier, 2016; Guo, Wei, Pan, & Li, 2010; Osorio, Moloney, Schmidt, & Monahan, 2011), and oil (Camin et al., 2010b; Camin, Bontempo, Ziller, Piangiolino, & Morchio, 2010a).

Cultivated plants have a large number of stable isotopes, which provide information on photosynthesis (e.g. carbon) or fertilization approaches (e.g. nitrogen), and geographical origins (e.g. oxygen and lead). Among them, C, N and O are the most common isotopic compositions and have been used to determine geographical origins of food (Nietner, Haughey, Ogle, Fauhl-Hassek, & Elliott, 2014). The isotopic abundance of individual organic compounds provides a more direct link to human-induced and environmental factors, including climatic drought, solar radiation, temperature, atmospheric pressure and stress (Mihailova, Abbado, Kelly, & Pedentchouk, 2015). Carbon isotopes in rice were discriminated via C3 photosynthesis, where carbon dioxide (CO_2) in the air was absorbed by plants to form sugars, which was based on Rubisco activity and stomata opening/closure. During the dispersion of CO_2 into the plants, the temperature, evapotranspiration and water usage led to fractionation changes (Hu, Cheng, & Tao, 2016; Long,

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Xiang, Xu, Su, & Kang, 2014; Zhou, Zhou, Hu, & Liao, 2013). The isotopic composition of nitrogen mainly depends on regional environment, agricultural fertilization, nitrogen fixation and land use patterns (Johnson & Berry, 2013; Meints, Shearer, Kohl, & Kurtz, 1975; Shearer & Legg, 1975; Yuan et al., 2012). In addition, fractionation of the nitrogen isotope during NH_4^+ uptake is important, and it relies on plant species and NH_4^+ concentrations (Yoneyama, Matsumaru, Usui, & Engelaar, 2001); fractionation of oxygen isotopes is mainly related to the amount of rainfall, water use efficiency, and evaporative effects in local areas (Korenaga, Musashi, Nakashita, & Suzuki, 2010). Moreover, there is a negative correlation between oxygen isotope values and relative humidity; when the relative humidity level is low, the oxygen isotopes in the organic matter of rice grains are greatly enhanced (Kaushal & Ghosh, 2018). Soil water conditions also exert a significant impact on rice. Mahindawansha et al. reported that in rice paddy systems, water uptake conditions could be reflected by stable isotopes in water (Mahindawansha et al., 2018). There was yet a positive linearity between $\delta^{18}O$ of rice grains and that of source water (Akamatsu, Suzuki, Nakashita, & Korenaga, 2014). Akamatsu et al. (2014) also revealed that the $\delta^{18}O$ value of rice exhibited a positive correlation with the mean minimum air temperature due to changes in the processes involved in amylose composition and translocation; lead isotopes ($^{207/206}Pb$ and $^{208/207}Pb$) produced as a result of soil mineralization reflected the origins of soil and geological ages, which increased the geographical resolution when the lithology varied according to regions (Armstrong, 1968; Capo, Stewart, & Chadwick, 1998; Zhang et al., 2002). To summarize, geographical isotopic fingerprints could serve as an efficient traceability tool for the origin of rice.

In food authenticity studies, chemometric statistical analysis is important in interpreting acquired data and generating a variable model for classifying samples of unknown origins (Oulhote, Le Bot, Deguen, & Glorennec, 2011). When inter-site differences are limited, it is not appropriate to use conventional univariate isotope discrimination analysis. Instead, multivariate principal component analysis (PCA) could be used for data improvement based on key principal components (PCs) that were preferentially selected for denoising and dimension reduction.

In this study, rice samples from six provinces in China (Heilongjiang, Jilin, Jiangsu, Zhejiang, Hunan and Guizhou) and four other Asian countries (Thailand, Malaysia, Philippines and Pakistan) were investigated and discriminated using multivariate stable isotope analysis. And then the index groups were screened and established for the geographical features of the selected samples based on chemometrics including PCA and discriminant analysis (DA), in order to set up a provenance prediction model and provide a sound technical solution for rice traceability.

2. Material and methods

2.1. Sample collection and preparation

A total of 189 authentic rice samples were collected from reliable sources. Among them, 55 were obtained from Thailand (TH), Malaysia (MA), Philippines (PH) and Pakistan (PA), and 134 were harvested from China (CH), including Heilongjiang (HLJ), Jilin (JL), Jiangsu (JS), Zhejiang (ZJ), Hunan (HN) and Guizhou (GZ) provinces. Detailed information about all the rice samples was listed in Table S1 (see Supplementary Material).

