

Determination of the Geographic Origin of Rice by Chemometrics with Strontium and Lead Isotope Ratios and Multielement Concentrations

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S Supporting Information

ABSTRACT: The objective of this study was to develop a technique for determining the country of origin of rice in the Japanese market. The rice samples included a total of 350 products grown in Japan ($n = 200$), the United States ($n = 50$), China ($n = 50$), and Thailand ($n = 50$). In this study, $^{87}\text{Sr}/^{86}\text{Sr}$ and Pb isotope (^{204}Pb , ^{206}Pb , ^{207}Pb , and ^{208}Pb) ratios and multielement concentrations (Al, Fe, Co, Ni, Cu, Rb, Sr, and Ba) were determined by high-resolution inductively coupled plasma mass spectrometry. By combining three chemometric techniques based on different principles and determination criteria, the countries of origin of rice were determined. The predictions made by 10-fold cross-validation were around 97% accurate. The presented method demonstrated the effectiveness of determining the geographic origin of an agricultural product by combining several chemometric techniques using heavy element isotope ratios and multielement concentrations.

KEYWORDS: rice, geographic origin, chemometrics, inductively coupled plasma (ICP), isotope ratio, Sr, Pb, multielement

INTRODUCTION

Rice (*Oryza sativa* L.) is a major source of calories for human beings, and a staple food in many countries, particularly in Asia. The expansion of global trade has also promoted the international trade of rice. As the global economy expands, including in Asia, agricultural products are increasingly labeled with their geographical origin in many countries. Geographical identification can be useful for branding strategy purposes and to help consumers in their selection of foodstuffs. For rice, this information is important to consumers in some countries. In particular, labeling that identifies the country of origin is mandatory in Japan, where the population pays close attention to this information. However, such labeling has sometimes been used fraudulently for financial gain. Therefore, techniques to prevent consumers or producers from suffering financial damage are required.

Studies have been conducted worldwide with the aim of determining the geographic origin of agricultural products; to this end, techniques based on various principles have been attempted. The major factors used for origin determination have been mineral composition, obtained by inductively coupled plasma (ICP) optical emission spectrometry (OES),^{1,2} ICP mass spectrometry (MS),^{3,4} and X-ray fluorescence analysis,^{5,6} the isotope composition of light elements,^{7–9} such as H, C, N, O, and S, and the ratios of heavy elements, such as strontium (Sr)^{10,11} and lead (Pb).¹² Studies that make use of a combination of these techniques for greater accuracy have also been conducted.^{13–15} Among these factors, the isotope ratios of heavy elements Sr and Pb have some advantages over the other factors for determining geographic origin. The isotopes of heavy elements are hardly fractionated in the terrestrial ecosystem. There is no difference in the isotope ratio between parts of a crop,^{16,17} or between a

crop and the exchangeable fraction in the soil,^{16,18} as long as the crop is grown under the same soil and water conditions. Voerkelius et al. reported that a $^{87}\text{Sr}/^{86}\text{Sr}$ database of surface water could be used to predict the geographic origin of honey and wheat.¹⁹ Once a database of one crop has been constructed, it would be applicable to new crops grown in the same area. Therefore, the Sr isotope ratio is an effective factor that inherits the geological character of a production area, and many studies on the use of this factor have been reported.^{10,11,18}

Lead has been utilized in various applications by humans since ancient times. The total amount of anthropogenic lead released into the environment was estimated to be 17 times higher than that from natural sources by Nriagu and Pacyna.¹⁹ Komáreck estimated that this amount is at least from 1 to 2 orders of magnitude higher.²⁰ Bollhöfer and Rosman reported the Pb isotope compositions in aerosols collected from across the world between 1994 and 1999,^{21,22} and different regions were characterized by the geographical variations found.^{21,22} These reports suggested the possibility that anthropogenic lead would affect the Pb isotope compositions in crops, and differences among countries were expected to be found. In spite of these advantages of Pb isotope ratios, their application to determining the origin of agricultural products has been limited because of the difficulty caused by the low concentration in a sample and the ease of contamination. However, Médina et al. studied the influence of atmospheric Pb pollution on wine and concluded that Pb isotope composition was a promising tool

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for certifying wine authenticity by taking advantage of the high transferability of Pb from the ambient surroundings of vineyards into grapes and wine.²³

