

# A pilot study of stable isotope ratio and elemental compositions in Leb Nok rice in the south of Thailand

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**Abstract.** In recent years, local Thai rice has been sharp-rising demands for consumers with high purchasing power who are willing to pay for nutritious grains and specialty rice. Leb Nok rice is traditional southern variety with high nutritious and popular rice grown in the south of Thailand. This study was done to investigate the isotopic compositions ( $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$ ) and the elemental compositions (%C, %N and %O) in rice samples obtained from different provinces of cultivation. Forty-one samples of Leb Nok rice were collected from Phatthalung and Nakhon Si Thammarat provinces. The  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ,  $\delta^{18}\text{O}$ , %C, %N and %O values were determined using elemental analyzer isotope ratio mass spectrometry (EA-IRMS). Our result found that  $\delta^{18}\text{O}$ , %O and %C in Leb Nok rice samples were significantly different but there was no evidence that  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and %N was significantly different among the provinces at 95% confidence interval. The  $\delta^{18}\text{O}$ , %O and %C values showed good potential in differentiating Leb Nok rice from different geographical provinces. This technique may be possible to trace the geographical origin of rice cultivated from different regions in Thailand.

## 1. Introduction

Rice is a staple cereal that nurtures the lives of the Thai people and more than half of the world's population [1]. Strains of rice in Thailand are diverse. Each region of the country has its own variety of local rice that are well recognised. It is considered to have economic value as they are popular among local and international consumers. Leb Nok rice is a local rice variety, mostly grown in the south of Thailand. It contains high nutrition and high fiber content which can prevent brain disease, constipation, reduce blood pressure and prevent cancer in the intestines.

The geographical origin of healthy rice product is an essential issue in order to prevent mislabeling and adulteration problem [2]. Generally, the isotopic compositions of plant materials reflect various factors such as isotopic compositions of source materials (e.g.,  $\text{CO}_2$ ,  $\text{H}_2\text{O}$  and  $\text{NO}_2$ ) and their assimilation processes as well as growth environments, especially the climate and altitude [3]. The carbon isotopic composition of plant materials strongly depends on the carbon fixation process such as the C-3, C-4 or crassulacean acid metabolism (CAM) cycle, while the nitrogen isotopic composition mainly depends on soil nutrition [4]. Therefore, characteristics of the isotopic compositions have been widely used to investigate the authenticity of food materials. Moreover, the geographical origin of food such as meat [5], dairy products [6], and rice [7] can also be test by EA-IRMS

In this work, stable isotope ratio and elemental compositions in Leb Nok rice cultivated from 2 provinces in the south of Thailand, Phatthalung and Nakhon Si Thammarat provinces were determined using elemental analyzer isotope ratio mass spectrometry (EA-IRMS). The t-test at 95% confidence interval was also evaluated.

## 2. Experimental

### 2.1. Rice samples

Forty-one rice samples were collected from paddy fields from Phatthalung (n=19) and Nakhon Si Thammarat (n=22) provinces. Samples were dried and pulverized to fine powder by agate mortar. Then, the samples were dried at 60 °C for 48 h in oven and kept in desiccator prior to analysis.

