

# Stable isotope ratio of local rice samples in Thailand

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**Abstract.** Stable isotopes including  $^{13}\text{C}$ ,  $^{15}\text{N}$  and  $^{18}\text{O}$  of local Thai rice samples were carried out using elemental analyzer isotope ratio mass spectrometry (EA-IRMS). Thai rice samples, Khaowong sticky rice, Pakaumpul local rice, and Jek Chuey Sao Hai rice were cultivated from Kalasin, Surin, and Saraburi provinces, respectively. In this study, we report the stable isotope ratio of rices for the isotopic characteristics of Thai rice cultivated from different provinces. The analysis of variance (ANOVA) and multiple comparisons using Least significant difference (LSD) were also investigated. Stable isotope ratio of  $^{13}\text{C}/^{12}\text{C}$  and  $^{18}\text{O}/^{16}\text{O}$  were good characteristic indicators for Thai rice that could be applied for the geographical origins of rice.

## 1. Introduction

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 [1]. However, many people concern the healthier options like the local rice which contains for nutritional and healthy benefits such as high protein, high vitamins, antioxidant and anticancer properties, etc. Pakaumpul local rice contains the highest protein in comparison with the other local rice cultivated in Surin province. Geographical indication or GI is a sign that reveals the origin (a province, region or country) of a product. Khaowong sticky rice in Kalasin Province and Jek Chuey Sao Hai rice in Saraburi province are GI rice products in Thailand. Khaowong sticky rice has a soft and fragrant nature that is not found in rice grown from other sources in Kalasin province. Jek Chuey Sao Hai Rice is a Jek Chuey rice variety and is produced in Sao Hai, Mueng, Nong Sang, Viharn Dang, NongKae, Nong Don, and Don Pud subdistricts in Saraburi province. When cooked, the rice is soft and rises well. It is not damp, hard, coarse or sticky. It is suitable to be consumed with curry. When old rice is cooked, there is no musty smell.

It is considered as rice is problematic to economic adulteration. Thus, tracing the geographical origin of rice is essential issue to prevent mislabeling and adulteration [2]. Nowadays, there are research interests related to identify the geographical origin of a wide range of agricultural food products. Tracing of food authenticity is important issue for quality control and food safety [3]. Various analytical techniques that have been used for discrimination of rice origins were inductively coupled plasma atomic emission spectrometry (ICP-AES), inductively coupled plasma mass spectrometry (ICP-MS), and elemental analyzer/ isotope ratio mass spectrometry (EA/IRMS) [1,2,4]. 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  $\text{C}_3$  or  $\text{C}_4$  cycle [5]. Nitrogen isotope mainly depends on soil nutrition [6].

In this study, we determined carbon, nitrogen, and oxygen isotopic compositions ( $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ,  $\delta^{18}\text{O}$ ) of some local Thai rice, Khaowong sticky rice, Pakaumpul local rice, and Jek Chuey Sao Hai rice cultivated in Kalasin, Surin, Saraburi provinces, respectively using elemental analyzer isotope ratio mass spectrometry (EA-IRMS). In order to investigate the differences in stable isotopic compositions that is possible to use as parameters for discrimination of the rice origin, ANOVA and LSD were also evaluated.

## 2. Experimental

### 2.1 Rice sample

Samples of three kinds of Thai rice samples were collected from paddy fields from different provinces in Thailand. Twenty-nine samples of Khaowong sticky rice, Pakaumpul local rice, and Jek Chuey Sao Hai rice were collected from Kalasin (n=13), Surin (n=7), and Saraburi (n=9) provinces, respectively. Samples were dried and ground to fine powder prior to the analysis.