These techniques for determining origins usually include multivariate analyses or chemometrics because the combination of several components is needed. Linear discriminant analysis (LDA), which is a supervised parametric pattern recognition technique, is the most popular chemometric technique in this field. This technique is based on simple principles and can be used to construct linear functions for discrimination models. Since the technique is available in many chemometric application software packages and is effective for use in studies in this field, numerous studies using this technique have been reported.^{1–4} Soft independent modeling of class analogy (SIMCA) is also a supervised parametric pattern recognition technique and is a popular technique.^{3,23} However, the models are constructed on the basis of principal component analysis²⁴ and are more complex than those based on LDA, and application software available for the technique is limited. *k* nearest neighbors (KNN) is a supervised nonparametric pattern recognition technique and is also a popular technique.^{25,26} Since this technique does not hypothesize a statistical distribution among samples, it does not call for symmetry in the sample distribution. Because these pattern recognition techniques that construct models for origin determination are able to predict the origin of an unknown sample, they have been used as effective chemometric techniques in this field.

In studies of origin determination in rice, Yasui and Shindo determined the multielement concentration of brown rice by ICPOES and ICPMS and classified particular districts in Japan by combining the values obtained using chemometrics.²⁷ Oda et al. applied ¹¹B/¹⁰B and ⁸⁷Sr/⁸⁶Sr ratios to brown rice samples by ICPMS and multiple-collector ICPMS and concluded that their countries of origin could be distinguished.¹⁰ Korenaga et al. reported that a statistical analysis using light element isotope ratios ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{16}\text{O}$) could be a useful tool for determining the country of origin of rice.⁹ However, these studies neither were completed nor showed sufficient reliability for the prediction of unknown samples because of their small sample sizes.

The objective of this study is to develop a technique for determining the country of origin of rice in the Japanese market that could be put into practical use. In the past, determining the Pb isotope ratio of rice had been difficult, particularly for polished rice because of the low concentration (0.1–2 ng g⁻¹). However, the method proposed in a previous study was able to determine not only the Pb isotope ratios of polished rice but also the Sr isotope ratio.^{28,29} The method can also determine those values simultaneously and easily, compared with conventional methods for performing Sr isotope ratio analysis alone.^{28,29} In this study, we determined multielement concentrations as well as Sr and Pb isotope ratios using the method proposed in the previous study by high-resolution (HR) ICPMS^{28,29} and conducted chemometrics analyses using the data obtained to determine the country of origin of rice. A criterion for determination consisting of three kinds of chemometric techniques was constructed and evaluated the effectiveness of determining the country of origin.

MATERIALS AND METHODS

Samples. We used 350 samples of kernel rice (*O. sativa* L.). The production areas of the samples were 44 prefectures in Japan (*n* =

200), the United States (*n* = 50), China (*n* = 50), and Thailand (*n* = 50). Since more than 99.9% of the rice available in the Japanese market originated from these countries in the years that this study was conducted (2009–2010) according to the Trade Statistics of Japan from the Ministry of Finance of Japan, we targeted the rice from these countries. The Japanese rice samples were obtained from producers or producer groups. The samples from the other countries were obtained from ships carrying rice from abroad at ports in Japan. Therefore, the samples were from certain countries and reflect the general population of the Japanese market, which are very important in this field of study. The detailed geographic origins of samples from the United States, China, and Thailand were not clear in their countries, except that samples from the United States were all from California and those from China were all from the northern areas. Since the samples from Thailand were indica rice with a long grain shape, the samples could be distinguished by appearance from others which were japonica rice with a short grain shape. For application to powdered rice or crushed rice, we targeted rice from Thailand as well. The samples were kernel grains of polished and brown rice. The details of the samples are given in the Supporting Information because the effectiveness and reliability of discrimination models depend on the samples used. The production areas of the Japanese samples were distributed throughout Japan; 44 prefectures out of 47 were included. Each of the samples was taken from a different lot.