The samples were numbered according to their locations and varieties. The collected rice was dried at a constant temperature of 60 °C for 48 h to constant weight, and subsequently shelled. Ten grams of each sample were ground in a mortar to obtain fine powder, and then the tube was enclosed by tin for determination.

2.2. C, N, and O isotopes obtained by EA-IRMS

All isotope samples were prepared and then analyzed according to earlier works (Chen et al., 2016; Liu et al., 2019). Stable isotopes of $\delta^{13}C$, $\delta^{15}N$ and $\delta^{18}O$ were analyzed by an elemental analyzer interfaced with an isotope ratio mass spectrometry (EA-IRMS, Thermo Fisher Scientific, USA). In this study, $\delta^{13}C$ (‰) was relative to the international standard Vienna Pee Dee Belimnite (VPDB); $\delta^{15}N$ (‰) was associated with atmospheric AIR; $\delta^{18}O$ (‰) were correlated with Standard Mean Ocean Water (SMOW). The ratios of the stable isotopes were adjusted against the following international standard references: USGS24 (Graphite) and IAEA600 (Caffeine) for the $\delta^{13}C$ value; USGS43 (Indian Human Hair) and IAEA600 for the $\delta^{15}N$ value; USGS42 (Tibetan Human Hair) for the $\delta^{18}O$ values. The analytical precision was lower than $\pm$ 0.3‰ for O, $\pm$ 0.2‰ for both C and N.

2.3. Pb-isotope analysis by ICP-MS

The lead isotope content in rice samples was determined by microwave digestion combined with inductively coupled plasma mass spectrometry (ICP-MS, Thermo Fisher X-Series II, USA). Approximately 0.2 g of powdered rice was placed into a polytetrafluoroethylene digestive tube, 10 mL of 65% nitric acid and 1 mL of hydrogen peroxide solution were added, and then they were digested in a microwave digestion system (CEM Corp., America). The microwave power increased from 0 W to 1200 W in 10 min, and then digestion was performed at 1200 W for 30 min. Subsequently, the cooled digested liquid was diluted to a 50 mL solution with ultrapure water (> 18.2 MΩ). Finally, the contents of ^{206}Pb, ^{207}Pb and ^{208}Pb were determined by ICP-MS. The Pb-isotope ratios of $^{206}Pb/^{207}Pb$ and $^{208}Pb/^{206}Pb$ were measured, and the elemental concentrations were determined in kinetic energy discrimination (KED) mode. The instrumental precision were lower than $\pm$ 0.04% for the ratio mode.

2.4. Statistical analysis

The statistical analysis was carried out with SIMCA 14.1 software (Umetrics, Umea, Sweden). First, essential discriminant information was extracted from all of the variables using multivariate statistical analysis such as PCA or linear discriminant analysis (LDA) for clear classification. Second, the variables including ratios and concentrations were measured by instruments, and a data matrix was built based on column-wise normalization and mean centralization to homogenize all of the information. Third, signal de-noising, dimension reduction and unsupervised PCA were performed. Finally, a supervised LDA model was constructed by selecting the first several orthogonal PCs rather than raw variables, and the accuracy of the model was verified by holdout cross-validation. In this entire process, 90% of the samples were randomly selected to build the model by the Monte-Carlo method and the other 10% were utilized to validate the data.

3. Results and discussion

3.1. Stable isotope results for rice of different origins

3.1.1. $\delta^{13}C$, $\delta^{15}N$ and $\delta^{18}O$ isotopes

For stable isotope ratios of rice from different countries, As illustrated in Table 1A, except rice from TH (-27.3 ± 0.6‰), rice from PH (-30.5 ± 0.3‰), PA (-28.0 ± 0.8‰) and MA (-30.5 ± 0.7‰) had more negative $\delta^{13}C$ values when compared to rice from CH (-27.3 ± 0.7‰). The carbon isotope composition of plants ($^{13}C/^{12}C$) is mainly related to CO_2-photosynthetic fixing pathways, such as the C3 or C4 cycle, and its fractionation information is mapped to plant and animal tissues through the food chain (Dawson, Mambelli, Plamboeck, Templer, & Tu, 2002; Smith & Epstein, 1971). Due to the conventional practice of rice cultivation, the $\delta^{15}N$ values of

Table 1

(A) Stable isotope results of polished rice from imported different countries, and (B) Stable isotope results of rice from different production regions in China using EA-IRMS and ICP-MS, respectively.

