Wageningen Academic Publishers

Verifying the provenance of rice using stable isotope ratio and multi-element analyses: a feasibility study

A. Li[1*], B. Keely[2], S.H. Chan[1], M. Baxter[3], G. Rees[3] and S. Kelly[3]

[1]Food Safety Laboratory, Applied Sciences Group, Health Sciences Authority, 11 Outram Road, 169078 Singapore, Singapore; [2]Department of Chemistry, University of York, Heslington, York YO10 5DD, United Kingdom; [3]The Food and Environment Research Agency, Sand Hutton, York YO41 1LZ, United Kingdom; angela_li@hsa.gov.sg

Received: 12 December 2013 / Accepted: 24 January 2014

RESEARCH ARTICLE

Abstract

Different varieties of rice grown in various countries around the world (Australia, China P.R., France, India, Italy, Japan, Korea, Malaysia, Myanmar, Pakistan, Spain, Taiwan, Thailand, USA and Vietnam) were analysed using isotope ratio mass spectrometry and inductively coupled plasma mass spectrometry. The stable isotope ratios of carbon, nitrogen, oxygen and hydrogen and the multi-elemental compositions were assessed as variables for discrimination of the geographical origins of the rice samples. The data were processed by canonical discriminant analysis (CDA) enabling classification according to geographical origin. Fifteen key variables were identified by CDA as providing the maximum discrimination between the rice samples across different rice types and categorised on the basis of broad geographical areas (Asia, Australia, Europe, India and Pakistan, North America and Southeast Asia), enabling 90.7% correct classification for the model generated. This feasibility study demonstrated that the methodology has good potential in identifying the geographical origin for different rice types and useful as a database for the examination of unknown rice samples.

Keywords: authenticity, canonical discriminant analysis, geographical origin, inductively coupled plasma mass spectrometry, isotope ratio mass spectrometry

1. Introduction

Rice is an important staple food for more than half of the world's population. It is also involved as an integral part of social rites, rituals and festivals in nearly all Asian countries. Rice farming can be traced back to about 10,000 years ago but the origin of its first development is still subject to much debate (Maclean *et al.*, 2002).

The fluctuations in the rice export prices over the years are reflections of the supply situation from the main rice producing countries. In addition, the introduction to the market of specialty varieties such as Basmati, Jasmine and Japonica rice has led to price differentiation between the general all-purpose long-grain rice and specialty rice. The premium price that consumers pay for such specialty rice has led to increasing emphasis on determining the geographical authenticity and traceability of such food commodities, because the superior quality is often attributed to a specific rice variety and/or production location e.g. basmati rice.

The prevalence of rice mislabelling and adulteration threatens the honest livelihood of rice traders and undermines both the consumer confidence in food quality and the reliability of labelling information. To overcome this problem, a variety of experimental approaches have been developed to determine the geographical origin of rice. As summarised by Vlachos and Arvanitoyannis (2008) in a review on rice authenticity, these analytical methods include isotope ratio mass spectrometry (IRMS), inductively coupled plasma mass spectrometry (ICP-MS), and inductively coupled plasma high resolution mass spectrometry (ICP-HRMS) (Kelly *et al.*, 2002; Yashui and Shindoh, 2000), multi-element, and multi-isotope analysis (Kelly *et al.*, 2005). In combination with the use

of multivariate analysis tools, the fingerprint or attribute that is unique to the country of origin of the rice could be differentiated. Notably, however, most of the studies concerning the geographical origins of rice were either limited to rice samples from a single country or were restricted to a few countries across different continents (Kawasaki *et al.*, 2002; Kelly *et al.*, 2002; Yasui and Shindoh, 2000). Others have reported their findings based only on a single variety of rice (Yasui and Shindoh, 2000; Kawasaki *et al.*, 2002; Suzuki *et al.*, 2008). With the current extent of globalisation in trade, there is a need to establish rice geographical origin data across the world, and incorporate information on different rice varieties, reflecting the disparities in rice consumption preferences globally. Such a database remains to be established.

In this paper, we expanded on the existing work done on rice geographical origin by extending the pool of rice samples in the study to span from countries in the East to the West, as well as across several rice varieties. The selected analytical approach involved the use of stable isotope and multi-elemental analysis by IRMS and ICP-MS, respectively. Finally, a chemometric study on the rice profiles was performed by applying canonical discriminant analysis (CDA).

2. Experimental

Rice samples

A total of 214 rice samples from 15 different countries were used in the study. The rice samples comprised of various rice types including japonica rice, jasmine rice, basmati rice, brown rice, wild rice, parboiled rice, glutinous rice, organic rice and broken rice. As these rice samples were mostly purchased from retailers in Singapore the information obtained for their countries of origin was based on the packaging label.

Stable isotope ratio analysis

Rice samples, 10-15 g, were ground to a fine powder using a Mixer Mill MM 400 (Retsch, Haan, Germany) at a milling frequency of 25 Hz for 30 seconds. The mill was carefully cleaned with deionised water and dried between each round of milling to avoid cross contamination between samples. One mg and three mg of the ground rice powder were then weighed into tin capsules (4×6 mm, obtained from Elemental Microanalysis, Cambridge, UK) for the respective carbon and nitrogen stable isotope ratio analyses, while two mg of the ground rice powder was weighed into similar silver capsules for the oxygen and hydrogen stable isotope ratio analyses.

Multi-elemental analysis

Rice grain samples (~0.5 g) were digested in 4.75 ml concentrated nitric acid (BDH Laboratory Supplies, Poole, UK) and 0.25 ml concentrated hydrochloric acid (Fisher Scientific, Loughborough, UK) using a microwave digestion system (PerkinElmer, Waltham, MA, USA) at a pressure of 75 bar. The sample digest was first diluted to 10 ml with high purity deionised water (Millipore, Billerica, MA, USA), after which 0.5 ml of this solution was further diluted with 4.5 ml of 1% nitric acid containing indium as an internal standard.

Isotopic and trace element reference materials

USGS 40 (L-glutamic acid), USGS 41 (L-glutamic acid), IAEA-601 (benzoic acid) and IAEA-602 (benzoic acid) were obtained from the International Atomic Energy Agency, Vienna, Austria. Beet sugar and wheat flour laboratory reference materials were obtained from the TRACE project. USGS 40 and USGS 41 have assigned $\delta^{15}N$ values of -4.5 and 47.6‰ versus air, respectively. IAEA-601 and IAEA-602 have assigned $\delta^{18}O$ values of 23.3 and 71.4‰ versus Vienna standard mean ocean water (VSMOW), respectively. Beet sugar has an inter-laboratory collated $\delta^{13}C$ value of -26.8‰ versus pee Dee belemnite (PDB) while wheat flour has an inter-laboratory collated $\delta^{2}H$ value of -43.2‰ versus VSMOW. Inductively coupled plasma multi-element standard solution was obtained from Glen Spectra (Middlesex, UK). The composition and concentration of the multi-element standard was as described in the accompanying certificate of analysis. Inductively coupled plasma single element standard solutions were obtained from VWR International (Leicestershire, UK), BDH (Poole, UK) and Johnson Matthey (London, UK). Trace element reference materials 1568a (rice flour) and 1547 (peach leaves) were obtained from National Institute of Standards and Technology (Gaithersburg, MD, USA). ZC 73013 (spinach) was obtained from China National Analysis Centre for Iron and Steel (Beijing, China P.R.). The certified values for constituent elements in the three trace element reference materials were detailed in the accompanying certificate.