Determination of Isotope Ratios and Multielement Concentrations. Determinations of Sr and Pb isotope ratios were carried out by following the procedure described in a previous study.^{28,29} The rice samples were used in the form of kernels without pulverization. Polished rice was used for multielement determination. Brown rice, which contains Pb at a higher concentration than polished rice, was also used if it was available to prepare a sample solution with as high a Pb concentration as possible. Each sample contained either 2.5 g of polished rice and 7.5 g of brown rice or 10 g of polished rice alone. Out of each 10 g sample, 2.5 g was put in each of four digestion vessels (DigiTUBE, SCP Science, Quebec, Canada), was washed with ultrapure water, and underwent acid digestion with HNO₃ and H₂O₂. A 50 μL volume of yttrium standard solution (20 mg L⁻¹) was added as an internal standard for multielement determination to the digestion vessel of the polished rice sample. A total rice sample of 10 g distributed in four digestion vessels was digested using an acid digestion system with an automated thermal control unit (DigiPREP, SCP Science). The digested polished rice sample was dissolved in 8 mol L⁻¹ HNO₃ to make a volume of 2.5 mL. A 50 μL volume of the solution was transferred into a 5 mL vial and diluted 40-fold with 1% HNO₃. The rest of the polished rice solution and the three digested solutions were transferred into a centrifuge tube and diluted to a concentration of 8 mol L⁻¹ HNO₃ in a volume of 12 mL. The solution was cooled to room temperature and centrifuged at 3000 rpm for 10 min. The supernatant solution was loaded into a column packed with Sr resin (50–100 μm , Eichrom Technologies, Lisle, IL) for extraction chromatography to separate and concentrate Sr and Pb.^{30,31} In extraction chromatography, Sr was eluted with 1.5 mL of 0.05 mol L⁻¹ HNO₃²⁸ after passing through 0.05 mol L⁻¹ HNO₃ to gain a higher concentration. Lead was eluted with 1.5 mL of 6 mol L⁻¹ HCl²⁸ after passing through 0.5 mL of HCl to gain a higher concentration. In this extraction chromatography method, no isotope fractionation was observed.^{28,29} The concentrations of Sr and Pb in the eluted solutions were measured using an HR-ICPMS instrument (Element2, Thermo Fisher Scientific, Waltham, MA). The Sr and Pb eluted solutions were diluted to concentrations of 100–500 $\mu\text{g L}^{-1}$ using 0.05 mol L⁻¹ HNO₃ and 1–10 $\mu\text{g L}^{-1}$ using 6 mol L⁻¹ HCl, respectively, to match those of the standard solutions.

The ⁸⁷Sr/⁸⁶Sr, ²⁰⁴Pb/²⁰⁶Pb, ²⁰⁷Pb/²⁰⁶Pb, and ²⁰⁸Pb/²⁰⁶Pb ratios were determined by HR-ICPMS following the procedure described in the previous study.^{28,29} In the isotope ratio measurements, standard reference material (SRM) 987 (National Institute of Standards and Technology (NIST), Gaithersburg, MD) for Sr and SRM 981 (NIST) for Pb were repeatedly measured until stable values were observed before the sample measurements were taken, and they were also measured after the sample measurements. The Sr and Pb isotope ratios

of the samples were calculated by linear correction using the obtained values of the SRMs.^{28,29} The concentrations of Al, Fe, Co, Ni, Cu, Rb, Sr, and Ba in the polished rice samples were determined using the same HR-ICPMS instrument by measuring the digested solutions diluted to 1/40 without extraction chromatography. The conditions for a multielement measurement were adjusted to produce the best performance once a day before a series of sample measurements in the following conditions: radio frequency (rf) power 1200–1300 W, sample gas flow rate 1.00–1.02 L min⁻¹, auxiliary gas flow rate 1.00 L min⁻¹, and cooling gas flow rate 16 L min⁻¹. The resolution performances employed for target isotopes were 300 for ⁸⁵Rb, ⁸⁸Sr, ⁸⁹Y, and ¹³⁷Ba and 4000 for ²⁷Al, ⁵⁶Fe, ⁵⁹Co, ⁶⁰Ni, ⁶³Cu, and ⁸⁹Y. Spectral interferences were avoided by performing measurements under these resolutions. The measurements were carried out by the internal standard method using γ . Calibration curves were constructed with three or four levels of multielement standard solutions including zero concentrations, depending on the concentrations. Each sample was analyzed just once.

The Sr and Pb concentrations of blank solutions for isotope ratio measurements were typically <2 and 0.1–0.2 $\mu\text{g L}^{-1}$, respectively. The Sr concentrations of extracted sample solutions were typically 100–500 $\mu\text{g L}^{-1}$. The Pb concentrations of extracted sample solutions were typically 1–10 $\mu\text{g L}^{-1}$. The bias of ⁸⁷Sr/⁸⁶Sr ratios estimated from blank values was typically <0.006%, because the isotope ratio of the blank solution was almost the same as the average value of rice (ca. 0.709). The biases of the Pb isotope ratios estimated from blank values were typically <0.04% for Japanese rice and <0.06% for rice from other countries, because the isotope ratios of blank solutions were close to that of Japanese rice.