(A)

Variable	Thailand (n = 20)	Malaysia (n = 22)	Pakistan (n = 6)	Philippines (n = 7)	China (n = 134)
$\delta^{13}C$/‰	-27.3 ± 0.6^c	-30.5 ± 0.7^a	-28.0 ± 0.8^b	-30.5 ± 0.3^a	-27.3 ± 0.7^c
$\delta^{15}N$/‰	5.1 ± 1.4^b	3.7 ± 1.1^a	4.9 ± 0.8^b	3.5 ± 0.5^a	5.8 ± 1.6^b
$\delta^{18}O$/‰	25.8 ± 0.9^c	21.5 ± 1.4^a	26.2 ± 2.9^c	22.6 ± 2.3^a	24.0 ± 1.8^b
$^{207}Pb/^{206}Pb$	1.00 ± 0.05	1.05 ± 0.11	0.98 ± 0.03	1.05 ± 0.19	0.96 ± 0.35
$^{208}Pb/^{207}Pb$	0.97 ± 0.04	0.97 ± 0.11	1.01 ± 0.03	0.79 ± 0.59	0.90 ± 0.75

(B)

Variable	Heilongjiang (n = 51)	Jilin (n = 4)	Jiangsu (n = 13)	Zhejiang (n = 10)	Hunan (n = 45)	Guizhou (n = 11)
$\delta^{13}C$/‰	-26.8 ± 0.4^c	-27.9 ± 0.5^{ab}	-26.7 ± 0.9^c	-28.2 ± 0.5^a	-27.7 ± 0.4^b	-28.0 ± 0.2^{ab}
$\delta^{15}N$/‰	$6.1 \pm 1.3b^c$	6.4 ± 1.4^c	5.0 ± 0.9^b	2.7 ± 0.8^a	6.2 ± 1.5^c	6.0 ± 0.9^{bc}
$\delta^{18}O$/‰	22.7 ± 0.7^b	21.0 ± 0.4^a	26.0 ± 0.5^d	24.7 ± 1.5^c	25.5 ± 0.9^d	22.0 ± 1.0^b
$^{207}Pb/^{206}Pb$	0.91 ± 0.56	0.93 ± 0.11	0.98 ± 0.03	0.98 ± 0.02	0.99 ± 0.12	1.03 ± 0.03
$^{208}Pb/^{207}Pb$	0.71 ± 1.17	0.95 ± 0.02	1.01 ± 0.02	1.01 ± 0.02	1.03 ± 0.30	1.00 ± 0.02

Note: Values represent means and standard deviations; different small letters represent significant different at $p = 0.05$ confidence level.

rice from the four countries (TH, MA, PA and PH) were all lower than those of the Chinese rice. The most striking comparison was between MA (3.7 ± 1.1‰), PH (3.5 ± 0.5‰) and CH (5.8 ± 1.6‰), which suggested more synthetic fertilizers were applied to MA and PH rice. Although the $\delta^{15}N$ values of rice from TH and PA (5.1 ± 1.4‰ and 4.9 ± 0.8‰, respectively) were not significantly different from those of CH, while this did not mean that synthetic fertilizers application in TH and PA was low, as samples from China were both conventional and organic. The nitrogen isotope in rice was generated by absorbing soil nutrients via the roots, mainly inorganic ions containing nitrogen, for example, NO_3^- and NH_4^+. The ions were transformed into plant proteins through biosynthesis. In addition, the $\delta^{15}N$ value of rice was extremely liable to agricultural practices, such as fertilizers (Kelly, Heaton, & Hoogewerff, 2005). Fertilizers synthesized by compressing air in the Haber method and those produced via biological nitrogen fixation were inclined to contribute to the $\delta^{15}N$ value by nearly 0‰ (N value in air) (Bateman & Kelly, 2007). Rice that was grown by adding more synthetic fertilizers would have a $\delta^{15}N$ value closer to 0‰ when compared to rice added with less synthetic fertilizers or organic manure. Other organic foods (e.g. fruits) have similar results (Camin et al., 2011). In the present study, the $\delta^{18}O$ values of rice from MA (21.5 ± 1.4‰) and PH (22.6 ± 2.3‰) were more negative than TH (25.8 ± 0.9‰), PA (26.2 ± 2.9‰) and CH (24.0 ± 1.8‰). Oxygen isotope in rice mostly arose from rain or irrigation water. A fractionation process altered the relative isotopic composition when the climate changed from warm to cool, the altitude changed from low to high, and the geographic feature changed from oceans to inland for water flows (Korenaga et al., 2010).