$\delta^{13}C$ and $\delta^{15}N$ determinations

The capsules containing the rice samples were placed in the autosampler of the elemental analyser (EA) (Fisons, Ipswich, UK) and dropped into a vertical quartz tube maintained at a temperature of 1,020 °C. During the EA combustion process the helium flow was temporarily enriched with oxygen and the sample was oxidised in an instantaneous 'flash' combustion reaction. Quantitative combustion was achieved by passing the gas mixture over a catalyst layer of chromium oxide (Pelican Scientific, Stockport, UK) and a halogen scrubber layer of silvered cobaltous oxide (Pelican Scientific). The combustion gases were then passed over

copper grains (Pelican Scientific) at a temperature of 650 °C, in a second quartz tube, to remove residual oxygen and convert nitrogen oxides to nitrogen. Water was removed from the gas stream using a chemical trap (10 mm i.d., length 0.2 m) containing anhydrous magnesium perchlorate (Elemental Microanalysis, Cambridge, UK). Following the water trap, the gas mixture comprising CO_2 and N_2 was separated on a chromatographic column (Porapak QS, 2 m, 6×5 mm) heated at 35 °C. The effluent was transferred from the elemental analyser to the IRMS (Isoprime, Cheadle, UK) with helium as the carrier gas (ca. 85 ml/min), and the signal from ions at m/z 44, 45 and 46 was monitored for the carbon isotope ratio while the signal from ions at m/z 28, 29 and 30 was monitored for the nitrogen isotope ratio. The rice samples were analysed in duplicate for $\delta^{13}C$ and triplicate for $\delta^{15}N$ determinations. The repeatability of the $\delta^{13}C$ and $\delta^{15}N$ measurements was 0.3‰, based on the standard deviation of repeated determinations of in-house reference materials.

$\delta^{18}O$ and $\delta^{2}H$ determinations

The capsules containing the rice samples were placed in the autosampler of the elemental analyser (EuroVector, Milan, Italy) and introduced into a quartz reactor where the pyrolysis of the samples occurred at a temperature of 1260 °C. As described by Kelly *et al.* (2002) on the EA pyrolysis process, the pyrolysis gases were carried by a flow of helium carrier gas (ca. 100 ml/min) through the glassy carbon (HTW Hochtemperatur-Werkstoffe, Thierhaupten, Germany) packing material in the quartz tube. Residual water vapour and carbon dioxide produced were removed by a chemical trap (10 mm i.d., length 0.2 m) containing magnesium perchlorate (Elemental Microanalysis, Cambridge, UK). The pyrolysis gases then passed through a chromatographic column (molecular sieve, 5Å, 4 mm i.d., length 2 m) heated at 80 °C for the separation of H_2, N_2 and CO. A portion of the effluent was eventually transferred to the IRMS (Isoprime, Cheadle, UK) for the detection of m/z 28, 29 and 30 for oxygen isotope ratio. The rice samples were analysed in triplicates for the $\delta^{18}O$ determination. The procedure for $\delta^{2}H$ determination was largely similar to the $\delta^{18}O$ determination apart from the elemental analyser and IRMS (Thermo Scientific, Waltham, MA, USA) used for the detection of m/z 2, 3 and 4 for hydrogen isotope ratio. The rice samples were analysed in triplicate for the $\delta^{2}H$ determination. The repeatability of the $\delta^{18}O$ and $\delta^{2}H$ measurements were 0.8 and 3‰, respectively, based on the standard deviation of repeated determinations of in-house reference materials.

ICP-MS analysis

The instrument was calibrated using acid matched single and multi-element standards. Quality control consisted of acid blanks, a spiked blank and reference materials to ascertain detection limits, recovery and accuracy, respectively. Normal in-house criteria were applied to assess the quality of the data; these were: (1) instrumental drift from beginning to end of batch within ± 20%; (2) recoveries within ± 40%, with at least 75% within ± 20%; and (3) reference material values within 40% of certified values. The concentrations of 66 elements were determined simultaneously, including macro (e.g. aluminium, calcium, magnesium, sodium and potassium), micro (e.g. manganese, iron, cobalt, nickel, copper and zinc) and trace (e.g. strontium, molybdenum, cadmium, lead and the rare earths).

3. Results and discussion

Evaluation of $\delta^{13}C$ measurements in rice

The stable C, N, O and H isotope composition of the rice samples are summarised in Table 1. The table shows the mean value for each isotope parameter by geographical origin and the number of samples (n) obtained for each country of origin.

The measured $\delta^{13}C$ values were found to be consistent with the range for plants utilizing the Calvin photosynthetic cycle (C_3 plant materials). The $\delta^{13}C$ variation of the rice samples ranged from -28.85‰ to -25.52‰ (Table 1). Using a boxplot (Figure 1A) it was observed that the rice samples are not significantly distinguishable by their countries of origin based on the carbon isotopic data alone.

Depending on the environmental conditions experienced by the rice plants during their cultivation, variations in the stable carbon isotopic composition in plants may be observed. An example of such sources of variations includes altitudinal gradients where Körner *et al.* (1988) reported that $\delta^{13}C$ increased with altitude. Morecroft and Woodward (1990) attributed the altitudinal gradient primarily to temperature effects on gas exchange, from the extrapolations made from controlled-environment studies. Marshall *et al.* (2007) also reported that genetic variations within species may contribute to the differences in the $\delta^{13}C$ observed. Populations within a species often vary in $\delta^{13}C$ when grown in similar environments. Another source of $\delta^{13}C$ variation among rice samples may be observed in the presence of temperature or light changes (Smith *et al.*, 1976). In addition, Korenaga *et al.* (2010) reported that the ^{13}C enrichment in rice can be attributed to the degree of dryness during the paddy cultivation. In general, fractionation processes tend to discriminate against the heavier ^{13}C isotope.

Furthermore, plants have a physiological response to water stress which can result in stomatal closure, leading to a decrease in photosynthesis, transpiration and leaf conductance (Farquhar and Sharkey, 1982). The consequence would be decreased discrimination against

Table 1. A summary of the stable C, N, O and H isotope composition of rice samples for each country represented in the study.