The uncertainties obtained from interday variances of analyses carried out seven times on separate days at the same laboratory using rice powder samples were 0.036%, 0.53%, 0.21%, and 0.17% as RSD for ⁸⁷Sr/⁸⁶Sr, ²⁰⁴Pb/²⁰⁶Pb, ²⁰⁷Pb/²⁰⁶Pb, and ²⁰⁸Pb/²⁰⁶Pb ratios, respectively²⁹ and 19%, 4.5%, 6.1%, 8.9%, 5.6%, 6.3%, 7.9%, and 10% as RSD for Al, Fe, Co, Ni, Cu, Rb, Sr, and Ba concentrations, respectively.

Chemometrics. One-way analysis of variance (ANOVA) was carried out to test the differences between mean values using the application software Statistica Pro (StatSoft Japan, Tokyo, Japan). The ANOVA was performed using the Kuruskal–Wallis method as a nonparametric analysis because many elements did not follow a normal distribution.

Principal component analysis (PCA) was carried out using the application software Statistica Pro. The data used were the standardized values for all the obtained data: ⁸⁷Sr/⁸⁶Sr, ²⁰⁴Pb/²⁰⁶Pb, ²⁰⁷Pb/²⁰⁶Pb, and ²⁰⁸Pb/²⁰⁶Pb ratios and Al, Fe, Co, Ni, Cu, Rb, Sr, and Ba concentrations.

Discrimination models of LDA were constructed for classifying rice from Japan, the United States, China, and Thailand using the application software Statistica Pro. All of the obtained data, namely, ⁸⁷Sr/⁸⁶Sr, ²⁰⁴Pb/²⁰⁶Pb, ²⁰⁷Pb/²⁰⁶Pb, and ²⁰⁸Pb/²⁰⁶Pb ratios and Al, Fe, Co, Ni, Cu, Rb, Sr, and Ba concentrations, were used as variables for modeling by LDA. Discrimination models of SIMCA were constructed in the same way using the application software Pirouette (version 2, Informetrix, Bothell, WA). This technique establishes a principal component model that is called a SIMCA box for each class. Next an unknown case is applied to each model and classified. In this study, we classified it into the class that it had the highest probability of belonging to. The probability is obtained from the F factor, which is the square of the distance from a class divided by the total residual variance of the class. Discrimination models of KNN were constructed in the same way using the application software Pirouette. An adequate value of k was established automatically by running the software.

RESULTS AND DISCUSSION

Multielement Concentrations. The concentrations of many elements were significantly different ($p < 0.05$) between countries as determined by multiple comparisons (Table 1). As a distinctive trend, the concentrations of Rb, Sr, Al, Fe, Co, and

Table 1. Analytical Results of Rice Samples from Each Country and Results of Multiple Comparisons between Countries^a

	Japan ($n = 200$), median	United States ($n = 50$), median	China ($n = 50$), median	Thailand ($n = 50$), median
[Rb]/ $\mu\text{g g}^{-1}$	0.82 a	1.85 b	3.17 c	4.17 c
[Sr]/ $\mu\text{g g}^{-1}$	0.040 a	0.087 b	0.084 b	0.072 b
[Ba]/ $\mu\text{g g}^{-1}$	0.048 a	0.027 b	0.028 ab	0.124 c
[Al]/ $\mu\text{g g}^{-1}$	0.048 a	0.102 b	0.106 b	0.092 b
[Fe]/ $\mu\text{g g}^{-1}$	0.84 a	1.04 b	1.08 b	0.87 a
[Co]/ $\mu\text{g g}^{-1}$	0.0025 a	0.0044 b	0.0034 b	0.0105 c
[Ni]/ $\mu\text{g g}^{-1}$	0.073 a	0.183 b	0.116 c	0.109 d
[Cu]/ $\mu\text{g g}^{-1}$	1.64 a	1.88 b	1.73 b	1.15 c
⁸⁷ Sr/ ⁸⁶ Sr	0.7079 a	0.7059 b	0.7103 c	0.7129 d
²⁰⁴ Pb/ ²⁰⁶ Pb	0.05517 a	0.05418 b	0.05495 a	0.05540 a
²⁰⁷ Pb/ ²⁰⁶ Pb	0.8615 a	0.8476 b	0.8584 a	0.8661 c
²⁰⁸ Pb/ ²⁰⁶ Pb	2.106 a	2.072 b	2.104 a	2.115 c

^aDifferent letters mean significantly different results ($p < 0.05$).

