Effects of Processing on Stable Isotope Compositions (δ^{13}C, δ^{15}N, and δ^{18}O) of Rice (*Oryza sativa*) and Stable Isotope Analysis of Asian Rice Samples for Tracing Their Geographical Origins

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Abstract

We evaluated the effects of processing (i.e., polishing, washing, boiling, and rice cake preparation) on the stable carbon (δ^{13}C), nitrogen (δ^{15}N), and oxygen (δ^{18}O) isotopic compositions of rice (*Oryza sativa*) to assess its geographical origin. There were no significant differences in their δ^{13}C, δ^{15}N, and δ^{18}O values before and after boiling and rice cake preparation, indicating that the database of the δ^{13}C, δ^{15}N, and δ^{18}O values of rice samples could be adapted and used to trace the geographical origin of rice used for cooking and rice cake preparation. Conversely, the δ^{13}C and δ^{18}O values were significantly altered after polishing. After the brown rice samples were polished at a polishing rate of 90%-92%, we determined the δ^{13}C, δ^{15}N, and δ^{18}O values of the polished rice samples from nine countries—Australia, Bangladesh, China, Japan, Malaysia, Myanmar, Philippines, Sri Lanka, and Thailand. The rice samples from Bangladesh, Malaysia, Philippines, and Sri Lanka had significantly lower δ^{13}C values than those from the other five countries. The Chinese, Japanese, and Philippine rice samples had lower δ^{18}O values than those from the other countries. The δ^{13}C and δ^{18}O values could be potential tools for tracing the geographical origin of Asian rice.

Discipline: Food
Additional key words: food authenticity, isotope fingerprinting

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Introduction

Rice is one of the most important crops in Asian food culture. The contribution of Asian countries to the global rice production reached approximately 90% in 2009. Rice imports and exports have increased not just between the Asian countries but also between other countries around the world. Rice cultivars and cultivation areas are important factors in determining the market value of rice. For example, Basmati rice, which is one of the most famous premium types of rice, is cultivated in the Himalayan foothill regions of India and Pakistan. It is sold at a price that is two to three times higher than that of other long-grain rice types (Bligh 2000). Thai Hom Mali rice is internationally known as Thai Jasmine Rice; this rice, which is grown in the Thung Kula Rong Hai area, is the highest-quality jasmine rice and is recognized by the European Union as a geographical indication product (Kukusamude & Kongsri 2018). Koshihikari is the most famous Japanese short-grain rice cultivar in Japan. Besides Japan, it is cultivated in various other countries, such as Australia and the USA. In the Chinese market, Koshihikari cultivated in Japan sells at approximately 50 times the price of ordinary Chinese rice (Kobayashi et al. 2018). Premium rice varieties have sometimes been targeted for mislabeling. Cultivation area or rice cultivars cannot be distinguished by image analysis, which leads to rice authenticity problems, such as mislabeling and the adulteration of premium rice by the addition of inferior rice. Therefore, in Asia, a rice traceability system is required to resolve these rice authenticity problems based on analytical methods that identify different cultivation area and varieties.

Stable isotope analysis has been widely used to trace the origin of food materials. Generally, the isotopic compositions of plant materials reflect various factors, such as the isotopic compositions of source materials (e.g., CO_2, H_2O, NH_4, and NO_2) and their assimilation processes, as well as their growth environments. Many studies have already reported the use of stable isotope analysis to identify the cultivation area of rice samples (Kelly et al. 2002, Suzuki et al. 2008, 2009a, Korenaga et al. 2010, Li et al. 2015, Chen et al. 2016, Chung et al. 2016, Kukusamude & Kongsri 2018). However, these studies have primarily focused on rice samples from one country or just a few countries. Li et al. (2015) reported the stable carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$), and oxygen ($\delta^{18}O$) values of rice samples belonging to 12 varieties of rice grown in 15 countries (Australia, China, France, India, Italy, Japan, Korea, Malaysia, Myanmar, Pakistan, Spain, Taiwan, Thailand, USA, and Vietnam). Their rice samples were mainly collected from the market.

Authentic samples are required to build a database for testing the labels of commercial products in the market (Camin et al. 2017). In addition, these previous studies reported the stable isotope compositions of either brown and/or polished rice. Rice polishing causes changes in the contents of nutritional components such as water, proteins, carbohydrates, lipids, and minerals. The loss of these materials from grains during processing is expected to cause isotopic fractionations. To build and share a database for tracing the geographical origin of rice, it is necessary to evaluate the effect of the polishing process on the $\delta^{13}C$, $\delta^{15}N$, and $\delta^{18}O$ values of rice. Moreover, since September 2017, the Japanese Food Labeling Law has demanded country of origin labeling for the main ingredient by weight in all processed foods manufactured in Japan. The development of analytical methods for tracing the geographical origin of rice and its products is required. The stable isotopic composition is relatively slightly affected by the cooking process. For example, no significant difference in the $\delta^{13}C$ and $\delta^{15}N$ values of meats was observed after boiling, baking, and steaming (Zhou et al. 2015). Therefore, stable isotope analysis has been applied to trace the geographical origin of processed foods such as liquor (especially wine), juice, and dairy products (Kelly et al. 2005). However, thus far, no study has evaluated the effect of the cooking process on the $\delta^{13}C$, $\delta^{15}N$, and $\delta^{18}O$ values of rice.

