Investigating differences in light stable isotopes between Thai jasmine rice and Sungyod rice

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Abstract. We report the differences in light stable isotopes between two kinds of Thai rice (Thai jasmine and Sungyod rice). Thai jasmine rice and Sungyod rice were cultivated in the northeast and the south of Thailand. Light isotopes including 13C, 15N and 18O of Thai jasmine rice and Sungyod rice samples were carried out using isotope ratio mass spectrometry (IRMS). Thai jasmine rice (Khao Dawk Mali 105) was cultivated from Thung Kula Rong Hai area, whereas Sungyod rice was cultivated from Phatthalung province. Hypothesis testing of difference of each isotope between Thai jasmine rice and Sungyod rice was also studied. The study was the feasibility test whether the light stable isotopes can be the variables to identify Thai jasmine rice and Sungyod rice. The result shows that there was difference in the isotope patterns of Thai jasmine rice and Sungyod rice. Our results may provide the useful information in term of stable isotope profiles of Thai rice.

1. Introduction
The most important crop in the world is rice, *Oryza sativa* L., providing up to 76% for the population of Southeast Asia [1]. Rice is the main source of calories and protein for human especially in Asia, America, and some European countries. It also a good source of energy, vitamins, and essential elements for human [2]. Thai jasmine rice (Khao Dawk Mali 105) is originated from Thailand, mostly grown in Thung Kula Rong Hai area in the northeast of Thailand [3]. Thung Kula Rong Hai area covers five provinces including Roi-Et, Mahasarakham, Sisaket, Yasothon, and Surin. It is considered as premium rice in the world market leading to economic adulteration problem. Sungyod is mostly grown in Phatthalung province, Thailand. Sungyod is recognized as Geographical Indication (GI) rice. Thus, tracing the geographical origin of rice is essential to prevent mislabeling and adulteration [3,4,5]. Nowadays, identification of the geographical origin of a wide range of agricultural food products is interested issue. Tracing of food authenticity is important for quality control and food safety.

Various analytical techniques for discrimination of rice origin such as inductively coupled plasma atomic emission spectrometry (ICP-AES), inductively coupled plasma mass spectrometry (ICP-MS), and elemental analyzer/isotope ratio mass spectrometry (EA/IRMS) [2,3,4,6] have been used. Stable isotope analyses have been widely used to trace the origin of organic materials in various fields such as geochemistry, biochemistry, archaeology, and petroleum chemistry [7,8]. It has become important issue as a solution for food authenticity problems. In fact, the isotopic compositions of plant reflect various factors. For instance, carbon isotopic composition of plant strongly depends on the carbon fixation process such as C₃ or C₄ cycle [9]. Nitrogen isotope mainly depends on soil nutrition [10].
In this study, we determined carbon, nitrogen, and oxygen isotopic compositions (\(\delta^{13}C\), \(\delta^{15}N\), \(\delta^{18}O\)) of Thai jasmine rice (Khao Dawk Mali 105) and Sungyo rice cultivated in the Thung Kula Rong Hai area and Phatthalung province, respectively using elemental analyzer isotope ratio mass spectrometry (EA-IRMS). In order to investigate the differences in stable isotopic compositions that can be used as parameters for discrimination of the rice origin, t-test was also evaluated.

2. Experimental

2.1. Rice samples

Samples of two kinds of Thai rice were collected from paddy fields from different regions (the Northeast and the South) in Thailand. The samples (n=49) named Khao Dawk Mali 105 from the Northeast located in Thung Kula Rong Hai area were collected. The samples (n=43) of Sungyo rice were collected from Phatthalung province in the South of Thailand. Samples were dried and ground to fine powder before analysis.

2.2. \(\delta^{13}C\), \(\delta^{15}N\), and \(\delta^{18}O\) analyses by Elemental analyzer/ isotope ratio mass spectrometry

All samples were analyzed using elemental analyzer isotope ratio mass spectrometry (EA-IRMS), Isoprime, UK in order to determine \(\delta^{13}C\), \(\delta^{15}N\), and \(\delta^{18}O\) isotope ratios. The powered rice samples were accurately weighed 4 mg into tin boats (4 mm x 4 mm x 11 mm). The boats were then folded and compressed in order to avoid air presented. For the analysis of stable nitrogen, the powered rice samples were (4 mg) was weighed and analyzed in a similar way as the stable carbon analysis. The samples for \(\delta^{13}C\) and \(\delta^{15}N\) analyses were performed in combustion mode by EA-IRMS. For \(\delta^{18}O\) analysis, the powered rice samples (0.3 mg) were accurately weighed into silver boats (4 mm x 4 mm x 11 mm) and analyzed using pyrolysis mode.