Ni in the Japanese samples were lower than those from the other three countries. The concentrations of Rb, Ba, Al, and Co in Thai samples were higher than those from the other three countries. In other words, Japanese rice had low element contents and Thai rice had high element contents. The difference in rainfall, soil type, cultivation environment, or cultivation method might affect these results.^{30,31}

⁸⁷Sr/⁸⁶Sr Ratio. The ⁸⁷Sr/⁸⁶Sr ratios of rice samples were significantly different between different countries of origin ($p < 0.05$) (Table 1). Since these observed values were likely reflected in the geochronological and lithological characteristics of the grown areas, the value was considered to be effective for discriminating the country of origin of rice. Because plots of ⁸⁷Sr/⁸⁶Sr ratio vs Sr concentration and ⁸⁷Sr/⁸⁶Sr ratio vs Rb concentration had particular distributions on samples from each country, these plots were constructed in Figures 1 and 2,

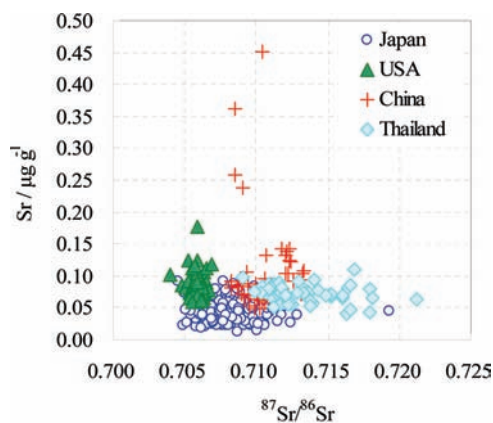


Figure 1. ⁸⁷Sr/⁸⁶Sr vs Sr concentration for rice samples.

respectively. Samples from the United States were distributed over small areas in both plots. The reason for this was thought to be that the samples were grown in limited farm fields in California. The low ⁸⁷Sr/⁸⁶Sr ratios were derived from the relatively young Mesozoic batholiths.³² The ⁸⁷Sr/⁸⁶Sr ratio of rice is mainly reflected in the exchangeable Sr of soil derived from bedrock or sedimentary rock formed by the sedimentation of materials at the earth's surface, because the concentration in

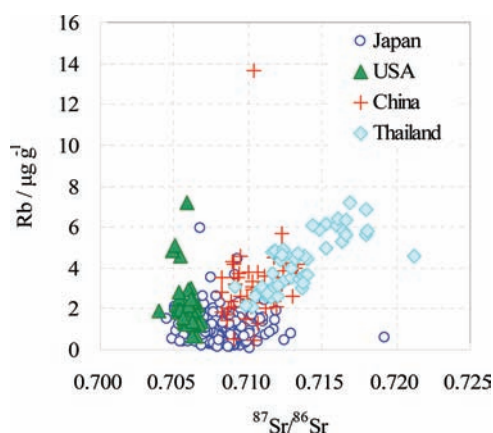


Figure 2. $^{87}\text{Sr}/^{86}\text{Sr}$ vs Rb concentration for rice samples.

soil is high enough not to be influenced by fertilizers or possible anthropogenic contamination. Kawasaki et al. reported that Japanese brown rice had $^{87}\text{Sr}/^{86}\text{Sr}$ ratios of 0.706–0.711 ($n = 44$).³³ In this study, many Japanese samples (92.5%) were within this range, but several Japanese samples had values higher (3%) or lower (4.5%) than this range, whereas Kawasaki et al. reported that Californian brown rice had $^{87}\text{Sr}/^{86}\text{Sr}$ ratios of 0.703–0.707 ($n = 15$),³³ and those of all the Californian rice used in this study were also within this range (0.704–0.707). The samples from China had $^{87}\text{Sr}/^{86}\text{Sr}$ ratios of 0.708–0.713. The values were within a relatively limited range considering the samples from China were distributed over a vast land area. The reason for this was that all of the samples were grown in the northern areas of China, although the specific provenance of each sample was unknown. The samples from Thailand had $^{87}\text{Sr}/^{86}\text{Sr}$ ratios of 0.709–0.721. The values were high and had a wide range; the samples were expected to be grown in various areas in the country.

