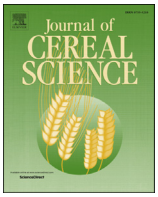
Journal of Cereal Science 65 (2015) 252–259

Contents lists available at ScienceDirect

Journal of Cereal Science

journal homepage: www.elsevier.com/locate/jcs



Discrimination of geographical origin of rice (*Oryza sativa* L.) by multielement analysis using inductively coupled plasma atomic emission spectroscopy and multivariate analysis

Ill-Min Chung [a], Jae-Kwang Kim [b], Jae-Keun Lee [c], Seung-Hyun Kim [a, *]

[a] *Department of Applied Bioscience, College of Life and Environmental Science, Konkuk University, Seoul 143-701, Republic of Korea*
[b] *Division of Life Sciences and Bio-Resource and Environmental Center, Incheon National University, Incheon 406-772, Republic of Korea*
[c] *Department of Applied Plant Sciences, College of Agriculture and Life Science, Kangwon National University, Chuncheon 200-701, Republic of Korea*

ARTICLE INFO

Article history:
Received 10 October 2014
Received in revised form
6 July 2015
Accepted 10 August 2015
Available online 10 August 2015

Keywords:
Rice geographical origin
Multielements
ICP-AES
Chemometrics

ABSTRACT

This study aims to determine the authenticity of the geographical origin of rice using inductively coupled plasma atomic emission spectroscopy (ICP-AES) and chemometrics. The profiles of 25 elements in brown rice measured by ICP-AES were subjected to data-mining processes, including principal component analysis (PCA) and partial least-squares discriminant analysis (PLS-DA). PLS-DA clearly discriminated the geographical origin of rice samples grown in three countries. Eleven elements (Cu, Ag, Zn, Cr, Ca, Ba, Cd, Bi, K, Pb, and In) significantly contributed to the ability to discriminate the geographical origin of the rice. These results demonstrate the use of multielement profiling combined with chemometrics as a tool for discriminating food origins. This study extends our knowledge about the applications of both multielement profiling and chemometrics for the determination of food authenticity, and thus can be useful for controlling the geographical origin of rice by governmental administration and protecting consumers from improper domestic labeling.

1. Introduction

Rice (*Oryza sativa* L.) is the third largest food crop next to wheat and corn, and its world annual production as a milled rice was 463,215 Kilotons between 2012 and 2013. China is the world's largest rice producing country and produced 142,000 Kilotons between 2012 and 2013 (USDA, 2014). Approximately 50% of the world's population uses rice as a main food source because of the calorie content and various nutrients including minerals and vitamins present in rice (Shimamoto, 1995; USDA, 2013; Wu et al., 2002). Furthermore, rice also contains various phytochemicals (i.e., γ-oryzanol, polyphenols), which are reported to have some health-promoting potentials, including antioxidant and anticancer properties (Cicero and Gaddi, 2001; Mao et al., 2003; Min et al., 2012).

In Korea, rice has been continually considered to be the most valuable food crop, and its production as a milled rice was 4300 Kilotons between 2012 and 2013, thereby making it the highest produced food crop (USDA, 2014). As per the regulations of the World Trade Organization (WTO), which was established in 1995, the rice market in Korea will open without a special protective tariff among different countries from 2015. However, in general, many Koreans believe domestic rice has better nutritional and/or organoleptic quality compared to rice cultivated in foreign countries. Thus, the determination of rice authenticity, i.e., geographical origin, has become a critical concern for government regulatory agencies and/or consumers (Heaton et al., 2008).