Origin	n	Mean value[1]			
		$\delta^{13}C‰_{PDB}$	$\delta^{15}N‰_{AIR}$	$\delta^{18}O‰_{VSMOW}$	$\delta^{2}H‰_{VSMOW}$
Australia	4	-26.87	5.61	26.70	-33.75
China P.R.	4	-26.92	5.98	17.36	-60.92
France	3	-26.86	4.18	19.84	-48.75
India	30	-27.49	3.23	21.72	-46.94
Italy	5	-26.78	4.90	20.86	-57.42
Japan	27	-26.98	3.25	19.58	-59.89
Korea	2	-25.92	4.14	21.27	-54.16
Malaysia	6	-28.42	1.29	19.89	-53.08
Myanmar	2	-27.26	1.84	19.07	-44.71
Pakistan	18	-27.37	2.83	22.32	-46.98
Spain	3	-26.14	8.76	21.90	-43.62
Taiwan	10	-27.26	3.56	20.66	-44.33
Thailand	64	-27.33	2.81	20.23	-52.25
USA	20	-26.62	2.61	21.90	-53.03
Vietnam	16	-27.69	1.80	19.35	-50.80

[1] PDB = pee Dee belemnite; VSMOW = Vienna standard mean ocean water.

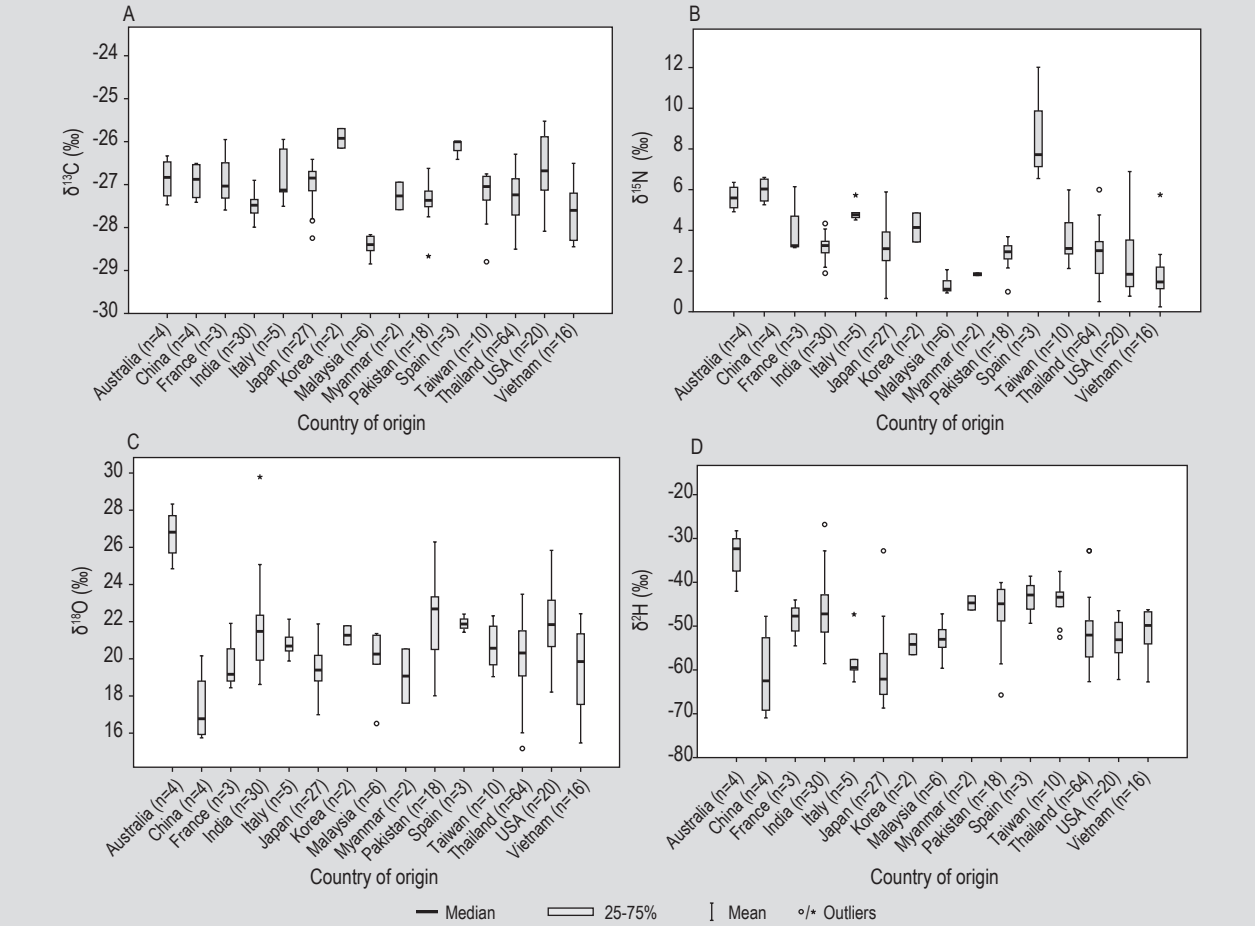


Figure 1. Box and whisker plot showing (A) $\delta^{13}C$, (B) $\delta^{15}N$, (C) $\delta^{18}O$ and (D) $\delta^{2}H$ variations for all rice types according to country of origin.

^{13}C and less negative $\delta^{13}C$‰ values. Water deficit stress in plants could occur due to insufficient soil moisture, an arid climate or a combination of both. Rice has been reported to be sensitive to drought stress, especially during the flowering stage, thus severe yield losses could result from water deficit (Liu *et al.*, 2006). Comparison between the mean $\delta^{13}C$ value in rice and the mean water vapour pressure of the cultivation regions of the respective countries (Table 2) during the rice growing months (Figure 2) was performed to test for a possible relationship (Figure 3). Water vapour pressure is defined as the atmospheric pressure which is exerted by water vapour and is one way of measuring the humidity of the air. For example, at a given temperature, an increase in water vapour in the air corresponds to an increase in the humidity of the air. The water vapour pressure information for the specific rice cultivation regions was obtained from the online climate estimator (FAO, 2002) from which the temperature data were obtained.

With reference to Figure 3, the general trend observed is that the greater the water vapour pressure, the more negative the $\delta^{13}C$ value of the rice samples. Thus, the higher the humidity, the more diminished the effect of water stress on the rice plant during growth. Hence, the photosynthetic and transpiration processes could result in discrimination against ^{13}C. It should also be noted that the some of the differences in the $\delta^{13}C$ values of rice within and between individual regions could be due to the different photosynthetic water use efficiencies (WUE). The relationship between $\delta^{13}C$ and WUE is attributed to the behavior of the carbon dioxide molecules as they enter a photosynthesising leaf (Marshall *et al.*, 2007). For example, if the decline in net photosynthesis is less than the decline in transpiration, WUE increases. The water vapour pressure information for the rice cultivation regions in Australia was unavailable; hence Australia was omitted from this comparison. The rice samples from Korea displayed the most marked deviation from this relationship, possibly due to its small sample size. Comparison with the exact growth location of the rice may clarify this deviation from the trend line.

Evaluation of $\delta^{15}N$ measurements in rice

The $\delta^{15}N$ variation of the rice samples ranged from 0.24 ‰ to 12.01 ‰ (Table 1). Using a boxplot (Figure 1B) it was observed that the variation in the $\delta^{15}N$ values is larger than that of the $\delta^{13}C$ values in general.