Pb Isotope Composition. In this study, since we observed four Pb isotopes (^{204}Pb , ^{206}Pb , ^{207}Pb , and ^{208}Pb), we could obtain six isotope ratios, except for replacements between the numerator and denominator. To visualize the difference in Pb isotope ratios between countries, we were able to construct 15 two-dimensional plots comprising these ratios. Plots of $^{208}\text{Pb}/^{206}\text{Pb}$ vs $^{208}\text{Pb}/^{204}\text{Pb}$ ratios and $^{208}\text{Pb}/^{206}\text{Pb}$ vs $^{206}\text{Pb}/^{207}\text{Pb}$ ratios are shown in Figure 3. The samples from Japan were distributed in a small area except for one sample, even though the samples were collected from all over Japan. The outlier sample might have been contaminated during cultivation for some reason. The distribution of Japanese rice was in the same area as those of Japanese barley and wheat; there were no differences between different crops.²⁸ The samples from the United States showed a distribution quite different from those of the other samples. This result showed that the Pb isotope composition was effective for discriminating Californian rice from rice grown in Japan, China, and Thailand that were available in the Japanese market. The samples from China and Thailand overlapped greatly with Japanese samples.

The Pb isotope compositions of aerosols in Japan and China, reported by Bollhöfer and Rosman, were in the same region as those of the rice samples from both countries obtained in this study.²³ Since the Pb isotope composition of rice is considered to be a reflection of anthropogenic sources, it is possible that aerosols in both countries might have affected the Pb isotope compositions of rice grown in both countries. The overlap of

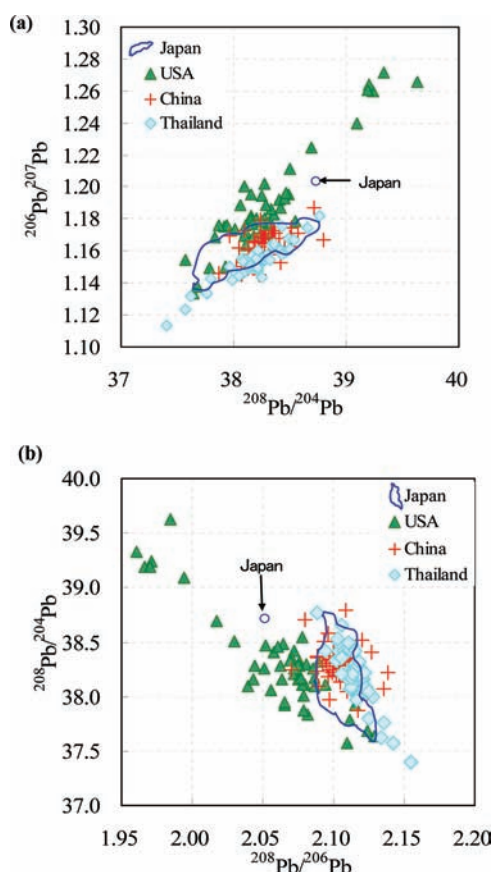


Figure 3. (a) $^{208}\text{Pb}/^{204}\text{Pb}$ vs $^{206}\text{Pb}/^{207}\text{Pb}$ and (b) $^{208}\text{Pb}/^{206}\text{Pb}$ vs $^{208}\text{Pb}/^{204}\text{Pb}$ for rice samples. Japanese samples are within the closed curve.

samples from Thailand as well as Japan and China might be caused by the similarity of the Pb isotope compositions between the anthropogenic sources in these countries. The Californian rice samples used in this study had a wider range of Pb isotope compositions than those of aerosols reported by Bollhöfer and Rosman, and the $^{206}\text{Pb}/^{207}\text{Pb}$ and $^{208}\text{Pb}/^{207}\text{Pb}$ ratios of the rice samples ranged from the values of aerosols collected in 1997–1999 to much higher values.²³ The anthropogenic sources in California might have changed.