In the present study, we first evaluated the effect of polishing, boiling, and rice cake preparation on the $\delta^{13}C$, $\delta^{15}N$, and $\delta^{18}O$ values of rice. Boiled rice and rice cakes were prepared using three different types of water with different $\delta^{18}O$ values to investigate whether the water used in the washing and boiling processes could affect the stable isotope ratio of rice. Because we found significant differences in stable isotope ratios between polished and brown rice, we needed to choose between polished and brown rice to build and share the database of stable isotope ratios. In general, most Asian people consume polished rice more than brown rice. Thus, we analyzed the $\delta^{13}C$, $\delta^{15}N$, and $\delta^{18}O$ values of polished rice samples from nine countries—Australia, Bangladesh, China, Japan, Malaysia, Myanmar, Philippines, Sri Lanka, and Thailand—to trace the geographical origin of Asian–Pacific rice. The International Atomic Energy Agency (IAEA) actively cooperates in the peaceful use of nuclear technology for food safety and authenticity. The agricultural projects of the IAEA in the Asia–Pacific region (RAS5062 and RAS5081) aim to develop analytical techniques and build a database of food safety and authenticity for that region. As part of the RAS5062 and RAS5081 projects, we collected authentic rice samples using this project network and examined the

possibility of tracing their geographical origin.

Materials and methods

1. Materials

To evaluate the effect of polishing on the $\delta^{13}C$, $\delta^{15}N$, and $\delta^{18}O$ values of rice grains, three brown rice samples of a nonglutinous cultivar (Rice #1: Koshihikari cultivated at Gifu Prefecture, Rice #2: Koshihikari cultivated at Yamanashi Prefecture, and Rice #3: Mori-no-kumasan cultivated at Kumamoto Prefecture) were collected from a market in Japan. To evaluate the effect of boiling and rice cake preparation on the $\delta^{13}C$, $\delta^{15}N$, and $\delta^{18}O$ values, one sample of unpolished nonglutinous rice cultivar (Koshihikari cultivated at Niigata Prefecture) and one sample of polished glutinous rice cultivar (Kitayukimochi cultivated at Hokkaido Prefecture) were also collected from the market in Japan.

To trace the geographical origin of Asia–Pacific rice samples, 327 authentic brown or polished rice samples were collected from nine countries. All samples were nonglutinous rice and included Indica and Japonica rice. We collected 5 samples from Australia (AUS), 21 samples from Bangladesh (BGD), 24 samples from China (CHN), 134 samples from Japan (JPN), 20 samples from Malaysia (MYS), 33 samples from Myanmar (MMR), 31 samples from the Philippines (PHL), 45 samples from Sri Lanka (LKA), and 14 samples from Thailand (THA).

2. Methods

(1) Sample preparation

To investigate the effect of polishing on the $\delta^{13}C$, $\delta^{15}N$, and $\delta^{18}O$ values, 150 g of brown rice was polished for 1, 2, and 3 min in half-milled rice mode using a home-use rice milling machine (MR-D720; Twinbird Industry, Niigata, Japan). The rice polishing rates were calculated using the following equation:

$$\text{Rice polishing rate (\%)} = \text{Weight of polished rice} \,/\, \text{Weight of brown rice} \times 100$$

The brown and polished rice were ground to a fine powder using a food processor (IFM-800; Twinbird Industry, Niigata, Japan).

(2) Polishing and cooking of rice

As per the 2015 Standard Tables of Food Composition in Japan (MEXT 2015), the average polishing rate of polished rice in the market ranged from 90 to 92%. In the present study, before boiling, 340 g of brown rice was polished with the setting for undermilled rice using a home-use rice milling machine. The rice polishing rates were calculated using the weight of rice before and after milling; it was confirmed that the rice polishing rates varied from 90 to 92%. For washing the polished rice, 300 g of this rice was placed into a stainless-steel bowl and 400 mL of water was added. The sample was stirred 20 times using a stainless-steel whisk. The washed rice was poured into a colander to drain the water out. The washed rice was then returned to the bowl. These steps were repeated four times. The washed rice was then placed in a rice kettle and water was added until the total weight of 800 g was obtained. The washed rice was cooked with water in a rice cooker (NJ-HM10; Mitsubishi Electric, Tokyo, Japan). The cooked rice samples were freeze-dried with FDU-2200 (Tokyo Rikakikai, Tokyo, Japan) for 24 h. Approximately 10 g of the dried rice samples were ground to a fine powder using a food processor.

