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# Elemental imaging and classifying rice grains by using laser ablation inductively coupled plasma mass spectrometry and linear discriminant analysis

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ABSTRACT

This study aims to investigate elemental imaging in a longitudinal section of single rice grain using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) and to classify rice according to their origins and types of 16 samples using LA-ICP-MS with linear discriminant analysis (LDA). The distributions of 8 essential elements (Ca, Cu, Fe, K, Mg, Mn, P and Zn) in a single rice grain were visualized as elemental images. Investigation of the elemental imaging of rice grain showed that essential elements were presented in large amounts in embryo and elevated level in endosperm. The elemental distributions of rice grain were not uniform. In addition, the concentration of 20 elements distributed in core endosperm was evaluated and used as chemical indicator to discriminate the origin and type of rice samples. The LDA can successfully differentiate rice samples according to their regions of origin (Northeast or South regions of Thailand) and types. Satisfied classifications are obtained with overall correct classification and cross-validation of 93.8% and 91.1% for origin classification and 100% and 97.9% for type classification.



## 1. Introduction

Rice (*Oryza sativa L.*) is the most important food crop in the world, providing over 21% of the calorie intake needs of the world's population and up to 76% for the population of South East Asia (Fitzgerald et al., 2009). It is an important source of energy, vitamins, essential elements and rare proteins for human. Brown rice is composed of four distinct tissues including bran (6–7% w/w), aleurone layer and embryo (2–3% w/w) and endosperm (90% w/w) (Chen et al., 1998). The bran is outermost part which is rich in oil containing oryzanol, tocotrinols, proteins, vitamin, and essential elements (Basnet et al., 2014; Shin et al., 1997). The aleurone layer and embryo are in inner grain which is rich of protein (6–12%w/w) and starch (Glimn-Lacy and Kaufman, 2006). Endosperm which consists of aleurone layer formed outer layer and starchy part, is rich in starch and proteins (Basnet et al., 2014). The nutrients mainly exist in embryo and bran layer which is minor content in rice grain.

Micronutrient deficiencies involving in iron (Fe) and zinc (Zn) are the most prevalent deficiency-related health disorders in the world. Nearly 3.7 billion people worldwide were iron-deficient and the problem was severe enough to cause anemia in 2 billion people (Gregorio et al., 1999). Rice grains which are main food, can be an important source of mineral for human. Therefore, many attentions are to increase amount and bioavailability of essential elements in rice grains. Elemental distribution information is important for improving rice quality. It provides the answer of the information on basic questions for biomedical research as well as enables bioaccumulation and bioavailability studies for ecological and toxicological risk assessment in humans, animals, and plants (Becker et al., 2014).

To gain elemental distribution information, several analytical techniques have been used such as X-ray fluorescence spectrometry (Gholap et al., 2010), synchrotron radiation X-ray fluorescence spectrometry (Wang et al., 2010), scanning electromicroscopy-energy dispersive X-ray spectrometry (Vázquez et al., 2013), glow discharge optical emission spectrometry (Gamez et al., 2012), auger electron spectroscopy (Sánchez-Amaya et al., 2012), secondary ion mass spectroscopy (Sui et al., 2015), and LA-ICP-MS (Wu and Becker, 2012). These techniques provide high spatial resolution

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(nm-μm) with good sensitivities (μg/g or ng/g) and possible quantification. In recent years, bioimaging using LA-ICP-MS has been focused due to its advantages such as minimal sample preparation, high sample throughput, access to isotopic information, the capability to quantify trace elements with high spatial resolution (1–100 μm), and the possibility of analyzing both conductive and non-conductive and opaque and transparent materials (Becker et al., 2014; Günther and Hattendorf, 2005). The LA-ICP-MS with spot ablation analysis was carried out to characterize the localization of arsenic (As) across the rice grain due to uniform carbon (C) signal in longitudinal section of single polished (white) and unpolished (brown) rice grain. The lowest As accumulation was found in core endosperm. However, the quantitative analysis for As accumulation was not performed (Meharg et al., 2008). LA-ICP-MS has been used to observe iron distribution in the endosperm of transgenic rice using single line scan ablation. The iron was accumulated in spots (Wirth et al., 2009). Spatial distributions of trace elements (As, Cd, Pb, Sb and Zn) in single rice grain from contaminated rice fields were imaged by using LA-ICP-MS (Basnet et al., 2014).