Principal Component Analysis. A plot of principal component (PC) 1 and PC 2 obtained by PCA is shown in Figure 4. The plot reflects the features of rice grown in each country. The rice samples from Thailand had high values for PC 1 and PC 2, and the rice samples from the United States had low values. The rice samples from China had values between those from Thailand and the United States, and the rice samples from Japan had low values for PC 2. PC 1 had a 30% contribution ratio and high factor loadings on the $^{204}\text{Pb}/^{206}\text{Pb}$, $^{207}\text{Pb}/^{206}\text{Pb}$, and $^{208}\text{Pb}/^{206}\text{Pb}$ ratios (Table 2). The high PC 1 values for samples from Thailand and the low values for the United States were reflected by high and low values, respectively, of these components. PC 2 had a 25% contribution ratio and high factor loadings on the $^{87}\text{Sr}/^{86}\text{Sr}$ ratio and Rb, Ba, and Co concentrations (Table 2). The high PC 2 values of rice samples from Thailand and the low values for Japan were reflected by high and low values, respectively, of these components. From these results, the $^{87}\text{Sr}/^{86}\text{Sr}$, $^{204}\text{Pb}/^{206}\text{Pb}$, $^{207}\text{Pb}/^{206}\text{Pb}$, and $^{208}\text{Pb}/^{206}\text{Pb}$ ratios and Rb, Ba,

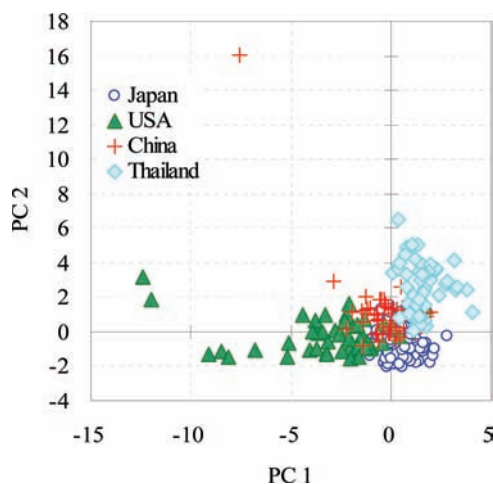


Figure 4. PC 1 vs PC 2 by PCA for rice samples.

Table 2. Factor Loading of Each Component on PC 1 and PC 2

	PC 1, contribution ratio 30%	PC 2, contribution ratio 25%
Rb	0.00022	0.24835
Sr	0.04131	0.11538
Ba	0.00914	0.15374
Al	0.09134	0.07596
Fe	0.04462	0.10131
Co	0.00529	0.14594
Ni	0.03576	0.00522
Cu	0.03925	0.02042
$^{87}\text{Sr}/^{86}\text{Sr}$	0.04320	0.12725
$^{204}\text{Pb}/^{206}\text{Pb}$	0.23158	0.00480
$^{207}\text{Pb}/^{206}\text{Pb}$	0.23513	0.00145
$^{208}\text{Pb}/^{206}\text{Pb}$	0.22317	0.00018

and Co concentrations were considered to be effective for determining the geographic origin.

Determination of Production Country by Chemometrics. LDA, SIMCA, and KNN were carried out as chemometric techniques, and their discrimination models were constructed to determine the production country using the obtained data of $^{87}\text{Sr}/^{86}\text{Sr}$, $^{204}\text{Pb}/^{206}\text{Pb}$, $^{207}\text{Pb}/^{206}\text{Pb}$, and $^{208}\text{Pb}/^{206}\text{Pb}$ ratios and Al, Fe, Co, Ni, Cu, Rb, Sr, and Ba concentrations. Since each chemometric technique is based on a different criterion, the combination of the techniques was expected to be effective. The criterion used in combination with the three kinds of chemometric techniques was as follows: the country determined by more than one chemometric technique was determined to be the country of origin. The results of 10-fold cross-validations by LDA, SIMCA, and KNN and their combination are shown in Table 3. Each technique had advantages and disadvantages for each country, and they also differed in terms of the frequency of type I errors and type II errors. In the 10-fold cross-validation, all SIMCA models had four factors, and the KNN models had different numbers of factors ranging from one to eight by model. The different numbers of factors were derived from differences in the sample data. However, the models with those numbers of factors showed different total error rates at a maximum of 3% for modeling samples. Variation of the error rate within 3% was expected by KNN modeling using different sample data.

Table 3. Ten-fold Cross-Validation Results (%) by Chemometric Analyses

country		LDA	SIMCA	KNN	combination ^a
Japan ($n = 200$)	type I error	3	10	2	3
	type II error	3	1	3	1
United States ($n = 50$)	type I error	4	8	10	2
	type II error	1	0	1	0
China ($n = 50$)	type I error	12	6	18	10
	type II error	4	8	1	1
Thailand ($n = 50$)	type I error	18	6	0	0
	type II error	1	1	2	1
total ($n = 350$)	error	7	8	5	3
	hit	93	92	95	97

^aThe country determined by more than one chemometric technique was determined to be the country of origin.