The nitrogen in soil that is available for plant utilisation is generally more enriched with the heavier isotope than atmospheric nitrogen. This is due to fractionation processes associated with the transformation of compounds of nitrogen in the soil environment. Biochemical and microbial processes such as nitrification, denitrification, colonisation of roots by mycorrhiza, ammonia volatilisation, and sorption of nitrogen to clay minerals are responsible for the preferential retention of ^{15}N relative to ^{14}N in soils (Husted *et al.*, 2004). As nitrogen is an essential element for plant growth, the supply of nitrogen from the naturally occurring indigenous sources (mainly soil and organic matter) is usually insufficient to give a high rice yield (IRRI, 2009). Addition of nitrogen fertiliser is generally an integral component of farming practice in irrigated and favourable rain fed rice farms in order to enhance yields and profit (IRRI, 2009). The nitrogen isotope ratio in plants can, to an extent, reflect the soil type in which the plant was grown. Since the actual agricultural practices applied to the rice samples are unknown, it can only be suggested that the intensive use of organic manures in Spain may have been likely to play an important role in the ^{15}N enrichment of those rice samples.

The observation that rice samples from Malaysia and Vietnam typically had $\delta^{15}N$ values lower than the mean whereas those from Australia and China P.R. typically had $\delta^{15}N$ values higher than the mean (refer to Figure 1B) can also be suggested to relate to agricultural practice. Organic fertilisers generally raise the ^{15}N content in soil and plants whereas the use of synthetic fertilisers decreases it (Bateman and Kelly, 2007). A higher $\delta^{15}N$ value was also reported in Australian rice by Suzuki *et al.* (2008). They noted that Australian farmers practice rotation of rice crops with pasture crops, thus allowing the grazing animals to fertilise the soil with their dung during the turn of pasture crops. As a result, the $\delta^{15}N$ value of the soil increases, which is consistent with the higher $\delta^{15}N$ values found in the Australian rice samples in this study.

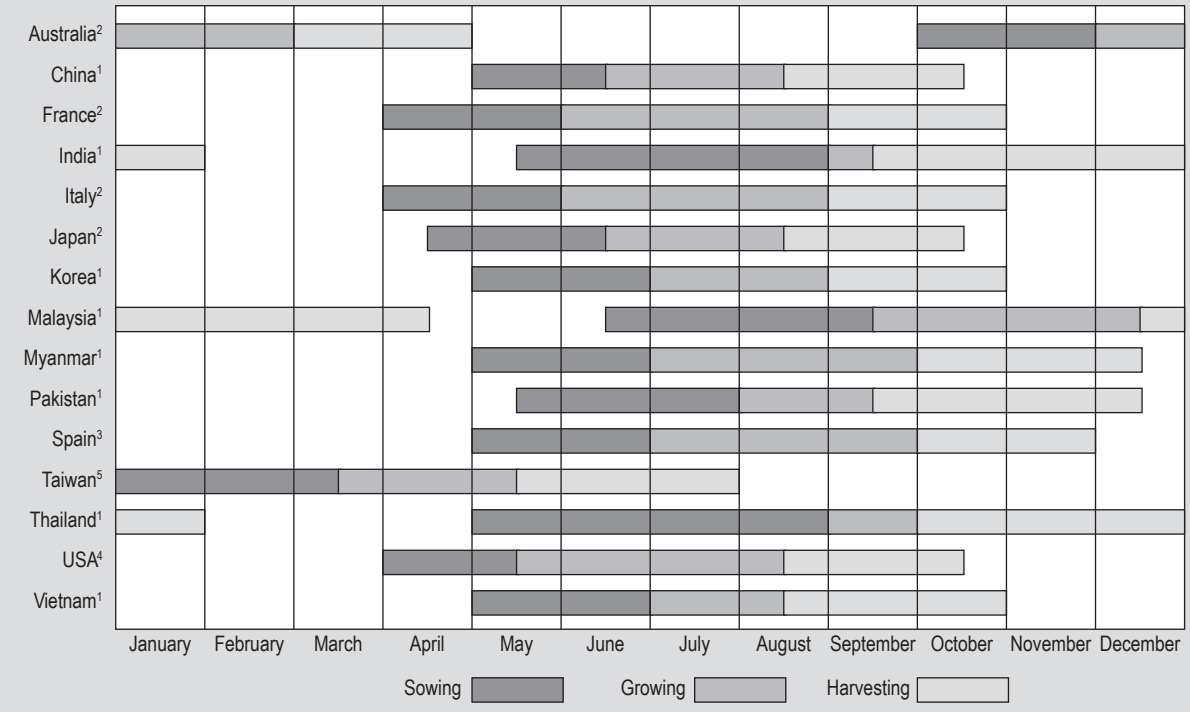
Evaluation of $\delta^{18}O$ measurements in rice

The $\delta^{18}O$ variation of the rice samples ranged from 15.17 ‰ to 29.79 ‰ (Table 1). In general, there was a significant variation in the $\delta^{18}O$ values found in the rice samples. With reference to Figure 1C, an interesting observation is that the rice samples from Australia possess noticeably higher $\delta^{18}O$ values than that from the other countries. The same phenomenon was reported by Suzuki *et al.* (2008) and Korenaga *et al.* (2010).

Water is not isotopically fractionated when taken up by plants (Dawson and Ehleringer, 1993). Thus, the water in plant tissues may possess a similar isotopic profile to the source water for plant growth. Waterhouse *et al.* (2002) reported a linear correlation between the oxygen isotope ratios in precipitation and in the α-cellulose of oak, assuming that the precipitation is the source water taken up by the roots. The same observations were demonstrated in plant stem cellulose by Sternberg *et al.* (2003) and in maize by Williams *et al.* (2005). As the actual source water used for the cultivation of the rice samples in this study is unknown, the predicted $\delta^{18}O$ value of the precipitation

Table 2. Rice cultivation regions of the countries in this study.