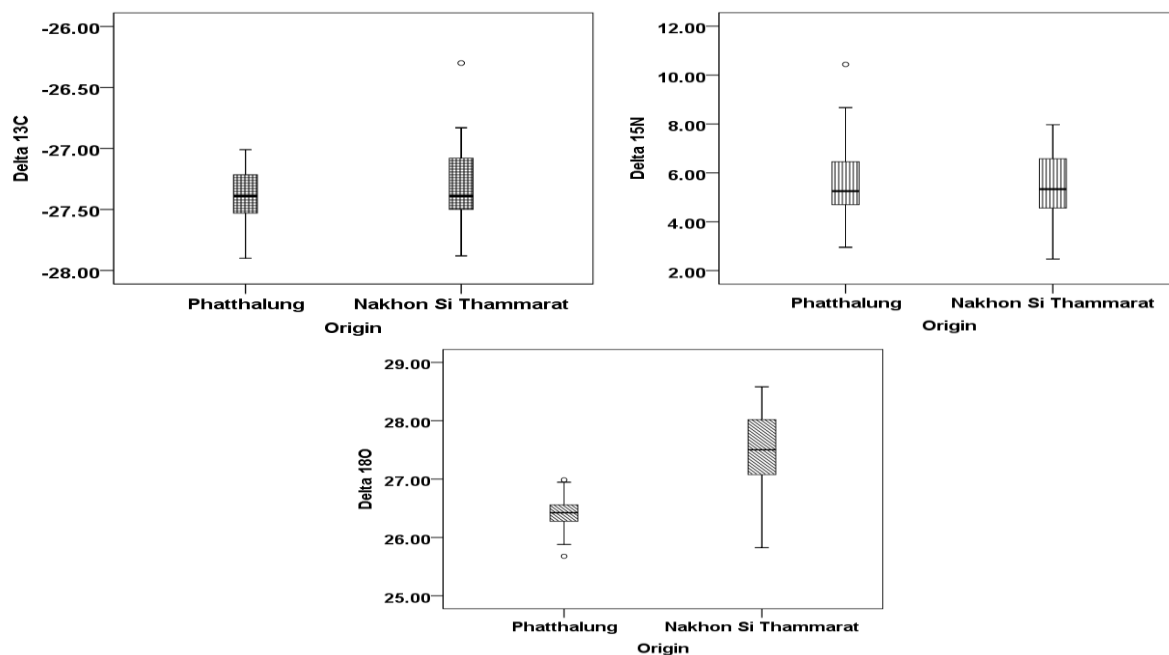
### 2.2. Isotope Ratio Mass Spectrometry (IRMS) analysis

The percentages (%) of carbon, nitrogen, and oxygen were determined by elemental analyzer (EA). The stable isotope ratio analysis ( $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  values) was performed by elemental analyzer isotope ratio mass spectrometry (EA-IRMS). The ground rice powder (3.50-4.50 mg) were weighed into tin capsules for carbon and nitrogen stable isotope ratio analysis. For oxygen, the powdered rice samples (0.30 mg) were weighed into silver capsules prior to EA-IRMS analysis in the pyrolysis mode. The experiment was performed in triplicate.

