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Geographic authentication of Asian rice (*Oryza sativa* L.) using multi-elemental and stable isotopic data combined with multivariate analysis

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ABSTRACT

Rice (*Oryza sativa* L.) is the world's third largest food crop after wheat and corn. Geographic authentication of rice has recently emerged as an important issue for enhancing human health via food safety and quality assurance. Here, we aimed to discriminate rice of six Asian countries through geographic authentication using combinations of elemental/isotopic composition analysis and chemometric techniques. Principal components analysis could distinguish samples cultivated from most countries, except for those cultivated in the Philippines and Japan. Furthermore, orthogonal projection to latent structure-discriminant analysis provided clear discrimination between rice cultivated in Korea and other countries. The major common variables responsible for differentiation in these models were δ^{34}S, Mn, and Mg. Our findings contribute to understanding the variations of elemental and isotopic compositions in rice depending on geographic origins, and offer valuable insight into the control of fraudulent labeling regarding the geographic origins of rice traded among Asian countries.

1. Introduction

Accurate determination of geographic authentication from mislabeling, falsification, and adulteration of foodstuffs is a critical issue for consumers, producers, and industries at all levels of the food production process worldwide for improving food safety and quality assurance (Lo Feudo, Naccarato, Sindona, & Tagarelli, 2010; Longobardi, Sacco, Casiello, Ventrella, & Sacco, 2015). The geographic authentication of agroproducts traded between countries in the European Union (EU) has been officially classified by the EU Council regulation as follows (Council regulation (EEC) No. 2081/92, 1992): Protected Denomination of Origin (PDO), Protected Geographical Indication (PGI), and Traditional Specialty Guaranteed (TSG). Cheese, beer, and meat products are the top three agroproducts registered as PDO, PGI, and TSG products in the EU, accounting for 73% of all EU products registered (Drivelos & Georgiou, 2012).

In Korea, the Agricultural Food Country of Origin Labeling system has been implemented with the accordance of the Foreign Trade Act of July 1991 to establish fair trade orders, and to protect both the agricultural producers and consumers from illegal distribution practices such as the indiscriminate importation of cheap foreign agricultural products that are disguised as domestic products. Furthermore, the Korean Geographical Identification and Agricultural Products Traceability certification systems were created to manage the safety and quality assurance of agroproducts in accordance with the Agricultural Products Quality Assurance Act established in 2002 (Ministry of Agriculture Food and Rural Affairs, 2016). In addition, labeling of the geographic origin of rice in Japan is obligatory to protect consumers and farmers from fraudulent labeling or imitation. The Japanese Agricultural Standard Law requires that certain information be included on the label, including the cultivation region, rice cultivar, and cultivation year of the polished rice produced and sold in Japan (Ariyama, Shinozaki, & Kawasaki, 2012; Suzuki, Chikaraishi, Ogawa, Ohkouchi, & Korenaga, 2008). Moreover, other agroproducts in Japan are voluntarily introduced from each region and each item, which has led to implementation of a product traceability field movement based on the National Agricultural Cooperative Federation.

Rice (*Oryza sativa* L.) is a valuable food crop used as a main source of calories by approximately 50% of the world's population (Ariyama et al., 2012). However, since a straightforward image/visual analysis of agroproducts, including rice cereal, does not provide the authenticity of their geographic origin, development of reliable analytical methods for

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validating the geographical authentication is an issue of increasing concern for consumers, producers, and industries in order to best regulate and standardize food safety and quality assurance (Suzuki et al., 2008). To date, discrimination of the rice geographic origin has mostly been performed using mass spectrometry (MS)-based techniques combined with chemometrics, including isotope ratio MS (IRMS) (Chung, Kim, Prabakaran, Yang, & Kim, 2016; Kelly et al., 2002; Suzuki et al., 2008; Wu et al., 2015), inductively-coupled plasma MS (ICP-MS) (Ariyama et al., 2012; Cheajesadagul, Arnaudguilhem, Shiowatana, Siripinyanond, & Szpunar, 2013; Kawasaki, Oda, & Hirata, 2002; Lagad, Singh, & Rai, 2017; Maione, Batista, Campiglia, Barbosa, & Barbosa, 2016; Song, Ryu, Shin, & Lee, 2014), ICP-optical emission spectrometry (OES) (Chung, Kim, Lee, & Kim, 2015; Gonzálvez, Armenta, & de la Guardia, 2011), and combinations of these techniques (Kelly et al., 2002; Yasui & Shindoh, 2000).