Country	Rice cultivation regions	Reference
Australia	New South Wales, Victoria	Ricegrowers' Association of Australia, http://www.rga.org.au/
China P.R.	Anhui, Beijing, Chongqing, Fujian, Gansu, Guangdong, Guangxi, Guizhou, Hainan, Hebei, Heilongjiang, Henan, Hubei, Hunan, Jiangsu, Jiangxi, Jilin, Liaoning, Nei Mongol Ningxia Hui, Qinghai, Shaanxi, Shandong, Shanghai, Sichuan, Tianjin, Xinjiang Uygur, Xizang, Yunnan, Zhejiang	IRRI, 1995
France	Camargue	Notteghem *et al.*, 1997
India	Andaman and Nicobar, Andhra Pradesh, Arunachal Pradesh, Assam, Bihar, Chandigarh, Chhattisgarh, Dadra and Nagar Haveli, Daman and Diu, Delhi, Goa, Gujarat, Haryana, Himachal Pradesh, Jammu and Kashmir, Jharkhand, Karnataka, Kerala, Lakshadweep, Madhya Pradesh, Maharashtra, Manipur, Meghalaya, Mizoram, Nagaland, Orissa, Puducherry, Punjab, Rajasthan, Sikkim, Tamil Nadu, Tripura, Uttar Pradesh, Uttaranchal, West Bengal	IRRI, 1995
Italy	Po Valley	Russo and Callegarin, 1997
Japan	Aichi, Akita, Aomori, Chiba, Ehime, Fukui, Fukuoka, Fukushima, Gifu, Gunma, Hiroshima, Hokkaido, Hyogo, Ibaraki, Ishikawa, Iwate, Kagawa, Kagoshima, Kanagawa, Kochi, Kumamoto, Kyoto, Mie, Miyagi, Miyazaki, Nagano, Nagasaki, Nara, Niigata, Oita, Okayama, Okinawa, Osaka, Saga, Saitama, Shiga, Shimane, Shizuoka, Tochigi, Tokushima, Tokyo, Tottori, Toyama, Wakayama, Yamagata, Yamaguchi, Yamanashi	IRRI, 1995
Korea	Busan, Chungcheongbuk-do, Chungcheongnam-do, Daegu, Daejeon, Gangwon-do, Gwangju, Gyeonggi-do, Gyeongsangbuk-do, Gyeongsangnam-do, Incheon, Jeju, Jeollabuk-do, Jeollanam-do, Seoul, Ulsan	IRRI, 1995
Malaysia	Johor, Kedah, Kelantan, Melaka, Negeri Sembilan, Pahang, Perak, Perlis, Pulau Pinang, Sabah, Sarawak, Selangor, Trengganu	IRRI, 1995
Myanmar	Ayeyarwady, Bago, Chin, Kachin, Kayah, Kayin, Magway, Mandalay, Mon, Rakhine, Sagaing, Shan, Tanintharyi, Yangon	IRRI, 1995
Pakistan	Baluchistan, N.W.F.P., Punjab, Sind	IRRI, 1995
Spain	Extremadura, Seville, Tarragona, Valencia	Herruzo and Morote, 1996
Taiwan	Changhua, Chiayi, Hsinchu, Hualien, Kaohsiung, Keelung, Miaoli, Nantou, Penghu, Pingtung, Taichung, Tainan, Taipei, Taitung, Taoyuan, Yilan, Yunlin	Council of Agriculture, Executive Yuan R.O.C. (Taiwan), 2008
Thailand	Amnat Charoen, Ang Thong, Bangkok Metropolis, Buri Ram, Chachoengsao, Chai Nat, Chaiyaphum, Chanthaburi, Chiang Mai, Chiang Rai, Chon Buri, Chumphon, Kalasin, Kamphaeng Phet, Kanchanaburi, Khon Kaen, Krabi, Lampang, Lamphun, Loei, Lop Buri, Mae Hong Son, Maha Sarakham, Mukdahan, Nakhon Nayok, Nakhon Pathom, Nakhon Phanom, Nakhon Ratchasima, Nakhon Sawan, Nakhon Si Thammarat, Nan, Narathiwat, Nong Bua Lam Phu, Nong Khai, Nonthaburi, Pathum Thani, Pattani, Petchaburi, Phangnga, Phattalung, Phayao, Phetchabun, Phichit, Phisanulok, Phra Nakhon Si Ayuttaya, Phrae, Phuket, Prachin Buri, Prachuap Khiri Khan, Ranong, Ratchaburi, Rayong, Roi Et, Sa Kaeo, Sakon Nakhon, Samut Prakan, Samut Sakhon, Samut Songkhram, Saraburi, Satun, Si Sa Ket, Sing Buri, Songkhla, Sukhothai, Suphan Buri, Surat Thani, Surin, Tak, Trang, Trat, Ubon Ratchathani, Udon Thani, Uthai Thani, Uttaradit, Yala, Yasothon	IRRI, 1995
USA	Arkansas, California, Louisiana, Mississipi, Missouri, Texas	USA Rice Federation, http://www.usarice.com/doclib/188/219/3674.PDF
Vietnam	An Giang, Ba Ria Vung Tau, Bac Giang, Bac Kan, Bac Lieu, Bac Ninh, Ben Tre, Binh Dinh, Binh Duong, Binh Phuoc, Binh Thuan, Ca Mau, Can Tho, Cao Bang, Da Nang, Dak Lak, Dak Nong, Dien Bien, Dong Nai, Dong Thap, Gia Lai, Ha Giang, Ha Nam, Ha Noi, Ha Tinh, Hai Duong, Hai Phong, Hau Giang, Ho Chi Minh, Hoa Binh, Hung Yen, Khanh Hoa, Kien Giang, Kon Tum, Lai Chau, Lam Dong, Lang Son, Lao Cai, Long An, Nam Dinh, Nghe An, Ninh Binh, Ninh Thuan, Phu Tho, Phu Yen, Quang Binh, Quang Nam, Quang Ngai, Quang Ninh, Quang Tri, Soc Trang, Son La, Tay Ninh, Thai Binh, Thai Nguyen, Thanh Hoa, Thua Thien Hue, Tien Giang, Tra Vinh, Tuyen Quang, Vinh Long, Vinh Phuc, Yen Bai	IRRI, 1995



[1]Global Information and Early Warning System (GIEWS) on food and agriculture, 2011.
[2]FAO, 2004.
[3]USDA, 2010.
[4]USDA, 1997.
[5]IRRI, 1995.

Figure 2. Crop calendar for rice.

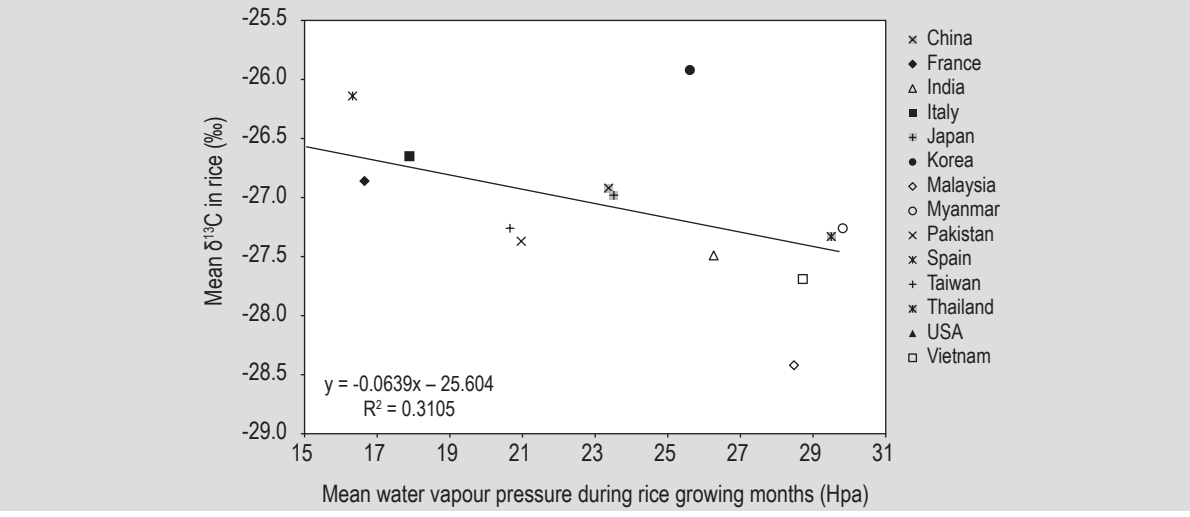


Figure 3. Comparison between the mean $\delta^{13}C$ value in rice and the mean water vapour pressure of the rice cultivation regions of the respective countries during the rice growing months. The water vapour pressures for the rice cultivation regions (listed in Table 2) were obtained from the online climate estimator (FAO, 2002).

was employed in this study. Estimated mean monthly oxygen isotope compositions of precipitation at specified locations were obtained from the online water isotope value calculator (Bowen, 2011). The mean predicted $\delta^{18}O$ values of the precipitation for the rice cultivation regions of the respective countries during the rice growing months show a generally linear relationship to the mean $\delta^{18}O$ values of the rice (Figure 4). It is possible that a better linear correlation may have been achieved if the specific rice cultivation location and growing conditions were available.