Because of the increasing interest/concern about food safety and authenticity, European Union (EU) countries have designated labels on agricultural products since September 2000. This regulation can help to confirm food authenticity and traceability, i.e., geographical origin (Boner and Forstel, 2004; Osorio et al., 2011). EU created the official classifications, “protected designation of origin (PDO)” and

---

List of abbreviation: AES, atomic emission spectroscopy; ICP-AES, inductively coupled plasma atomic emission spectroscopy; ICP-MS, inductively coupled plasma mass spectrometry; IR, Infrared spectroscopy; IRMS, isotope ratio mass spectrometry; LOD, limit of detection; LOQ, limit of quantitation; NMR, nuclear magnetic resonance; LSD, least significant difference; PCA, principal component analysis; PDO, protected designation of origin; PGI, protected geographical indications; PLS-DA, partial least-squares discriminant analysis; VIP, Variable importance in the projection.

* Corresponding author.
E-mail address: kshkim@konkuk.ac.kr (S.-H. Kim).

http://dx.doi.org/10.1016/j.jcs.2015.08.001

"protected geographical indications (PGI)", to confirm the authenticity and quality of foodstuffs traded between the EU countries. PDO is a brand used for foodstuffs with a strong regional identity that are produced, processed, and prepared in a specific geographical area using particular techniques. PGI relates to agricultural products and foodstuffs closely linked to a geographical area in at least one of the stages of production, preparation, or processing (Furia et al., 2011).

The discrimination of the geographical origin of various foodstuffs has been achieved using a combination of multivariate analysis and/or several analytical methods like mass spectrometry (i.e., IRMS, ICP-MS), spectroscopic techniques (i.e., NMR, IR, AES), separation (i.e., HPLC, GC), and other (i.e., sensory or DNA analysis) techniques (Benabdelkamel et al., 2012; Luykx and van Ruth, 2008). For example, the analysis of either trace elements or secondary metabolite profiles, like carotenoids, was applied to determine the geographical origin of orange juice, lemon juice, or valencia juice (McHard et al., 1979; Mouly et al., 1999). Furthermore, stable isotope ratio analysis of H, C, N, S, and $^{87}Sr/^{86}Sr$ was also able to discriminate the geographical origin of orange juice (Rummel et al., 2010). In addition, recently, multielement analysis using ICP-MS or ICP-AES has been effectively used to discriminate the geographical origin of various agricultural products. This technique has been successfully applied to the determination of the geographical origin of vegetables, nut, tea, olive oil, milk, juice, and wine because the elemental patterns in agricultural products are related to the patterns seen in soil (Benabdelkamel et al., 2012; Benincasa et al., 2008; Furia et al., 2011; Lo Feudo et al., 2010; Luykx and van Ruth, 2008).

Some prior studies reported a difference in the elemental pattern of rice cultivated in different countries or provinces within the same country. They found that the $\delta^{13}C$, $\delta^{18}O$, B, Ho, Mg, Rb, Se, and/or $^{87}Sr/^{86}Sr$ in rice grains were critical markers for discriminating the geographical origin of rice (Kawasaki et al., 2002; Kelly et al., 2002). Compared to other foodstuffs like juice, wine, and milk, however, there is still insufficient information related to the determination of the geographical origin of rice. Therefore, the present study aims to provide elemental (/or chemical) markers to identify the rice cultivated in different Asian countries in the same year. Thus, in the present study, 25 elemental composition analyses of the same brown rice cultivated in Korea, China, and the Philippines in 2012 were conducted using inductively coupled plasma atomic emission spectroscopy (ICP-AES). Furthermore, the present study also examined the annual variations in the 25 elemental patterns in brown rice cultivated in Korea from the year 2010–2012. In addition, we used principal component analysis (PCA) and partial least square-discriminate analysis (PLS-DA) to discriminate rice samples according to geographical origin. PCA has been used to perform a preliminary inspection of the data information distribution. PLS-DA is a well-established chemometric approach for supervised analyses based on a PLS model in which the dependent variable represents class membership. Hence, this preliminary study can provide a useful elemental marker for determining the geographical origin of the rice cultivated in different Asian countries. This study may be helpful in controlling the geographical origin of rice by governmental administration and/or for protecting consumers from improper domestic labeling.