Before preparing the rice cake (Mochi), 280 g of polished glutinous rice was washed as described above. The washed rice was placed into a stainless-steel bowl, and 240 mL of water was added. The washed rice sample was steamed, kneaded, and pounded in the Mochi pounding mode using a bread maker (PY-E731W; Twinbird Industry, Niigata, Japan). The rice cake samples were freeze-dried with FDU-2200 for 24 h. Approximately 10 g of the dried samples were ground to a fine powder using a food processor.

Each step in the boiling and rice cake preparation processes was conducted using three different types of water with different $\delta^{18}O$ values. Water #1 was bottled mineral water from Yamanashi Prefecture, Water #2 was tap water from Musashino City in Tokyo, and Water #3 was bottled deep-sea water from the depth of 344 m off the coast of Muroto in Kochi Prefecture.

(3) Stable isotope analysis

For carbon and nitrogen isotope analysis, fine rice powders were weighed into approximately 5.0 mg aliquots into tin capsules (5.0 mm × 9.0 mm). Subsequently, each sample was analyzed via elemental analyzer/isotope ratio mass spectrometry (IRMS) using IsoPrime 100 (Elementar UK, Manchester, UK) interfaced with an Elementar vario PYRO cube (Elementar UK, Manchester, UK) to determine the $\delta^{13}C$ and $\delta^{15}N$ values. The measured $\delta^{13}C$ and $\delta^{15}N$ values were normalized using five isotope-known amino acid standards (histidine: $\delta^{15}N$ = −7.6‰ and $\delta^{13}C$ = −10.1‰, L-alanine: $\delta^{15}N$ = −1.06‰ and $\delta^{13}C$ = −19.94‰, glycine: $\delta^{15}N$ = +1.2‰ and $\delta^{13}C$ = −30.5‰, L-alanine: $\delta^{15}N$ = +10.1‰ and $\delta^{13}C$ = −19.6‰, and L-alanine: $\delta^{15}N$ = +20.0‰ and $\delta^{13}C$ = −19.6‰) purchased from Shoko Science (Saitama, Japan). These standards were calibrated using international standards IAEA-N-1, IAEA-N-2, and NBS 19-limestone (NIST RM #8544). The five working standards were analyzed after every 12 samples to confirm the reproducibility of

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the measurements. Analytical errors in the $\delta^{13}C$ and $\delta^{15}N$ values were less than ±0.2‰ and ±0.3‰, respectively.

For the oxygen isotope analysis of the rice samples, approximately 1.0 mg of fine rice powders were weighed into silver capsules (3.3 mm × 5.0 mm). For the oxygen isotope analysis of the water samples, approximately 0.2 μL of water were syringed and sealed in silver smooth wall capsules (1.06 mm × 5.5 mm). Subsequently, the oxygen isotope was analyzed using IRMS interfaced with an Elementar vario PYRO cube. The measured $\delta^{18}O$ values of the rice samples were normalized using four working standards: dibenzo-24-crown-8 (−15.7‰), dibenzo-18-crown-6 (+1.7‰), β-D-galactose pentaacetate (+12.7‰), and D-(+)-sucrose octaacetate (+26.8‰). These four working standards were calibrated using isotope-known benzoic acid standards (+71.4‰ and +23.2‰) purchased from Indiana University (State of Indiana, USA). The four working standards were analyzed after every 12 samples to confirm the reproducibility of the measurements. The measured $\delta^{18}O$ values of the water samples were normalized using isotope-known water standards (S1= −0.066‰, S2= −10.8‰, and S3= −14.5‰) purchased from Shoko Science. The analytical error of the isotope measurement was less than 0.3‰ and 0.2‰ for the rice and water samples, respectively. The isotopic composition was reported in the δ notation:

$$\delta\ (‰) = (R_{sample} / R_{standard} - 1) \times 1{,}000,$$

where R_{sample} is the isotope ratio (i.e., $^{13}C/^{12}C$, $^{15}N/^{14}N$, and $^{18}O/^{16}O$) of the sample and $R_{standard}$ is the isotope ratio of the international standards described as follows: for carbon, Vienna Pee Dee Belemnite; for nitrogen, air; and for oxygen, Vienna Standard Mean Ocean Water. The isotope ratios were expressed per mil (‰).

(4) Statistical analysis

A paired t-test was performed to test the significance of the differences in the $\delta^{13}C$, $\delta^{15}N$, and $\delta^{18}O$ values between the rice samples before and after processing or cooking. One-way analysis of variance was performed to test the significance of the differences in the $\delta^{13}C$, $\delta^{15}N$, and $\delta^{18}O$ values among the rice samples obtained from nine countries. For all tests, $P< 0.05$ indicated statistical significance. All analyses were conducted using IBM SPSS Statistics 23.0 (IBM).