Nowadays, there is growing interest in research related to identification of the geographic origin of a wide range of agricultural food products. The determination of food authenticity is important issue in quality control and safety of food. The applications of multi-element analysis for discrimination of rice grain have been used to classify the origin or type using several analytical methods and data interpretations. The inductively coupled plasma atomic emission spectrometry (ICP-AES), ICP-MS and flame atomic absorption spectrometry (FAAS) with principal component analysis (PCA) were used to classify various rice samples collected in Australia and Vietnam by 14 elements (Al, As, Ca, Cd, Cu, Fe, K, Mn, Mo, Na, Ni, P, and Zn) (Kokot and Dong Phuong, 1999). The elemental analyzer/isotope ratio mass spectrometry (EA/IRMS) with radar plot was carried out to classify rice from Australia, Japan and USA (Suzuki et al., 2008). The high resolution inductively coupled plasma mass spectrometry (HR-ICP-MS) in coupled with radar plot, PCA and discriminant analysis (DA) was used for the discrimination of the origin of rice samples in Thai jasmine rice samples and foreign rice samples from France, India, Italy, Japan and Pakistan by 21 elements (Al, As, B, Ba, Cd, Co, Cr, Cs, Cu, Fe, Mg, Mn, Mo, Ni, Pb, Rb, Se, Sr, Ti, V and Zn) (Cheajesadagul et al., 2013). The ICP-AES and chemometrics with PCA and partial least-squares discriminant analysis (PLS-DA) was carried out to determine the authenticity of the geographical origin of rice from China, Korea and Philippines by 11 elements (Ag, Ba, Bi, Ca, Cd, Cr, Cu, In, K, Pb and Zn) (Chung et al., 2015). Solution based analytical techniques were carried out to quantify multielement composition in rice grain samples for discriminant analysis. Interestingly, solid based analytical techniques such as LA-ICP-MS has been successfully reported to provide element concentration of various samples due to its advantages over other techniques including direct analysis of solid sample, reduced laboratory preparation and ability to provide information on trace element isotopes (Basnet et al., 2016; Cheajesadagul et al., 2011; Halicz and Günther, 2004; M-M et al., 2011). Thus, LA-ICP-MS is a good option for quantification of multielement composition in rice grain samples for discriminant analysis. In Thailand, rice product is a major agriculture which provides around 30 million tons per year. Major rice agriculture regions are the Northeast and South. The two third of rice production is reserved for nationwide consumption while the rest is exported worldwide. Some rice varieties are more popular and thus more expensive than the others which are grown in specific area in the Northeast while some specific variety is only grown in the South. Therefore, it is essential to investigate the elemental and authenticity information in rice grain from the Northeast and South regions of Thailand.

To obtain information of elemental images and authenticity in rice grain, our purposes are (1) to investigate elemental distribution in single rice grain containing embryo and endosperm by elemental imaging using LA-ICP-MS and (2) to classify rice grain samples from the Northeast (NE) and the South (S) regions of Thailand according to their origins and types using data obtained from LA-ICP-MS and LDA. From our knowledge, this is the first time that the information obtained from LA-ICP-MS has been used to classify origin and type of rice grain samples.

## 2. Materials and methods

### 2.1. Sample information and preparation

For elemental imaging, the white rice grain sample from NE was cut in a longitudinal section by ceramic knife.

For elemental composition in core endosperm, 16 rice grain samples from 2 regions of Thailand were collected including 8 samples from NE and 8 samples from S, which were separated by types according to their colors. There are 8 white rice samples from NE and 5 white, 1 black, 1 red and 1 yellow rice samples from S. The rice grain samples were embedded in 2.5 cm diameter of resin. Epo-Kwick resin (Buehler, Lakebruff, IL, USA) was used. The rice grain samples were placed on sticky tape then covered with resin block. The Epo-Kwick solution was added into resin block for overnight to obtain rigid resin. The block was polished by polishing system, LaboPol-5 (Struers, Ballerup, Denmark). The samples were polished to get flat surface at the middle of grain readily to be analyzed by LA-ICP-MS.

### 2.2. Quantification

Quantification was obtained by using rice flour standard reference material, NIST 1568a, from the National Institute of Standard and Technology (Gaithersburg, MD, USA) as an external standard which is capable of providing acceptable accuracy and precision. Matrix matched standard is used to correct fractionation effects and also compensate for matrix effects produced in the ICP-MS (Hare et al., 2012). The external standard was prepared by mixing with Teflon powder (VHG Labs, Manchester, NH, USA) and pressing as a pellet. For bioimaging, $^{13}C$ was used as an acceptable internal standard element to compensate the conditions change (Hare et al., 2012).

ICP-MS data obtained after the ablation were exported to Microsoft Office Excel 2007 (Microsoft Cooperation) for all data treatment. Data reduction was performed using method reported by Longerich et al. (Longerich et al., 1996). The concentration of the analyte element in sample ($C_{A,SAM}$) is given by the count rate for the analyte in the sample ($R_{A,SAM}$) divided by the normalized sensitivity (S), as follows:

$$C_{A,SAM} = \frac{R_{A,SAM}}{S}$$

The normalized sensitivity (S) is the sensitivity, determined on a calibration standard (STD), corrected for the volume (mass) of sample ablated. When using naturally occurring internal standards, the sensitivity (cps per unit of concentration) normalized to the mass of the sample (SAM) ablated in the determinations is:

$$S = \frac{R_{A,STD}}{C_{A,STD}}\left(\frac{R_{IS,SAM}}{R_{IS,STD}}\frac{C_{IS,STD}}{C_{IS,SAM}}\right)$$

where $R_{A,STD}$ is the count rate of the analyte in the standard