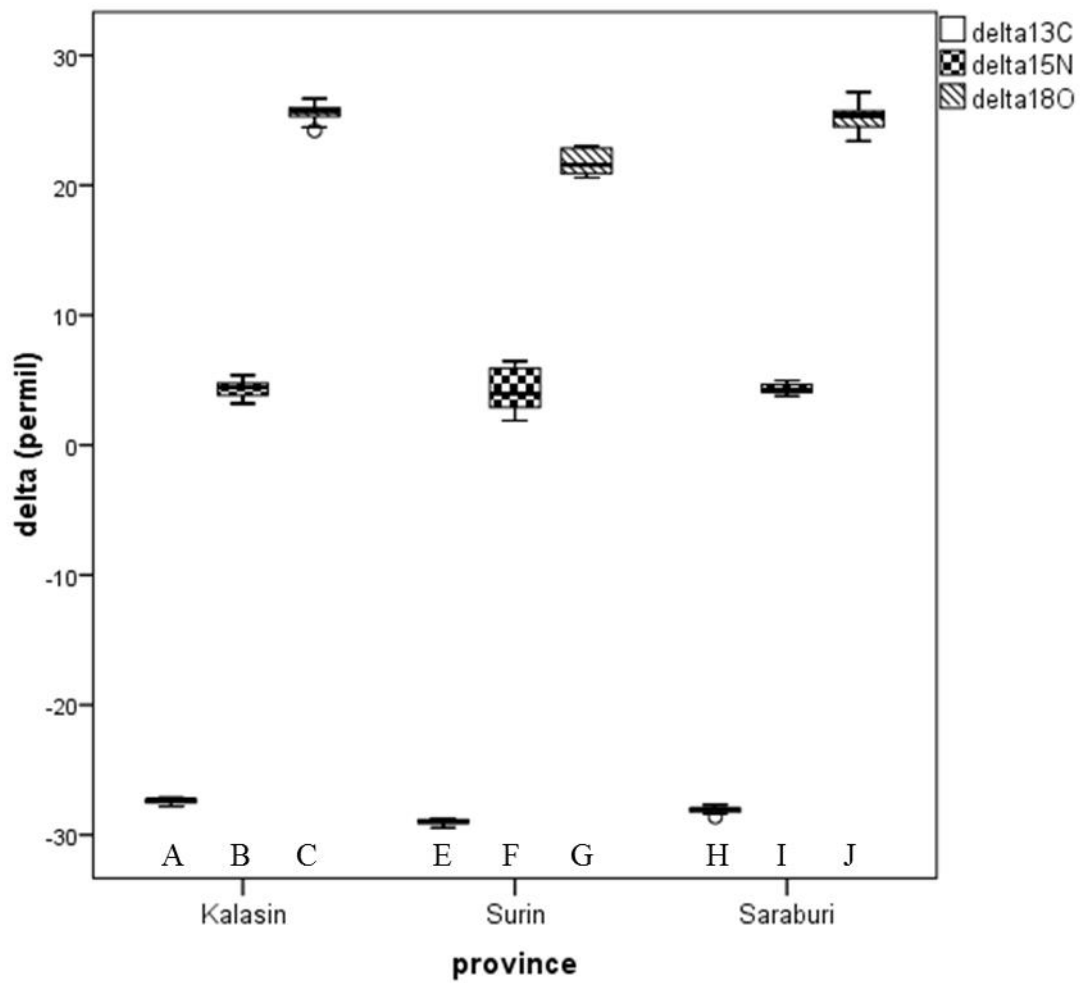
### 2.2 Analysis by elemental analyser isotope ratio mass spectrometry (EA-IRMS)

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

## 3. Results and discussion

The isotope ratio was described in term of  $\delta$  (per mil, ‰) defined as the following equation:  $\delta (\text{‰}) = (R_{\text{sample}} - R_{\text{standard}})/R_{\text{standard}} \times 1000$ , where  $\delta (\text{‰})$  is the isotope composition,  $R_{\text{sample}}$  and  $R_{\text{standard}}$  are the isotope ratio (i.e.,  $^{13}\text{C}/^{12}\text{C}$ ,  $^{15}\text{N}/^{14}\text{N}$ , and  $^{18}\text{O}/^{16}\text{O}$ ) 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 Kalasin, Surin, and Saraburi provinces were collected. The carbon, nitrogen, and oxygen isotopic compositions of rice were analyzed by EA-IRMS.

The results of  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and  $\delta^{18}\text{O}$  values of Khaowong sticky rice, Pakaumpul local rice, and Jek Chuey Sao Hai rice obtained from Kalasin, Surin, and Saraburi provinces, respectively are summarized in Fig. 1. Box plots A, E and H represent  $\delta^{13}\text{C}$  values Khaowong sticky rice, Pakaumpul local rice, and Jek Chuey Sao Hai rice from Kalasin, Surin, and Saraburi provinces, respectively, whereas box plots (B, F and I) and (C, G and J) represent  $\delta^{15}\text{N}$ , and  $\delta^{18}\text{O}$  values, correspondingly. The  $\delta^{13}\text{C}$  values of Khaowong sticky rice, Pakaumpul local rice, and Jek Chuey Sao Hai rice ranged from -27.82‰ to -27.14‰, -29.45‰ to -28.72‰, and -28.62‰ to -27.70‰, respectively. However, it was found that the  $\delta^{13}\text{C}$  values of all rice samples were in the range of  $\delta^{13}\text{C}$  values of C-3 plants (-22‰ to -33‰) [7]. The  $\delta^{15}\text{N}$  values of Khaowong sticky rice, Pakaumpul local rice, and Jek Chuey Sao Hai rice were between +3.20‰ to +5.37‰, +1.88‰ to +6.46‰, and +3.77‰ to +4.98‰, respectively. The  $\delta^{18}\text{O}$  values in Khaowong sticky rice, Pakaumpul local rice, and Jek Chuey Sao Hai rice ranged from +24.21‰ to +26.68‰, +20.58‰ to +22.99‰, and +23.41‰ to +27.18‰, respectively. The heaviest one was obtained in Jek Chuey Sao Hai rice sample cultivated in Saraburi province whereas the lightest one was Pakaumpul local rice sample cultivated in Surin province.