Results and discussion

1. Effects of the polishing process on the $\delta^{13}C$, $\delta^{15}N$, and $\delta^{18}O$ values of rice

Figure 1 shows the $\delta^{13}C$, $\delta^{15}N$, and $\delta^{18}O$ values of the brown and polished rice samples. There were significant

differences in these values between brown and polished rice ($P< 0.05$). In particular, the $\delta^{13}C$ and $\delta^{18}O$ values of the rice samples increased as the rice polishing rates

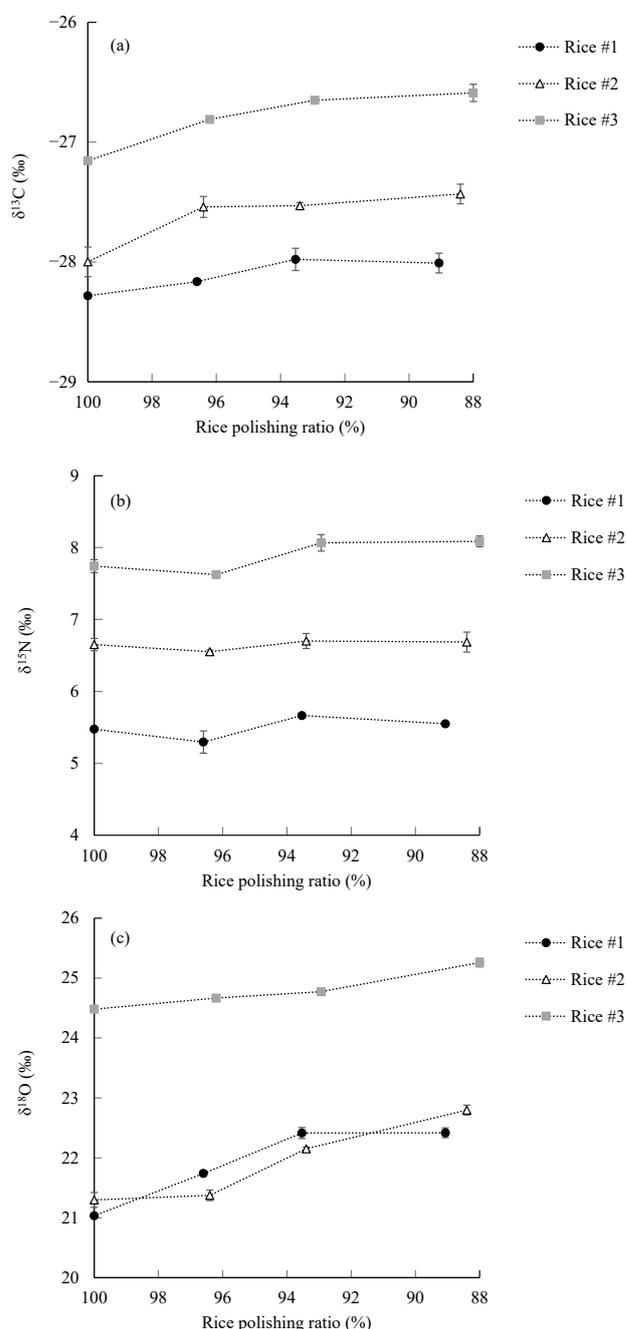**Fig. 1. $\delta^{13}C$ (a), $\delta^{15}N$ (b), and $\delta^{18}O$ (c) values of brown and polished rice samples**
The error bars indicate standard deviations (σ) of each sample (n = 3).
Rice #1: Koshihikari cultivated at Gifu Prefecture; Rice #2: Koshihikari cultivated at Yamanashi Prefecture; Rice #3: Mori-no-kumasan cultivated at Kumamoto Prefecture

decreased. According to the 2015 Standard Tables of Food Composition in Japan (MEXT 2015), the lipid content in paddy rice types is as follows: 2.7% in brown rice, 1.8% in half-milled rice, and 1.5% in polished rice. In contrast, rice bran has a high lipid content of 19.6%. The $\delta^{13}C$ values of lipids are significantly lower than those of other nutritional components of food (Park & Epstein 1961). Lipids have 3‰-8‰ lower $\delta^{13}C$ values than those of the bulk plants because lipids contain isotopes that are "lighter" than those of carbohydrates. The differences in lipid content may cause the differences in isotope values between brown and polished rice. In the present study, rice brans had lower $\delta^{13}C$ values than brown and polished rice samples (−30.5‰ in Rice #1, −30.6‰ in Rice #2, and −29.8‰ in Rice #3). These results suggested that the standardization of the rice polishing rate is required to build the database of rice samples. In the Japanese market, polished rice with a polishing rate of 90%-92% is very popular for consumption. Thus, in the present study, we analyzed the polished rice samples with a polishing rate of 90%-92% to build a database that could be used to trace their geographical origin.