For stable isotope ratios of rice from different provinces of China, as illustrated in Table 1B, except rice from JS (−26.7 ± 0.9‰), the $\delta^{13}C$ average values of rice from the regions along Yangtze River (HN, GZ and JS) were more negative than the northeast production regions (HLJ and JL) of China. For the same variety of rice, the $\delta^{15}N$ values Japonica from HLJ and JL (organic farming) were more positive than JS, ZJ and GZ (conventional farming). The $\delta^{18}O$ values of rice from the mid-lower reaches of the Yangtze river (JS, ZJ and HN) were more positive than inland areas (HLJ, JL and GZ), The same result was also reported in the literature (Liu et al., 2019).

3.1.2. $^{206}Pb/^{207}Pb$ and $^{208}Pb/^{207}Pb$ ratios

The lead isotope is an important lithologic and mineral indicator, characterized by varied geological and soil conditions, and it could be employed for geographical traceability of agro-products.

Lead isotopes derived from a variety of countries showed vast disparities ($P < 0.05$) without any distinguishable rules to follow. The $^{206}Pb/^{207}Pb$ and $^{208}Pb/^{207}Pb$ ratios of rice harvested from the JS, ZJ, HN and GZ provinces of China were largely similar, while rice from HLJ and JL featured slightly lower Pb ratios, which might be contributed by similar geographic characteristics of the Yangtze River Basin where the JS, ZJ, HN and GZ provinces are located.

3.2. Stable isotope results for rice of different cultivars and farming methods

Rice was collected and sampled to investigate the indicator changes for different categories. All rice samples were divided into two categories, including indica and japonica, following the classification

Table 2

(A) Stable isotope results of polished rice for different varieties from imported different countries and rice from different production regions in China, and (B) Stable isotope results of polished rice for different varieties from different production regions in China.

(A)

	N		$\delta^{13}C$/‰	$\delta^{15}N$/‰	$\delta^{18}O$/‰	$^{206}Pb/^{207}Pb$	$^{208}Pb/^{207}Pb$
Japonica	76	Mean ± SD	-27.0 ± 0.6	5.8 ± 1.4^b	23.0 ± 1.5	0.94 ± 0.46	0.81 ± 0.96
Indica	113	Mean ± SD	-28.4 ± 1.5	5.1 ± 1.7^a	24.6 ± 2.1	1.00 ± 0.10	0.99 ± 0.24

(B)

	N		$\delta^{13}C$/‰	$\delta^{15}N$/‰	$\delta^{18}O$/‰	$^{206}Pb/^{207}Pb$	$^{208}Pb/^{207}Pb$
Japonica	76	Mean ± SD	-27.0 ± 0.6^b	5.8 ± 1.4	23.0 ± 1.5^a	0.94 ± 0.46	0.81 ± 0.96
Indica	58	Mean ± SD	-27.7 ± 0.7^a	5.8 ± 1.6	25.4 ± 1.0^b	0.99 ± 0.10	1.03 ± 0.26

Note: Different small letters represent significant difference at $p = 0.05$ confidence level.

Table 3
Stable isotope results of polished rice for different Planting Pattern from different production regions in China.

	N		$\delta^{13}C$/‰	$\delta^{15}N$/‰	$\delta^{18}O$/‰	$^{206}Pb/^{207}Pb$	$^{208}Pb/^{207}Pb$
C	74	Mean ± SD	− 27.7 ± 0.6[a]	5.2 ± 1.3[a]	24.9 ± 1.6[b]	0.99 ± 0.09	1.02 ± 0.23
O	60	Mean ± SD	− 26.8 ± 0.5[b]	6.5 ± 1.2[b]	22.9 ± 1.3[a]	0.93 ± 0.52	0.75 ± 1.08

Note: C: conventional practice; O: organic practice; different small letters represent significant difference at $p = 0.05$ confidence level.

method of the Japanese scholar Kato Masahiro. For the rice samples from different countries (Table 2A), there were significant differences in the $\delta^{15}N$ values for the two varieties of rice ($P < 0.05$), while the values of $\delta^{13}C$, $\delta^{18}O$, $^{206}Pb/^{207}Pb$ and $^{208}Pb/^{207}Pb$ did not shown significant differences among varieties. Among the stable isotopes of indica and japonica rice in China, $\delta^{13}C$ and $\delta^{18}O$ values of japonica rice were significantly lower than those of indica rice (Table 2B).