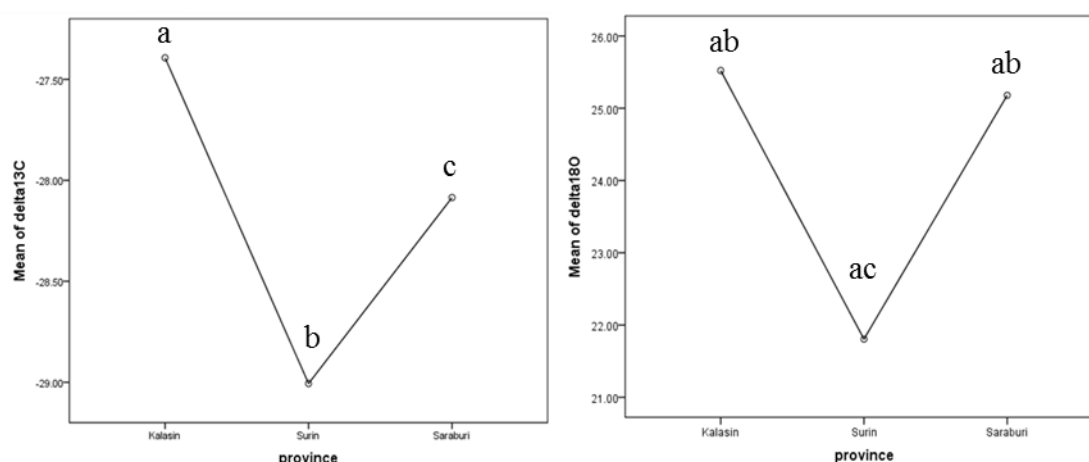


**Figure 1.** Box plot of  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and  $\delta^{18}\text{O}$  values of rice originated from Kalasin, Surin, and Saraburi provinces.

**Table 1.** Descriptive statistic and ANOVA for Thai rice samples from different provinces.

Isotope ratio	Province	Mean	S.D.	Sig.
$\delta^{13}\text{C}$	Kalasin	-27.39	0.19	0.000
	Surin	-29.01	0.24	
	Saraburi	-28.09	0.28	
$\delta^{15}\text{N}$	Kalasin	4.35	0.66	0.987
	Surin	4.28	1.84	
	Saraburi	4.36	0.41	
$\delta^{18}\text{O}$	Kalasin	25.52	0.71	0.000
	Surin	21.81	1.07	
	Saraburi	25.18	1.18	

From ANOVA test at  $p < 0.05$ , the significant values for  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and  $\delta^{18}\text{O}$  were 0.000, 0.987, and 0.000, respectively. This indicates that there was no significant difference in  $\delta^{15}\text{N}$  values whereas the mean values of  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  found in Thai rice samples were significantly different in the three different cultivation area at 95% confidence interval. Multiple comparison or post hoc was further analyzed using LSD test. The significant different in the mean values of  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  was depicted in Fig. 2. The values of  $\delta^{13}\text{C}$  in Khaowong sticky rice, Pakaumpul local rice, and Jek Chuey Sao Hai rice were significantly different from each other. The values of  $\delta^{18}\text{O}$  in Pakaumpul local rice obtained from Surin province in those in Khaowong sticky rice and Jek Chuey Sao Hai rice. The values of  $\delta^{15}\text{N}$  local Thai rice in three different cultivation area could be distinguished to each other.



**Figure 2.** Average stable isotope ratio of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in Thai rice cultivated in Kalasin, Surin, and Saraburi provinces. Data are analyzed by ANOVA using LSD test and different letters above each average point showed significant difference at  $p < 0.05$ .

#### 4. Conclusion

Three isotopes ( $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and  $\delta^{18}\text{O}$ ) in Khaowong sticky rice, Pakaumpul local rice, and Jek Chuey Sao Hai rice cultivated from Kalasin, Surin, and Saraburi provinces, respectively were determined by EA-IRMS. The values of  $\delta^{13}\text{C}$  in Khaowong sticky rice, Pakaumpul local rice, and Jek Chuey Sao Hai rice cultivated from three different provinces were significantly different from each other. The values of  $\delta^{18}\text{O}$  in Pakaumpul local rice were significantly different from those in Khaowong sticky rice and Jek Chuey Sao Hai rice at 95% confidence interval. Our results may provide preliminary information for discrimination of rice samples and it may be possible to trace the geographical origin of rice cultivated from different regions.

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