2. Effects of rice boiling and rice cake preparation on the $\delta^{13}C$, $\delta^{15}N$, and $\delta^{18}O$ values

Table 1 shows the $\delta^{13}C$, $\delta^{15}N$, and $\delta^{18}O$ values of polished nonglutinous rice, polished glutinous rice, boiled nonglutinous rice, and rice cakes prepared using three different types of water with different $\delta^{18}O$ values. The $\delta^{18}O$ values of Water #1, #2, and #3 were −10.1‰, −7.9‰, and −0.5‰, respectively. There was no significant difference in the $\delta^{13}C$, $\delta^{15}N$, and $\delta^{18}O$ values of rice before and after boiling or rice cake preparation ($P = 0.08$-0.57 for the $\delta^{13}C$ values, $P = 0.11$-0.31 for the $\delta^{15}N$ values, and $P = 0.09$-0.52 in the $\delta^{18}O$ values). Organisms mainly consist of C, N, O, and H, and it can be expected that the $\delta^{13}C$, $\delta^{15}N$, and $\delta^{18}O$ values of the solid structural components of tissues would not change after cooking. Suzuki et al. (2009b) analyzed the $\delta^{13}C$, $\delta^{15}N$, and $\delta^{18}O$ values of raw and processed eel products to evaluate the effects of steaming, boiling, and seasoning with sauces on the stable isotope ratio. They found no significant differences in the $\delta^{13}C$, $\delta^{15}N$, and $\delta^{18}O$ values before and after cooking. Similar to the results of their study, the processes of cooking rice and rice cake preparation in the present study had little effect on the $\delta^{13}C$, $\delta^{15}N$, and $\delta^{18}O$ values of rice. In particular, there were no significant differences in the $\delta^{18}O$ values of boiled rice and rice cake before and after cooking regardless of the use of water with different $\delta^{18}O$ values. These results suggested that the level of oxygen exchange between water and solid structural components of rice was low during the cooking

of rice and preparation of rice cake. Thus, the database of the $\delta^{13}C$, $\delta^{15}N$, and $\delta^{18}O$ values of polished rice samples are adequate for tracing the geographical origin of rice even after cooking rice and preparing rice cake.

3. $\delta^{13}C$, $\delta^{15}N$, and $\delta^{18}O$ values of rice samples from nine Asia–Pacific countries

The variations in $\delta^{13}C$ values among the polished rice samples from nine Asia-Pacific countries are shown in Figure 2a in the form of a boxplot. The average $\delta^{13}C$ values of the rice samples in the countries were as follows: −26.7‰ in AUS, −28.5‰ in BGD, −27.3‰ in CHN, −27.3‰ in JPN, −28.5‰ in MYS, −27.8‰ in MMR, −28.3‰ in PHL, −28.2‰ in LKA, and −26.6‰ in THA. The rice samples from BGD, MYS, LKA, and PHL had significantly lower $\delta^{13}C$ values than those from AUS, CHN, JPN, MMR, and THA ($P < 0.001$). Generally, there was a correlation between the $\delta^{13}C$ values and the use of water in plant photosynthesis (Saurer et al. 1995, Cui et al. 2009). The stomata are not only the gates for carbon dioxide diffusion into the leaf but also control water loss through the diffusion of water vapors from the leaf (transpiration). The opening and closing of the stomata depend on the amount of moisture available to

Table 1. $\delta^{13}C$, $\delta^{15}N$, and $\delta^{18}O$ values of polished nonglutinous rice, boiled nonglutinous rice, polished glutinous rice, and rice cake

	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{18}O$ (‰)
Polished nonglutinous rice[a]	−27.3 ± 0.1	3.5 ± 0.3	22.5 ± 0.2
Polished nonglutinous rice[a] boiled in			
Water #1[b]	−27.3 ± 0.1	3.7 ± 0.1	22.4 ± 0.2
Water #2[b]	−27.3 ± 0.2	3.2 ± 0.3	22.5 ± 0.4
Water #3[b]	−27.1 ± 0.1	3.7 ± 0.1	22.3 ± 0.3
	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{18}O$ (‰)
Polished glutinous rice[a]	−28.4 ± 0.1	2.4 ± 0.2	19.2 ± 0.3
Rice cake made with polished glutinous rice[a] and			
Water #1[b]	−28.4 ± 0.1	2.6 ± 0.2	19.1 ± 0.3
Water #2[b]	−28.4 ± 0.2	2.5 ± 0.1	19.2 ± 0.4
Water #3[b]	−28.4 ± 0.2	2.6 ± 0.1	19.2 ± 0.5