For the Japanese samples, LDA and KNN showed error rates of only 2–3%, but SIMCA showed a high type I error rate (10%), although it had a low type II error rate (1%). The combination of these techniques performed well, as the type I and type II error rates were 3% and 1%, respectively. The outlier Japanese sample in the Sr isotope ratio (Figure 1) was misclassified as rice from Thailand, but the plot of $^{87}\text{Sr}/^{86}\text{Sr}$ ratio vs Rb concentration was far from the areas not only in Japan but also Thailand. Although the low Rb concentration was too low to be rice from Thailand, the total composition of the sample was within Thailand. By constructing a database of outliers and particular criteria by which to define outliers, misclassification can be decreased. For the samples from the United States, LDA performed well, as the type I and type II error rates were 4% and 1%, respectively. Each technique had a low type II error rate (0–1%), so rice from the countries other than the United States will rarely be misclassified as coming from the United States. The combination performed well, as the type I and type II error rates were 2 and 0%, respectively, due to a synergy effect. For the samples from China, the performance was not particularly good, with KNN showing a particularly high type I error rate (18%). The combination showed type I and type II error rates of 10 and 1%, respectively. The high type I error rate means that some Chinese rice (10%) might be misclassified as having been produced in other countries, although the low type II error means that rice from countries other than China will rarely be misclassified as having originated in China. The outlier Chinese sample with a high Rb concentration (Figure 2) was not determined to be Chinese rice, since the value was too high for Chinese rice. For the samples from Thailand, the performance differed greatly between the chemometric techniques used. LDA showed a poor performance, as the type I and type II error rates were 18% and 1%, respectively. KNN also showed a good performance, as the type I and type II error rates were 0% and 2%, respectively. The combination showed an excellent performance as the type I and type II error rates were 0% and 1%, respectively. Overall, the performance of the combination method was good, with an accuracy rate of about 97%. Better performance was demonstrated by the combination of techniques than by the use of a single chemometric technique.

Each chemometric technique showed a particular performance as stated above. LDA and SIMCA are supervised parametric pattern recognition techniques; models constructed by LDA are simple linear functions, and models constructed by

SIMCA are more complex. LDA classifies samples by the plane surface, and SIMCA classifies samples on the basis of their distance from a model representing each country. KNN is a supervised nonparametric pattern recognition technique. To determine the origin accurately in KNN, sample data for a country are not required to show a normal distribution. Differences between the performances of the chemometric techniques were likely dependent on the distribution of sample data for each country. Some Chinese samples were somewhat inconsistent with the major Chinese distribution as seen in Figures 1–3. The distributions of data were broad in spite of the limited sample number, and many were not normal distributions. This was likely the reason that chemometric analyses performed poorly. For the samples from the United States, KNN did not perform well. The reason may have been that some sample data showed discrete distributions. For the samples from Thailand, LDA showed a good performance, but KNN showed a poor performance. This was because many of the sample data did not have normal distributions, but the distributions were continuous. The combination of chemometric techniques showed a synergy effect, and the error rates obtained by the combination were around the lowest error rates of each of the three single techniques.

In conclusion, the combination of three chemometric techniques based on different principles worked well enough. The method was about 97% accurate according to 10-fold cross-validation, although the performances of the techniques differed by country. Heavy element isotope ratios are reliable and effective factors for use in this field. However, Sr and Pb isotope composition, by coincidence, did not show clear differences between the target countries in this study. Those isotope compositions will be effective when other countries with different isotope ratios are targeted, as in the previous study on barley and wheat which targeted Australia and Canada.²⁹ In this study, the concentrations of eight elements were utilized as factors for more accurate determination. To improve the reliability and accuracy of the method, the utilization of many more elements can be effective at the sacrifice of high operability. Operability also can be important depending on the intended use. The method used in this study has high operability, the results of which can be obtained by HR-ICPMS alone in 3–4 days. This analytical method and the combination of chemometrics in the manner presented in this study can be applied to other agricultural products, although it would be important to select adequate elements in addition to Sr and Pb isotope ratios depending on the purpose.

■ ASSOCIATED CONTENT

■ Supporting Information

Details of the samples used in this study (Tables S1–S4). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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