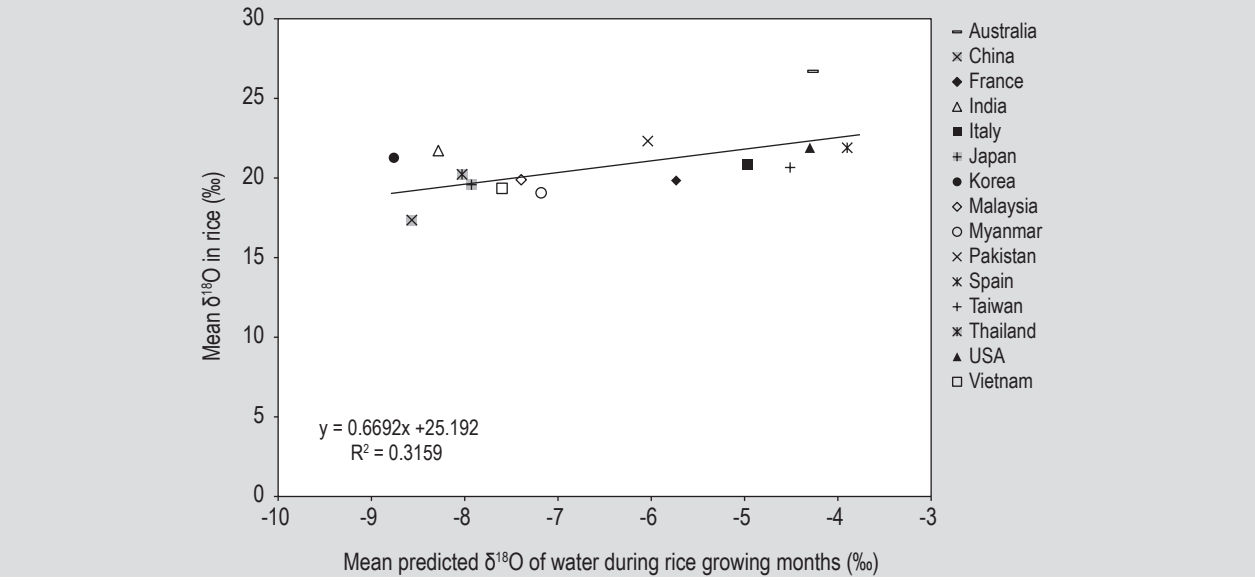


Figure 4. Comparison of the mean $\delta^{18}O$ value in rice and the mean predicted $\delta^{18}O$ value of precipitation at the rice cultivation regions of the respective countries during the rice growing months.

Several interesting points were made by Korenaga *et al.* (2010) on the $\delta^{18}O$ values in Australian, American and Japanese rice was in relation to the irrigation water used for the rice cultivation in these countries. They explained that Californian rice cultivation makes use of surface water supplied from a dam that stores meteoric water precipitated during the winter. As such, the water is more depleted in ^{18}O than precipitation at the growing period. As for the Japanese rice, the irrigation water used comprises snowmelt water which also has a much lighter $\delta^{18}O$ value than the usual precipitate. Therefore, the $\delta^{18}O$ value in rice is a reflection of the regional irrigation water used for cultivation. The same trend in $\delta^{18}O$ was found in this study where Australian rice had a higher $\delta^{18}O$ value, followed by American rice and then Japanese rice, suggesting that the irrigation practices in these geographic locations exert a strong control on the $\delta^{18}O$ values of the rice produced there.

The altitude effect on the $^{18}O/^{16}O$ in rice was also investigated in this study, using only those rice samples with specified rice cultivation regions on their packaging. Although the precise rice farm location is unavailable, a closer estimation of the cultivation conditions could be derived for comparison. The altitude of the rice cultivation regions (Table 2) was obtained from the online water isotope value calculator mentioned earlier (Bowen, 2011). The relationship between the mean $\delta^{18}O$ value in rice and altitude is illustrated in Figure 5 where the rice samples became more enriched in ^{18}O with increasing altitude. This observation may seem to oppose the theoretical elevation effect where $\delta^{18}O$ decreases with increasing altitude (Marshall *et al.*, 2007). In reality, however, the $\delta^{18}O$ in plants can be a result of the interplay of multiple factors. A possible explanation for the observed phenomenon in this study is that higher altitudes often lead to a lower atmospheric pressure. This, in turn, increases gas diffusivity and, with stomata conductance being dependent on gas diffusivity, plant transpiration rates would be substantially increased under hypobaric conditions (Gohil *et al.*, 2011). Since plant transpiration involves the loss of water into the environment, more ^{16}O will be lost from the leaves as the lighter isotope has a preference for the vapour phase. In this case, the altitude effect probably had a significant impact on the plant transpiration rate hence the rice samples became more enriched in ^{18}O at higher altitudes.

Evaluation of δ^2H measurements in rice

The δ^2H variation of these rice samples ranged from -70.95‰ to -26.81‰, as shown in Table 1. In general, there was a noticeable variation in the δ^2H values among the rice samples (Figure 1D) and this variation is similar to the $\delta^{18}O$ values found in the same rice samples. As discussed earlier, the hydrogen and oxygen isotopic profile of the source water for plant growth influences the δ^2H and $\delta^{18}O$ values in plant materials, hence the hydrological changes to the source water can lead to the same relative isotopic enrichment or depletion to both the hydrogen and oxygen isotopes.