The values are expressed as means ± standard deviations (n = 3).
[a] Nonglutinous rice: Koshihikari cultivated at Niigata Prefecture; glutinous rice: Kitayukimochi cultivated at Hokkaido Prefecture
[b] Water #1: bottled mineral water from Yamanashi Prefecture ($\delta^{18}O = -10.1$‰); Water #2: tap water from Musashino City in Tokyo ($\delta^{18}O = -7.9$‰); Water #3: bottled deep-sea water from the depth of 344 m off the coast of Muroto in Kochi Prefecture ($\delta^{18}O = -0.5$‰)

the plants. Water use efficiency (WUE) is defined as the ratio of the assimilation of carbon dioxide to the loss of water by the plants, and it represents the effectiveness of water use in plants. There was a positive correlation between the WUE and the $\delta^{13}C$ values of plant tissues. Under hot and dry conditions, the stomata must close to prevent transpiration from the leaves, thereby increasing the WUE and $\delta^{13}C$ values of plants. Suzuki et al. (2009a) investigated the $\delta^{13}C$ values of rice grown in paddy and upland fields to verify the relationship between the $\delta^{13}C$ values of rice and the water conditions. The $\delta^{13}C$ values of rice from the upland fields were approximately 1‰ higher than those from the paddy fields. Kohn (2010) reported the relationships between the $\delta^{13}C$ values of C_3

plants and mean annual precipitation; the $\delta^{13}C$ values of C_3 plants tended to increase with the decrease in mean annual precipitation. Based on meteorological statistical information from the Japan Meteorological Agency (2020), the mean annual precipitation in BGD, MYS, PHL, and LKA ranged from 1,500 to 3,000 mm/year, and the average $\delta^{13}C$ values of plants from these countries were lower than −28‰. Thus, the high level of precipitation could have been a factor influencing the decrease in the $\delta^{13}C$ values of rice from these areas. These results suggested that the $\delta^{13}C$ values of the rice samples reflected the moisture conditions of the growing areas and could be an indicator for tracing the geographical origin of rice.

The variations in $\delta^{15}N$ among the polished rice samples from nine Asia–Pacific countries are shown in Figure 2b in the form of a boxplot. The average $\delta^{15}N$ values of the rice samples in the countries were as follows: +7.2‰ in AUS, +2.7‰ in BGD, +3.9‰ in CHN, +4.4‰ in JPN, +3.8‰ in MYS, +2.9‰ in MMR, +4.1‰ in PHL, +2.5‰ in LKA, and +3.6‰ in THA. The Japanese rice had a wide range of $\delta^{15}N$ values, from −0.3‰ to +13.4‰. The $\delta^{15}N$ values of rice from AUS were relatively higher than those from the other countries. In general, the $\delta^{15}N$ values of plants mainly depend on soil nutrition. Suzuki et al. (2009a) reported the $\delta^{15}N$ values of rice and soil samples from rice fields; there were positive correlations between the $\delta^{15}N$ values of the rice and soil. Amundson et al. (2003) reported the estimated geographical distribution of soil $\delta^{15}N$ values. Based on this global pattern, the $\delta^{15}N$ value of soil in AUS and THA ranged from +7.6‰ to +9.0‰. These values were relatively higher than those from the other countries (+2.1‰ to +6.2‰ in CHN; +3.5‰ to +6.2‰ in JPN; +4.8‰ to +6.2‰ in MMR and PHL; and +6.2‰ to +7.6‰ in BGD, MYS, and LKA). Moreover, Suzuki et al. (2009a) reported that organic rice samples had higher $\delta^{15}N$ values than conventional rice. In AUS, pasture crop rotation in rice cultivation has been effective in maintaining high soil fertility (Faour et al. 2005). During the cultivation of pasture crops, the fields are fertilized with the dung of grazing animals. Generally, organic fertilizers such as cow, chicken, and pig manure have higher $\delta^{15}N$ values than chemical fertilizers (Kohl et al. 1973, Meints et al. 1975, Suzuki & Nakashita 2013). The high $\delta^{15}N$ value of Australian rice depends on the $\delta^{15}N$ value of the soil and the $\delta^{15}N$ value of the dung excreted by the animals grazing in rice fields. Thus, the $\delta^{15}N$ values of rice samples can reflect the soil $\delta^{15}N$ value in rice fields and agricultural practices in each country.