However, the accurate discrimination of rice geographical authentication remains a critical issue because of the unaccounted influences of genetic and environmental variations (Cheajesadagul et al., 2013; Gonzálvez et al., 2011).

Therefore, the aim of this study was to examine valuable elemental or isotope indicators for the determination of geographical authentication for rice samples collected from six Asian countries. The light-element (C, N, O, S) isotope ratios and compositions of 25 elements in rice were measured by IRMS and ICP-MS, respectively. Furthermore, to evaluate the discriminative power of the chemical markers measured in this study, the results were analyzed using chemometric techniques, including principal components analysis (PCA) and orthogonal projection to latent structure-discriminant analysis (OPLS-DA). Hence, this study demonstrates the feasibility of using the combination of multi-elements and light-element isotope ratios to discriminate rice geographic origins in Asian countries, which is the most predominant rice production and consumption region in the world. Moreover, these results can be useful for the regulation and management of the improper or fraudulent labeling of the geographic origin of rice traded among Asian countries, with potential broader applications for other countries.

2. Materials and methods

2.1. Rice samples, chemicals, and reagents

A total of 59 rice samples were obtained from Cambodia ($n = 14$), China ($n = 6$), Japan ($n = 10$), Korea ($n = 12$), the Philippines ($n = 13$), and Thailand ($n = 4$) from agricultural fields or local markets in 2015. The rice samples evaluated in this study were either non-glutinous rice varieties or F1 hybrid types and were characterized as either a brown rice or a milled rice type. Furthermore, all of the rice samples collected from China, Japan, and Korea were the japonica type, whereas those collected from other countries were the indica type (Fig. 1). The collected rice samples were stored in a freezer at −70 °C until the measurements of elements and stable isotope ratios.

Nitric acid (HNO_3), hydrogen peroxide (H_2O_2) solution, and ultrapure water for the trace analysis were purchased from Sigma-Aldrich Korea (*Trace*SELECT level for trace analysis, Seoul, Korea). Multi-element calibration standards of 10 $mg{\cdot}L^{-1}$ (part #: 8500–6942 and 8500–6940), including ^{9}Be, ^{11}B, ^{23}Na, ^{24}Mg, ^{27}Al, ^{39}K, ^{43}Ca, ^{48}Ti, ^{51}V, ^{52}Cr, ^{55}Mn, ^{56}Fe, ^{59}Co, ^{60}Ni, ^{65}Cu, ^{66}Zn, ^{75}As, ^{78}Se, ^{88}Sr, ^{98}Mo, ^{107}Ag, ^{114}Cd, ^{138}Ba, ^{205}Tl, and ^{208}Pb, were purchased from Agilent Technology (Seoul, Korea). Furthermore, the internal standard mixture solution of 10 $mg{\cdot}L^{-1}$ (part #: 5186-4681), including ^{45}Sc and ^{159}Tb, was obtained from Agilent Technology.

2.2. Sample preparation and IRMS measurements

All rice samples were lyophilized at −45 °C for 3 days and then grinded with a pulverizer (AGM-05, Angel Electronics Industry Co., Ltd., Gyeonggi-do, Korea) to obtain a fine powder (< 400 μm particle size) prior to the analyses of multi-elements and stable isotope ratios. The rice powder was enclosed in a tin capsule (Sn, 3.5 mm × 17 mm, IVA Analysentechnik e. K., Dusseldorf, Germany) for determinations of the C, N, and S stable isotope ratios (δ^{13}C, δ^{15}N, δ^{34}S) or in a silver capsule (Ag, 3.5 mm × 5.0 mm, Elemental Microanalysis, Okehampton, UK) for determination of the O stable isotope ratio (δ^{18}O). To obtain reliable measurements, ~5 mg rice powder was used for simultaneous δ^{13}C and δ^{15}N analyses, ~0.2 mg was used for δ^{18}O analysis, and ~20 mg was used for δ^{34}S analysis. The encapsulated rice samples were placed in a desiccator until the measurements of the C, N, O, and S stable isotope ratios were conducted (Chung et al., 2016).