3. Results and discussion

The isotope ratio was described in term of \(\delta\) (per mil, ‰) defined as the following equation: \(\delta\) (‰) = \((\text{R}_{\text{sample}} - \text{R}_{\text{standard}}) \times 1000\), where \(\delta\) (%) is the isotope composition, \(\text{R}_{\text{sample}}\) and \(\text{R}_{\text{standard}}\) are the isotope ratio (i.e., \(\delta^{13}C/\delta^{12}C\), \(\delta^{15}N/\delta^{14}N\), and \(\delta^{18}O/\delta^{16}O\)) of the sample and isotope ratio of international standard, respectively. In order to obtain the differences in stable isotopes according to the geographical origins, rice samples cultivated from Thung Kula Rong Hai area and Phatthalung province were collected. Thung Kula Rong Hai covers 5 provinces including Sisaket, Yasothon, Roi Et, Surin, and Mahasarakham provinces. The carbon, nitrogen, and oxygen isotopic compositions of rice were analyzed by EA-IRMS.

The results of \(\delta^{13}C\), \(\delta^{15}N\), and \(\delta^{18}O\) values of Thai jasmine and Sungyo rice obtained from Thung Kula Rong Hai area and Phatthalung province are summarized in Fig. 1. Box plots A and D represent \(\delta^{13}C\) values Thai jasmine rice from Thung Kula Rong Hai area and Sungyo rice from Phatthalung provinces, respectively, whereas box plots (B and E) and (C and F) represent \(\delta^{15}N\), and \(\delta^{18}O\) values, correspondingly. The \(\delta^{13}C\) values of Thai jasmine rice ranged from -28.04‰ to -26.31‰ (n=49), while those of Sungyo rice ranged from -28.41‰ to -26.81‰ (n=43). It is found that the \(\delta^{13}C\) values of all rice samples were in the range of \(\delta^{13}C\) values of C-3 plants (-22‰ to -33‰). The \(\delta^{15}N\) values of Thai jasmine rice were between +1.50‰ to +6.47‰. The \(\delta^{15}N\) values found in Sungyo rice ranged from +3.06‰ to +5.97‰. The S.D. of those values was 1.07 and 0.64, respectively. From Fig. 2 (B, C, E, and F), the results showed that data were highly deviated compared with the \(\delta^{13}C\) values. This is probably because rice samples were collected from the paddy fields used both organic and inorganic
fertilizers. Moreover, Thai jasmine rice and Sungyod rice were cultivated from different regions of Thailand. The $\delta^{18}O$ values in Thai jasmine rice and Sungyod rice ranged from $+23.44\%$ to $+26.69\%$ and $+23.50\%$ to $+29.50\%$, respectively. The heaviest was obtained in Sungyod rice sample cultivated in Phatthalung province whereas the lightest was the Thai jasmine rice sample cultivated in Thung Kula Rong Hai area.

![Box plot of $\delta^{13}C$, $\delta^{15}N$, and $\delta^{18}O$ values of rice originated from Thung Kula Rong Hai area and Phatthalung province.](image)

**Figure 1.** Box plot of $\delta^{13}C$, $\delta^{15}N$, and $\delta^{18}O$ values of rice originated from Thung Kula Rong Hai area and Phatthalung province.

| Isotope | Average (Thai jasmine rice) | Average (Sungyod rice) | S.D. (Thai jasmine rice) | S.D. (Sungyod rice) | P (T<=t) |
|---------|-----------------------------|------------------------|--------------------------|---------------------|---------|
| $\delta^{13}C$ | -27.24 | -27.71 | 0.32 | 0.37 | <0.0001 |
| $\delta^{15}N$ | +3.93 | +4.34 | 1.07 | 0.64 | 0.027 |
| $\delta^{18}O$ | +25.28 | +26.36 | 0.84 | 1.39 | <0.0001 |

**Table 1.** Descriptive statistic and t-test for Thai jasmine rice and Sungyod rice.

The average and S.D. of the interested elements found in Thai jasmine rice and Sungyod rice cultivated in Thung Kula Rong Hai area and Phatthalung province are illustrated in the Table 1. The difference in isotopic composition between Thai jasmine rice and Sungyod rice was carried out by t-
test. The obtained p-values of $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{18}\text{O}$ were <0.0001, 0.027 and <0.0001, respectively. It indicated that all studied isotopes including $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{18}\text{O}$ were significantly different between Thai jasmine rice and Sungyod rice samples at 95% confidence interval. This is possibly due to there was different in the geographical origin and water resource that used for cultivation of rice. This implied that the carbon, nitrogen, and oxygen isotopes could be possible to identify rice samples cultivated Thung Kula Rong Hai area (in the northeastern region of Thailand) and Phatthalung province. Our results may be useful database of Thai jasmine rice and Sungyod rice and may provide preliminary information for discrimination of rice samples that grown in different regions in Thailand.

4. Conclusion

Three isotopes ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{18}\text{O}$) in Thai jasmine and Sungyod rice samples cultivated from Thung Kula Rong Hai area and Phatthalung province were determined by EA-IRMS. All studied isotopes were significantly different between Thai jasmine rice and Sungyod rice samples at 95% confidence interval. Our results may provide preliminary information for discrimination of rice samples and it is possible to trace the geographical origin of rice cultivated from different regions in the further study using stable isotopes.

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