Rice in China was collected according to traditional and organic farming methods. After comparing the effects of different farming methods on stable isotopes (Table 3), we found that the values of $\delta^{13}C$, $\delta^{15}N$ and $\delta^{18}O$ of rice collected in traditional and organic farming methods were largely different, among which the $\delta^{15}N$ value of rice in organic cultivation was greatly higher when compared to the traditional farming method, showing a consistent result with the literature report (Yuan et al., 2018). This is because during the storage and processing of organic fertilizers, isotopes were fractionated from NH_3 volatilization so that $\delta^{15}N$ was generally enriched in organic fertilizers in comparison with synthetic fertilizers (Bateman, Kelly, & Woolfe, 2007; Rogers, 2008).

3.3. Principal component analysis of stable isotopes in rice

To visually understand the effects of isotope indexes on rice produced in different countries and different areas of China, PCA was carried out for the carbon, nitrogen, oxygen and lead isotopes in rice. The cumulative variance contribution rates of the first five PCs were shown in Fig. S1a and b, respectively. A scatter plot based on the first two PCs was shown in Fig. 1A to classify the samples from different countries. The first two PCs (PC1 and PC2) contributed 34.68% and 31.60% of the geochemical variances, respectively. MA/PH and CH/TH/PA samples could be differentiated with the combination of PC1 and PC2, but in contrast, MA and PH, as well as CH, TH, and PA samples were mixed and difficult to separate. Due to a large number of samples in China with their sources in both coastal and inland areas, their elevations, longitudes and latitudes spanned over a wide range, and it was difficult to identify their characteristics from other countries by PC2.

For samples derived from different provinces in China, the first two PCs (PC1 and PC2) contributed 35.24% and 25.71% of the geochemical variances, respectively. PC1 divided rice into two parts: South China region (JS, ZJ and HN) and northeastern region (HLJ and JL). Rice from the GZ province was indistinguishable from rice harvested from the northeastern region. From the perspective of the second PC, ZJ samples were generally separated from those in the northeastern and GZ provinces (Fig. 1B).

3.4. Discriminant analysis of stable isotopes in rice

In order to understand the role of each stable isotope index in the traceability of rice origins, DA was used to analyze the values of $\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$, $^{206}Pb/^{207}Pb$ and $^{208}Pb/^{207}Pb$ in rice in sequence. From the analytical results, the discrimination rates of single isotope indexes for rice producing areas were relatively low, with the highest being only 58.18% (Table S2a), when the isotopes were combined for DA, the discrimination rates of the isotope combinations for rice producing areas were obviously improved. The combination of the three isotopes of carbon, oxygen and nitrogen increased the discrimination rates of the origins to 78.43% (Table S2b). Therefore, the three isotope indexes of
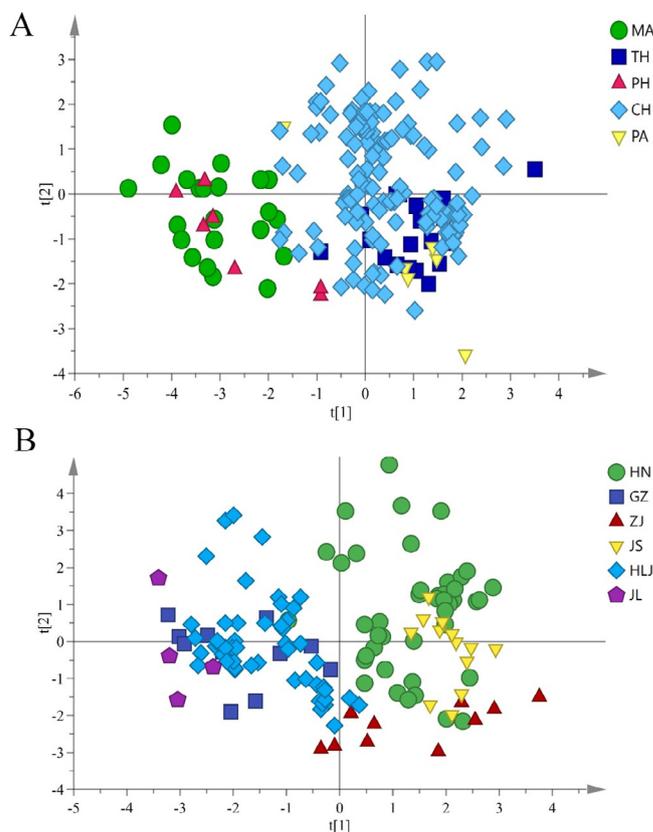