The variations in $\delta^{18}O$ among the polished rice samples from nine Asia–Pacific countries are shown
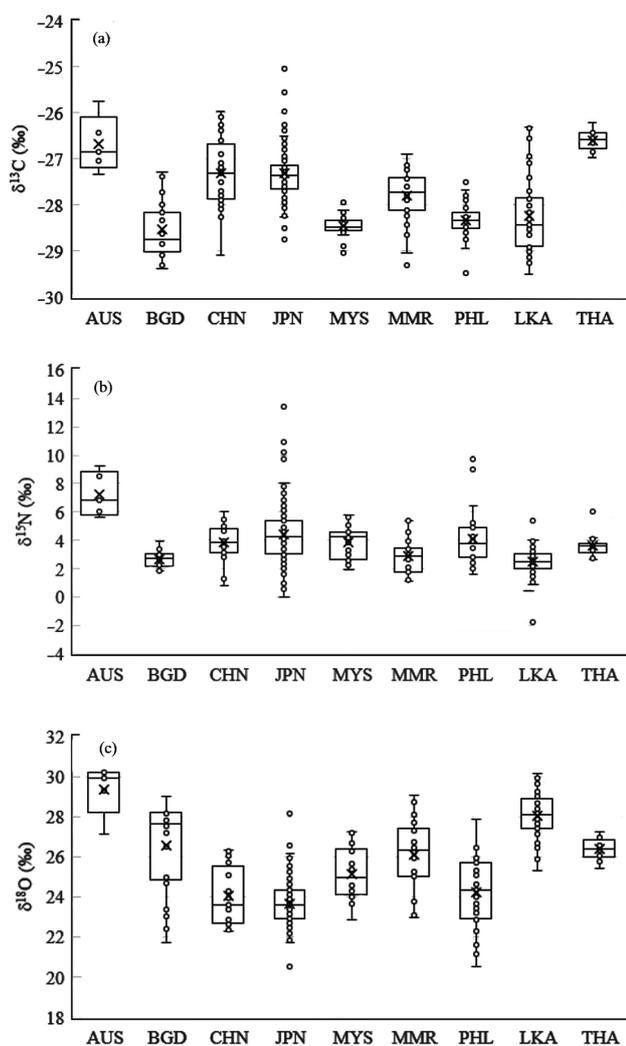
Fig. 2. Box and whisker plots showing the $\delta^{13}C$ (a), $\delta^{15}N$ (b), and $\delta^{18}O$ (c) variations in polished rice from nine Asia–Pacific countries
AUS: Australia; BGD: Bangladesh; CHN: China; JPN: Japan; MYS: Malaysia; MMR: Myanmar; PHL: Philippines; LKA: Sri Lanka; THA: Thailand

in Figure 2c in the form of a boxplot. The average $\delta^{18}O$ values of the rice samples from the countries were as follows: +29.4‰ in AUS, +26.6‰ in BGD, +24.1‰ in CHN, +23.7‰ in JPN, +25.1‰ in MYS, +26.1‰ in MMR, +24.2‰ in PHL, +28.1‰ in LKA, and +26.4‰ in THA. The rice samples from CHN, JPN, and PHL had lower $\delta^{18}O$ values than those from the other countries ($P < 0.001$). The $\delta^{18}O$ values of rice mainly reflect the type of water available to the plants. Suzuki et al. (2009a) reported the $\delta^{18}O$ values of rice and water collected from rice fields, and there were positive correlations between their $\delta^{18}O$ values. The $\delta^{18}O$ values of rainwater and groundwater depend on geographical factors such as latitude, altitude, and inland effect (Craig 1961, Gat 1996). WaterIsotopes.org (2003-2019) provides a global map of long-term, precipitation-amount weighted annual average $\delta^{18}O$ values estimated using data from the Global Network of Isotopes in Precipitation. Based on this map, the $\delta^{18}O$ values of precipitation in CHN, JPN, and PHL ranged from −12.7‰ to −5.8‰. These values were relatively lower than those from the other countries (−5.8‰ to −4.1‰ in AUS; −2.4‰ to −9.2‰ in BGD, MMR, and LKA; and −9.2‰ to −5.8‰ in MYS and THA). This result suggested that the $\delta^{18}O$ values of rice can reflect the $\delta^{18}O$ values of precipitation. Thus, the $\delta^{18}O$ values of rice samples could be used as indicators for tracing the geographical origin of rice.

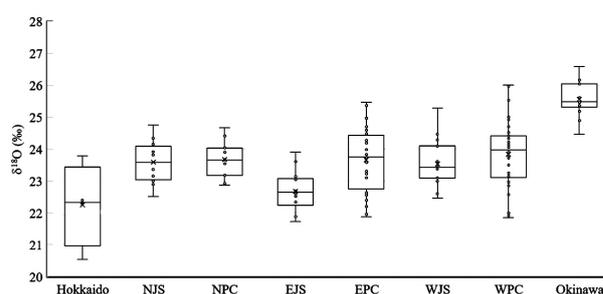## 4. $\delta^{18}O$ variation among polished rice samples from Japan