Simultaneous measurement of δ^{13}C and δ^{15}N in rice was conducted on a PDZ Europa 20-20 isotope ratio mass spectrometer (IRMS, Sercon Ltd., Cheshire, UK) linked to a PDZ Europa ANCA-GSL elemental analyzer (Sercon Ltd.). The δ^{18}O in rice was measured on an Elementar PyroCube system (Elementar Analysensysteme GmbH, Hanau, Germany) interfaced with a PDZ Europa 20-20 IRMS. The δ^{34}S in rice was investigated with an Elementar vario ISOTOPE cube and a pre-concentration unit interfaced with a continuous-flow SerCon 20-22 IRMS (Sercon Ltd.). For scale normalization and quality assurance, some reference materials with similar isotope ratios against the rice samples measured in this study were also analyzed together with the rice samples of interest during the IRMS analysis. The final C, N, O, and S isotopic ratios of the rice samples are expressed as parts per thousand (‰) against the international reference standards as follows (Sharp, 2007): δ^{13}C, δ^{15}N, δ^{18}O, or $\delta^{34}S_{\text{unknown}} = [(R_{\text{unknown}} - R_{\text{standard}})/R_{\text{standard}}]$, where R_{unknown} is the C, N, O, or S stable isotopic ratio of the unknown sample (rice samples in this study) and R_{standard} are those of the international reference standards (Vienna PeeDee Belemnite for C, atmospheric N_2 for N, Vienna Standard Mean Ocean Water for O, and Vienna Canyon Diablo Troilite for S isotope ratios). In this study, the analytical precision (± standard deviation) based on the analysis of the laboratory reference materials was found to be ≤ ± 0.3‰ for δ^{13}C, ≤ ± 0.2‰ for δ^{15}N, ≤ ± 0.3‰ for δ^{18}O, and ≤ ± 0.3‰ for δ^{34}S measurements.

2.3. Sample digestion and ICP-MS measurement

Rice sample digestion was conducted prior to ICP-MS analysis using a previously reported method based on the microwave-assisted digestion method (Chung et al., 2015). To avoid unexpected contaminations during the sample preparation, all materials, including the Teflon digestion vessels, were cleaned with a 2% (v/v) nitric solution by soaking for 24 h and then rinsed with ultrapure water prior to use. Here, 10 mL of ≥69% HNO_3 was added to 0.5 g of the pulverized/homogenous rice sample and mixed to achieve a homogenous dispersion. Thereafter, 1 mL of H_2O_2 was added to the mixture, and then the Teflon reactor was placed in the microwave oven under the programmed temperature. The programmed digestion temperature was gradually increased from 30 °C to 200 °C for 23 min, and then kept at 200 °C for an additional 20 min. Complete digestion of the rice samples of interest was realized when the sample mixture became transparent. After cooling at room temperature (~25 °C), the resultant solution was transferred to a polyethylene tube and the volume was finally adjusted to 50 mL with ultrapure water. At least one blank sample for every nine rice samples was prepared in the same manner as described for rice sample digestion.

An Agilent 7900 ICP-MS instrument (Agilent Technology, Seoul, Korea) was utilized for the measurement of the total 25 elements in the rice samples of interest. The main analytical conditions of the ICP-MS were an RF power of 1600 W, RF matching of 1.9 V, cell entrance of −50 V, cell exit of −60 V, integration time of 1 s, and carrier and nebulizer gas (Ar) flow rate of 1.1 $L{\cdot}min^{-1}$. Based on the amounts required for plant growth and development, the 25 elements were classified according to the macro (^{24}Mg, ^{39}K, ^{43}Ca), micro (^{11}B, ^{55}Mn, ^{56}Fe, ^{65}Cu, ^{66}Zn, ^{98}Mo), and trace elements (^{9}Be, ^{23}Na, ^{27}Al, ^{48}Ti, ^{51}V, ^{52}Cr, ^{59}Co, ^{60}Ni, ^{75}As, ^{78}Se, ^{88}Sr, ^{107}Ag, ^{114}Cd, ^{138}Ba, ^{205}Tl, ^{208}Pb).