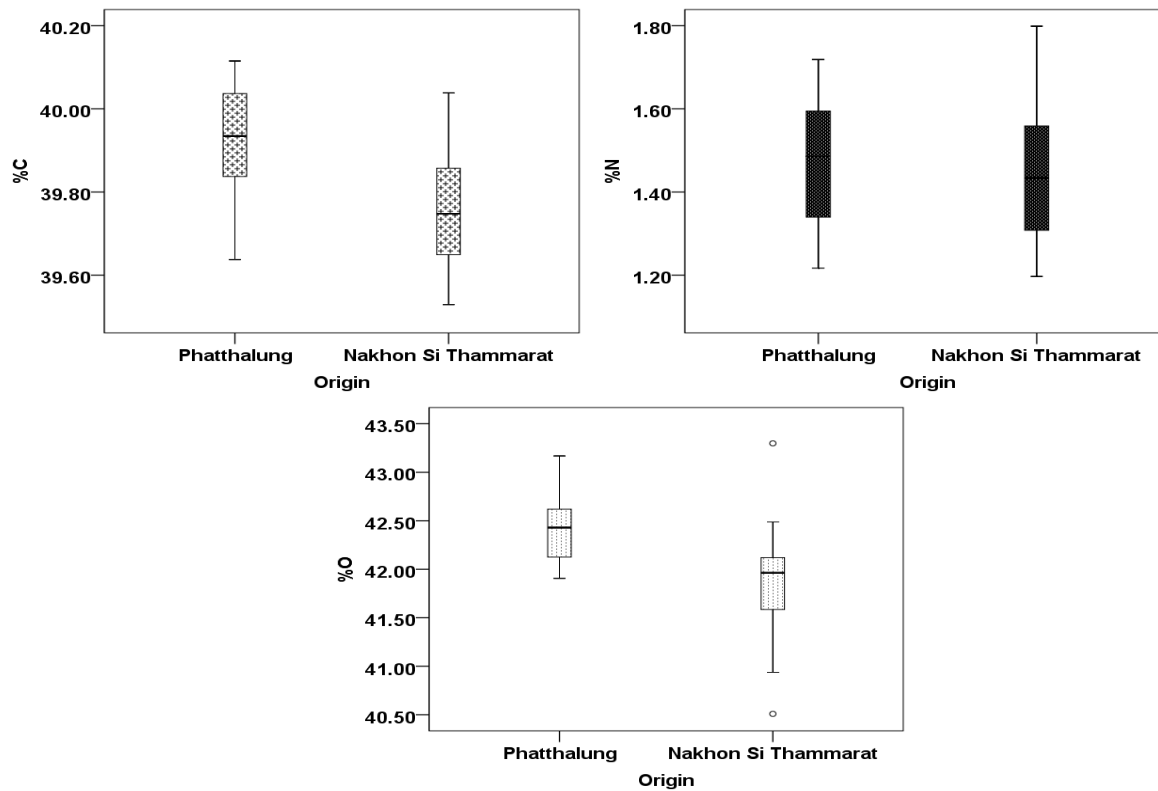
## 3. Results and discussion

The results are presented as box plots in Fig.1 and 2. Different elements found are presented by different symbols. The ranges of elements, maximum, minimum, and median found in the samples are also depicted. 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  $\delta$  (‰) 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  $^{18}\text{C}/^{16}\text{C}$ ) of the sample and isotope ratio of international standard, respectively.

The average and RSD of the interested elements found in Leb Nok rice samples in 2 provinces of Thailand are illustrated in the Table 1. T-test was evaluated for testing the difference of the isotopic compositions and the percentages of carbon, nitrogen and oxygen in Phatthalung and Nakhon Si Thammarat provinces. The p-values of %C, %N, %O,  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and  $\delta^{18}\text{O}$  were 0.001, 0.454, 0.001, 0.396, 0.630, and <0.0001, respectively. It indicates that %C, %O and  $\delta^{18}\text{O}$  were significantly different but there was no significant difference in %N,  $\delta^{13}\text{C}$ , and  $\delta^{15}\text{N}$  found in Leb Nok rice samples between Phatthalung and Nakhon Si Thammarat provinces at 95% confidence interval. The different in  $\delta^{18}\text{O}$  values between Phatthalung and Nakhon Si Thammarat provinces may probably due to the different source of water used in rice cultivation. This was implied that %C, %O and  $\delta^{18}\text{O}$  could be good indicators and possible to trace the geographical origin of rice cultivated from 2 provinces in Thailand.



**Figure 1.** Box plots of the  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  values in rice samples



**Figure 2.** Box plots of the %C, %N and %O values in rice samples

**Table 1** Descriptive statistical analysis of the isotopic compositions and the elemental compositions in Leb Nok rice samples.

Element	Average (Phatthalung)	Average (Nakhon Si Thammarat)	RSD (Phatthalung)	RSD (Nakhon Si Thammarat)	P(T≤Two-tail)
C (%)	39.916	39.758	0.141	0.135	0.001
N (%)	1.480	1.442	0.152	0.167	0.454
O (%)	42.426	41.887	0.329	0.576	0.001
$\delta^{13}\text{C}$ (‰)	-27.395	-27.308	0.255	0.375	0.396
$\delta^{15}\text{N}$ (‰)	5.739	5.491	1.841	1.432	0.630
$\delta^{18}\text{O}$ (‰)	26.409	27.517	0.320	0.721	<0.0001

#### 4. Conclusion

Six variables including %C, %N, %O,  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and  $\delta^{18}\text{O}$  values in Leb Nok rice samples cultivated in Phatthalung and Nakhon Si Thammarat provinces in the south of Thailand were determined by elemental analyzer isotope ratio mass spectrometry (EA-IRMS). %C, %O and  $\delta^{18}\text{O}$  values found in Leb Nok rice samples were significantly different between Phatthalung and Nakhon Si Thammarat provinces at 95% confidence interval. There was no significant difference in %N,  $\delta^{13}\text{C}$ , and  $\delta^{15}\text{N}$  values between Leb Nok rice cultivated in Phatthalung and Nakhon Si Thammarat provinces at 95% confidence interval. It may be possible to trace the geographical origin of rice cultivated from different regions in Thailand for the further study. Our results provide preliminary information for Thai rice samples according to stable isotope ratio and rice origins.

#### References

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