Evaluation of the multivariate analysis of the multi-element and stable isotope data

The rice samples were subjected to microwave-assisted acid digestion prior to analysis by ICP-MS for simultaneous quantitative determination of 66 elements. All of the elements analysed, with the exception of vanadium (V), were either found in some or all of the rice samples in this study. High levels of magnesium (Mg), potassium (K) and

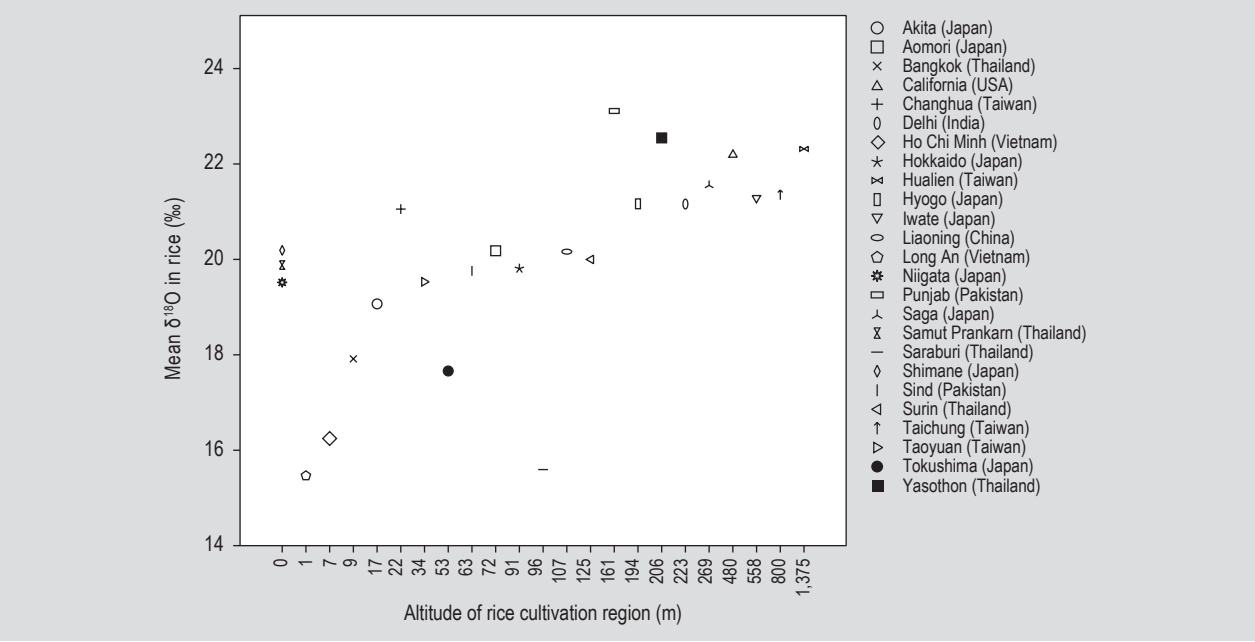


Figure 5. Comparison of the mean $\delta^{18}O$ value in rice and the altitude of the rice cultivation regions of the respective countries.

calcium (Ca) were observed in all the rice samples, similar to the reported findings of mineral nutrients in rice by Parengam *et al.* (2010). The distributions of the elemental composition among all the rice samples were generally found to be varied, and these differences observed may be attributed to variations in the rice species, the disparate abilities of the rice plant species to uptake trace elements from soil, the heterogeneous geochemical soil composition as well as contributions from surrounding environmental pollution (D'Ilio *et al.*, 2002).

The interrogation of the stable isotope ratios and multi-element data in rice by CDA was performed by a 'stepwise' analysis to select the most useful discriminating variables using the broad groups of Asia (China P.R., Japan, Korea and Taiwan), Australia, Europe (France, Italy and Spain), India and Pakistan, North America (USA), and Southeast Asia (Malaysia, Myanmar, Thailand and Vietnam).

The results of the CDA, including the group centroids, are shown in Figure 6A. The results of the CDA are represented by the group centroids of the respective country groups with 90.7% of the original grouped cases correctly classified and 87.9% of the cross-validated grouped cases correctly classified. The 15 elements selected by the software for this multivariate discrimination were $\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$, magnesium (Mg), aluminium (Al), potassium (K), manganese (Mn), iron (Fe), cobalt (Co), copper (Cu), zinc (Zn), arsenic (As), selenium (Se), molybdenum (Mo) and cadmium (Cd). Function 1 (51.7% of variance) which provided the main separation between the sample groups was primarily correlated with Mg, K, Mn, Zn, As and Mo while Function 2 (27.0% of variance) was mainly associated with $\delta^{13}C$, Mg, K, Cu and Zn (Figure 6A). The rice samples from Southeast Asia and India and Pakistan were found to be better discriminated from the rest.

In an attempt to enhance the discrimination of the rice samples grouped under the categories of Asia, Australia, Europe and North America, a separate model was constructed by the 'stepwise' CDA procedure using only the rice samples from these four categories. The CDA cross-plot for the Asian, Australian, European and North American rice samples shows group centroids of the respective country groups with 85.9% of the original grouped cases correctly classified and 82.1% of the cross-validated grouped cases correctly classified (Figure 6B). The 6 elements selected by the software for this multivariate discrimination were $\delta^{15}N$, $\delta^{18}O$, lithium (Li), aluminium (Al), palladium (Pd) and cadmium (Cd). Function 1 (63.7% of variance) which provided the main separation between the sample groups was primarily correlated with $\delta^{15}N$, $\delta^{18}O$ and Cd while Function 2 (29.7% of variance) was mainly associated with $\delta^{15}N$, Al and Pd. A significant improvement was achieved in the model when the Asian, Australian, European and North American rice samples were isolated from the other geographic locations for CDA. The $\delta^{15}N$ and $\delta^{18}O$ values, which were identified as important discriminating factors for the Asian, Australian, European and North American rice samples, are likely to be related to the differences in agricultural practices and the climate conditions responsible for the hydrological cycle, as discussed earlier.

An additional investigation into the discriminant study of the rice samples by their respective rice types was performed on the Aromatic and Japonica rice types. The Aromatic rice