2. Materials and methods

2.1. Chemicals and reagents

Nitric acid (HNO_3) was purchased from Showa Chemical Industry Co. Ltd. (Tokyo, Japan), and hydrogen peroxide (H_2O_2) solution was purchased from Sigma–Aldrich (Seoul, Korea). Ultrapure distilled water was obtained from a Zeneer power 1 system (Human Corporation, Seoul, Korea). Multielement calibration standards (product #: N9300233, N9300235) including Al, Ba, Ca, Cd, Co, Cu, Fe, Li, Mg, Mn, Ni, Pb, Se, In, Cr, Ti, V, Zn, Ag, Be, Bi, K, Na, Ge, and/or As were purchased from Perkin Elmer (Seoul, Korea). Furthermore, another multielement stock solution (Quality Control Standard 26, Catalog No. IV-26) was purchased from Inorganic Ventures (Christiansburg, VA). All other reagents used for sample digestion and instrumental analysis were of analytical grade. All materials, including glassware, flasks, and tubes, involved in sample preparation and measurement processes were cleaned with nitric acid (2%, v/v) by soaking overnight and rinsed with ultrapure water prior to use.

2.2. Brown rice source

The same brown rice samples, namely Hwacheong, Tong-il, Miryang ($n = 3$/cultivar), are obtained from Suwon, Korea (37°16′ N, 126°59′ E, 28.5 m a.s.l), Shanghai, China (30°54′ N, 121°24′ E, 5.6 m a.s.l), and Los Banos, Philippines (14°10 ′ N, 121°15′ E, 16.3 m a.s.l) in 2012 (Fig. 1). At Suwon, Korea, the same brown rice was also collected from 2010 to 2012 to investigate the annual variation in the 25 elements. The collected brown rice samples were stored at 8 °C and 45% relative humidity until analysis. All management, including the treatment of N, P, and K fertilizer and chemical pesticide/herbicide, was performed on the basis of the local recommendations during the cultivation period.

2.3. Digestion of the rice sample

Rice grain usually contains ~14% moisture; therefore, the harvested rice was lyophilized at −45 °C and then pulverized prior to digestion. The pulverized brown rice was pre-digested in nitric acid (HNO_3) as described by the EPA method (US Environmental Protection Agency, 2007.) Briefly, 500 mg of the pulverized rice sample was added to 7 mL of 70% HNO_3 and was kept at room temperature for 6 h. Then, 1 mL of H_2O_2 was added prior to the digestion, which was carried out using a microwave oven. The digestion temperature was gradually increased from 30 °C to 180 °C in 50 min, and the digestion was completed when the digested sample was colorless. Finally, the digested sample volume was adjusted to 50 mL with ultrapure distilled water. Blank samples were prepared in the same way.

2.4. Instrumentation and quantitation

An ICP-AES (Optima 7300 DV, Perkin Elmer, Korea) was used for the determination of the 25 elemental compositions and contents in brown rice. Brief operation conditions and optics of the ICP-AES used in the present study are as follows: axial mode plasma: 15 L min^{-1}, auxiliary: 0.2 L min^{-1}, nebulizer: 0.65 L min^{-1}, RF powder: 1300 W, flow rate: 1.5 mL min^{-1}. All elements, except K and Na (axial mode of ICP-AES), were measured in the radial mode of the ICP-AES.

An external calibration curve method was used for the quantitation of 25 elements in brown rice. Three to seven-point calibration curves, with an appropriate dilution, were used for quantitation, and the concentration ranges for the 25 elemental STDs are shown in Table 1. All the calibration curves had a good linearity ($R^2 > 0.994$) in the range investigated in the present study. The limit of detection (LOD) and limit of quantitation (LOQ) for the 25 elements were determined for each calibration curve as follows: $LOD = 3 \times SD/S$, $LOQ = 10 \times SD/S$, where, SD is the standard