Fig. 1. PCA classification results of rice samples: (A) Scatter plot of the first two principal score components (PC1 and PC2) for polished rice from imported different countries; *Note:* TH – Thailand, MA – Malaysia, PH – Philippines, PA – Pakistan (PA) and CH-China. Some data tags were covered with each other due to the sample volume. (B) Scatter plot of the first two principal score components (PC1 and PC2) for polished rice from different production regions in China. *Note:* HLJ – Heilongjiang JL – Jilin, JS – Jiangsu, ZJ – Zhejiang, HN – Hunan, GZ – Guizhou. Some data tags were covered with each other due to the sample volume.

carbon, oxygen and nitrogen in rice were selected for the discriminant model of rice samples in different countries. The discriminant model established by these three indicators was used to test the rice samples from different countries, and the results were illustrated in Table 4A. Due to the wide range of sample sources in China, the correct discrimination rate is the lowest (58.21%).

According to the DA results, using any single element among the isotope indexes of $\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$, $^{206}Pb/^{207}Pb$ and $^{208}Pb/^{207}Pb$ in rice from different provinces could not achieve satisfactory discrimination effects. The correct discrimination rates of other isotope indexes were less than 45% except for carbon and oxygen (Table S3a). When various isotope indexes were combined for DA, the correct discrimination rate of the isotope combination used for the rice from different producing areas was significantly higher than that of single isotopes. The combination of the three isotopes of carbon, oxygen and nitrogen and the four-element combination of the carbon, oxygen, nitrogen and lead isotopes achieved correct discrimination rates of over 80% (Table S3b).

According to the significance test at the 0.01 level, three of the four

Table 4
(A) Discriminant accuracies of rice imported from different countries, and (B) Discriminant accuracies of polished rice from different production regions in China using LDA model.

(A)

Sample source	Discriminant attribution					Discriminant accuracy (%)
	Pakistan	Philippines	Malaysia	Thailand	China	
Pakistan	4	0	0	2	0	66.67
Philippines	0	7	0	0	0	100.00
Malaysia	0	2	17	0	3	77.27
Thailand	2	0	0	18	0	90.00
China	18	0	3	35	78	58.21

(B)

Sample source	Discriminant attribution						Discriminant accuracy (%)
	Guizhou	Heilongjiang	Hunan	Jilin	Jiangsu	Zhejiang	
Guizhou	9	2	0	0	0	0	81.82
Heilongjiang	1	50	0	0	0	0	98.04
Hunan	0	0	42	0	2	1	93.33
Jilin	0	1	0	3	0	0	75.00
Jiangsu	0	0	1	0	11	1	84.62
Zhejiang	0	0	0	0	2	8	80.00

indicators were introduced into the discriminant model in the sequence of δ^{13}C, δ^{18}O, δ^{15}N. Using the established discriminant model, the rice samples were back-tested. As we could see from Table 4B, 11 of the 134 samples were misjudged, and the overall discriminant rate was 85%.

4. Conclusion

In summary, this study demonstrated that there were significant differences in the values of δ^{13}C, δ^{15}N, δ^{18}O and Pb for rice from different countries and different provinces of China, which might be caused by climates, altitudes, latitudes longitudes, farming methods and rice varieties. In this study, according to the principal component analysis (PCA) results, δ^{13}C, δ^{15}N and δ^{18}O had relatively strong discrimination power for the geographical origins of rice, while the discriminative power of lead was relatively weak. In addition, the combination of δ^{13}C, δ^{15}N and δ^{18}O had a correct discriminating rate of 78.43% for rice cultivated in different countries, and that for different provinces in China was over 85%, which could be used as an effective indicator system for the traceability of rice origins. However, due to the limitation of the sample size, further correction and verification are required for the accuracy, reliability and practicability of the established discriminant model in the future.

Author contributions

Yan Zhao, Jishi Wang and Tianjin Chen designed the experiments and Tianjin Chen, Jishi Wang, and Weixing Zhang performed the experiments. Tianjin Chen and Jishi Wang analyzed the data. Shuming Yang, Ailiang Chen and Weixing Zhang contributed materials/analysis tools; Jishi Wang and Yan Zhao wrote and reviewed the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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