The $\delta^{18}O$ variations among the polished rice samples from Japan are shown in Figure 3 in the form of a boxplot. Japan is a long archipelago made up of four main islands: Hokkaido, Honshu, Shikoku, and Kyushu. It is also divided into 47 prefectures. Ichiyanagi et al. (2016) reported the $\delta^{18}O$ and δ^2H values of precipitation at 56 sites across Japan. The sources of evaporated moisture in Japan are divided into three regions: the Pacific Ocean, Sea of Japan, and the East China Sea. They are further categorized into six regions according to regional weather forecasting classifications defined by the Japan Meteorological Agency (2020): the Northern Japan/Sea of Japan side (NJS), Northern Japan/Pacific Oceanside (NPC), Eastern Japan/Sea of Japan side (EJS), Eastern Japan/Pacific Oceanside (EPC), Western Japan/Sea of Japan side (WJS), and Western Japan/Pacific Oceanside (WPC). In the present study, Japanese rice was divided into eight regions, separating Hokkaido (northernmost) and Okinawa (southernmost) prefectures from the aforementioned six regions. The average $\delta^{18}O$ values of the rice in the studied regions were as follows: +22.2‰ in Hokkaido, +23.6‰ in NJS, +23.7‰ in NPC, +22.7‰

in EJS, +23.7‰ in EPC, +23.5‰ in WJS, +23.8‰ in WPC, and +25.6‰ in Okinawa. There was a significant difference between the $\delta^{18}O$ values from Hokkaido and those from Okinawa ($P < 0.001$). The $\delta^{18}O$ values of the Japanese rice samples tended to increase as the latitude decreased. There were similar trends in the $\delta^{18}O$ values of precipitation in Japan. The average $\delta^{18}O$ values of precipitation in the regions were as follows: −10.40‰ in NJS, −9.27‰ in NPC, −9.10‰ in EJS, −8.18‰ in EPC, −7.64‰ in WJS, and −7.79‰ in WPC (Ichiyanagi et al. 2016). The average $\delta^{18}O$ values of precipitation in the areas of the Pacific Oceanside were higher than those in the areas of the Sea of Japan side. The rice samples from EJS tended to have lower $\delta^{18}O$ values than those from EPC. However, there were no significant differences in the $\delta^{18}O$ values of the rice samples from NJS, NPC, EJS, EPC, WJS, and WPC. These results suggested that the regional origin Japanese polished rice samples within Japan cannot be significantly distinguished based on oxygen isotopic data alone. To improve the discrimination of Japanese rice samples, a combination of other analytical methods, such as trace element analysis, is required (Ariyama et al. 2012, Chung et al. 2015).

Conclusions

We evaluated the effects of the polishing process on the $\delta^{13}C$, $\delta^{15}N$, and $\delta^{18}O$ values of rice samples. Polishing alters the $\delta^{13}C$ and $\delta^{18}O$ values of the rice samples due to the difference in the lipid content between the surface tissues and the endosperm. This result suggested that the

Fig. 3. Box and whisker plots showing the $\delta^{13}C$, $\delta^{15}N$, and $\delta^{18}O$ variations in Japanese polished rice samples from eight regions in Japan
Hokkaido: Hokkaido Prefecture; NJS: the Northern Japan/Sea of Japan side; NPC: the Northern Japan/Pacific Oceanside; EJS: the Eastern Japan/Sea of Japan side; EPC: the Eastern Japan/Pacific Oceanside; WJS: the Western Japan/Sea of Japan side; WPC: the Western Japan/Pacific Oceanside; Okinawa: Okinawa Prefecture

rice polishing rate must be standardized for building the database of the δ^{13}C, δ^{15}N, and δ^{18}O values for tracing the geographical origin of rice. We chose polished rice because it is more popular compared with brown rice in the Asian market. To examine the applicability of tracing the geographical origin of processed rice products using stable isotope analysis, we evaluated the effects of the cooking process on the δ^{13}C, δ^{15}N, and δ^{18}O values of rice samples. Boiling and rice cake preparation had small or no effects on the δ^{13}C, δ^{15}N, and δ^{18}O values when compared with those of the samples before and after cooking. This result suggested that the database of the δ^{13}C, δ^{15}N, and δ^{18}O values of polished rice samples could be used to trace the geographical origin of rice even after cooking rice and preparing rice cake. As part of the IAEA's agricultural projects in the Asia–Pacific region (RAS5062 and RAS5081), we collected authentic rice samples using this project network and examined the possibility of tracing their geographical origin. We analyzed the δ^{13}C, δ^{15}N, and δ^{18}O values of polished rice samples from nine Asia–Pacific countries, adjusting the polishing rate from 90% to 92% to seek the possibility of tracing their geographical origin. There were significant differences in the average δ^{13}C and δ^{18}O values among the rice samples from the Asia–Pacific countries. These results suggested that the δ^{13}C and δ^{18}O values could be potential tools for tracing the geographical origin of Asian rice samples. The RAS5081 project is ongoing and will last until 2021, and the δ^{13}C, δ^{15}N, and δ^{18}O values of rice samples collected over a multiyear period should be analyzed for evaluating their annual variations. Unfortunately, the rice samples from some geographically close countries, such as Bangladesh and Sri Lanka or China and Japan, have very similar δ^{13}C and δ^{18}O values. Therefore, the combination of other analytical methods, such as trace element and strontium isotope ratio analyses, is required to be able to discriminate between these samples.

Acknowledgements

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