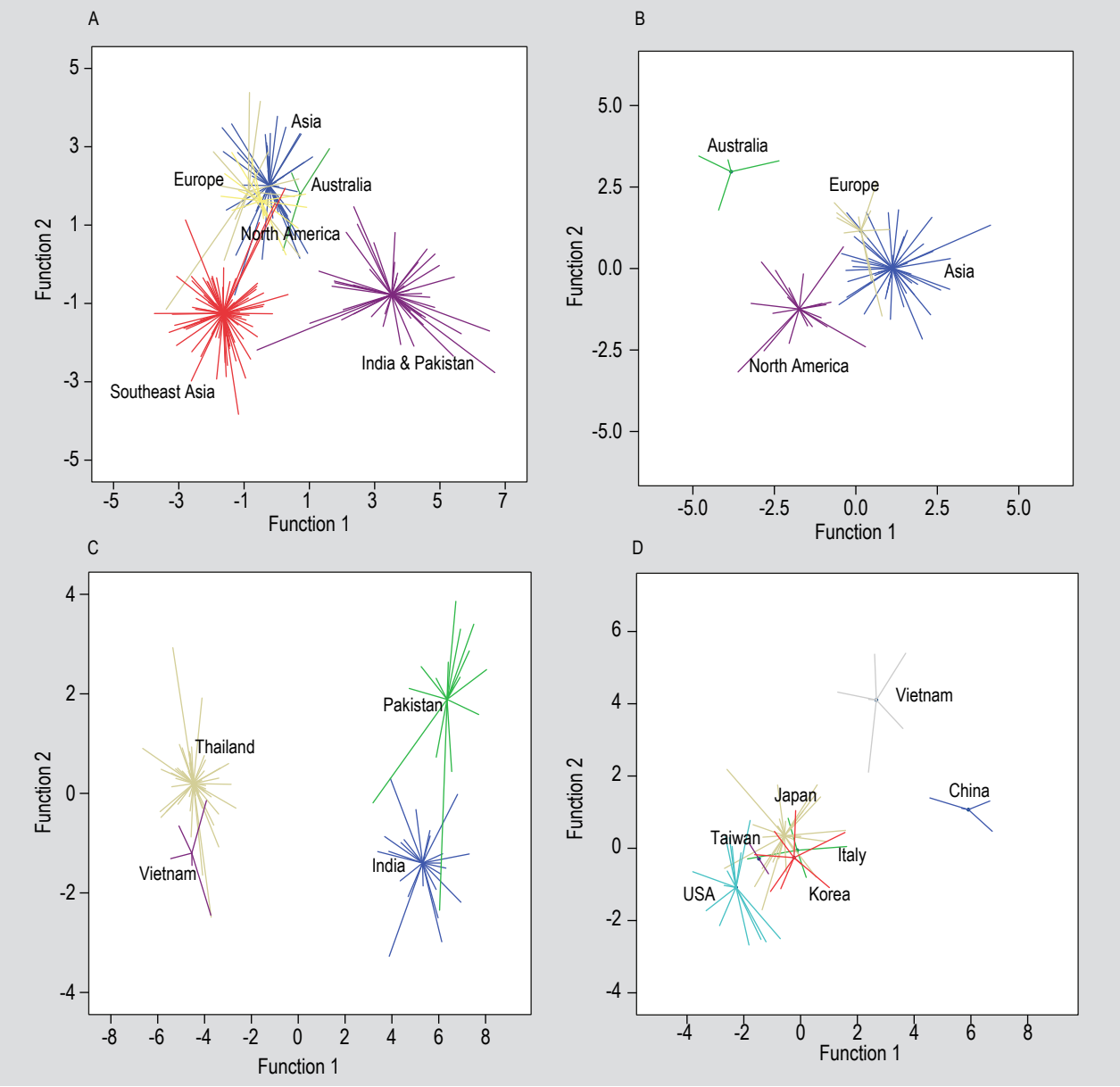


Figure 6. A cross-plot showing the first two discriminant functions obtained from the stepwise canonical discriminant analysis for stable isotope ratio and multi-element data (A) using a detailed classification by group of countries, (B) for the Asian, Australian, European and North American rice samples, (C) for the Aromatic rice type, and (D) for the Japonica rice type.

type refers to Jasmine and Basmati rice while the Japonica rice type refers to the short grain rice. Among all the rice samples in this study, four countries, Thailand, Vietnam, India and Pakistan, contributed to the pool of Aromatic rice types. Figure 6C shows the 'stepwise' CDA cross-plot for the Aromatic rice type. The results of the CDA are represented by the group centroids of the respective country groups with 95.7% of the original grouped cases correctly classified and 89.1% of the cross-validated grouped cases correctly classified. The 12 elements selected by the software for this multivariate discrimination were $\delta^{13}C$, $\delta^{15}N$, lithium (Li), aluminium (Al), iron (Fe), zinc (Zn), arsenic (As), rubidium (Rb), molybdenum (Mo), samarium (Sm), dysprosium (Dy) and iridium (Ir). Function 1 (92.7% of variance) provided the main separation between the sample groups was primarily correlated with $\delta^{15}N$, Fe, Zn, As, Rb, Mo and Ir while function 2 (4.5% of variance) was mainly associated with $\delta^{13}C$, Al, Sm and Dy. A fairly good differentiation could be observed for these Aromatic rice samples with relatively well separated country centroids.

For the Japonica rice type; seven countries were represented in the dataset for this study. Figure 6D shows the 'stepwise' CDA cross-plot for the Japonica rice type. The results of the CDA are represented by the group centroids of the respective country groups with 77.3% of the original

grouped cases correctly classified and 69.7% of the cross-validated grouped cases correctly classified. The 10 elements selected by the software for this multivariate discrimination were $\delta^{15}N$, $\delta^{18}O$, lithium (Li), aluminium (Al), calcium (Ca), copper (Cu), zinc (Zn), caesium (Cs), ytterbium (Yb) and osmium (Os). Function 1 (38.5% of variance) provided the main separation between the sample groups was primarily correlated with $\delta^{15}N$, Li, Ca, Zn, Cs and Os while function 2 (36.5% of variance) was mainly associated with $\delta^{18}O$, Li, Ca, Zn and Os. For the Japonica rice samples, it was observed that only the Chinese and Vietnamese rice samples could be clearly differentiated from those from other countries. In addition, the classification percentages obtained for the CDA procedure of the Japonica rice type are lower than for the Aromatic rice type, thus indicating that the latter model has greater robustness and reliability.

4. Conclusions

The stable isotope ratio and multi-element compositions of various rice types from different geographical origins were determined by IRMS and ICP-MS, respectively. Rice samples from India and Pakistan and Southeast Asia were differentiated from the rice samples from Asia, Australia, Europe and North America by applying CDA to their stable isotope ratio and multi-elemental profile, enabling a 90.7% of the original grouped cases correctly classified. The fifteen variables, $\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$, magnesium, aluminium, potassium, manganese, iron, cobalt, copper, zinc, arsenic, selenium, molybdenum and cadmium, were identified by the discriminant treatment to possess the best information for the differentiation of the geographical origins of rice. The empirical models constructed from the discriminant study were used to predict the geographical origin of unknown samples.

The stable isotope ratios of carbon, nitrogen, oxygen and hydrogen have been demonstrated to display some useful correlations to the geographical origin of the rice samples. With reference to the stable isotope ratios of carbon, nitrogen, oxygen and hydrogen in the rice samples from different countries (Figures 1A, 1B, 1C and 1D), conclusive geographical differentiation of rice could not be achieved, perhaps with the exception of the Malaysian rice samples ($\delta^{13}C$ values), Spanish rice samples ($\delta^{15}N$ values) and Australian rice samples ($\delta^{18}O$ and $\delta^{2}H$ values). As the rice samples in this study were sourced commercially, where the rice traceability information is unavailable to consumers, several assumptions have been made throughout the study to estimate the rice growing conditions at all the possible rice cultivation locations. Seasonal variations have a critical role in influencing some of these variables, hence, it is recommended that the discriminant model is reviewed periodically using up-to-date authentic rice samples in order to improve the reliability of the prediction results for unknown rice samples. Besides, the inclusion of the $\delta^{2}H$ values into the rice dataset for the CDA procedure did not contribute to any significant effect on the output results, thus indicating that the hydrogen isotope information did not offer any additional discrimination to that obtained with oxygen isotope data. As a result, the $\delta^{2}H$ data was omitted from the geographical origin discriminant model for rice.

Several elements have been selected as influential indicators of the geographical origin of the rice samples in this feasibility study due to their noticeable differences in soil concentrations which are in turn reflected in the rice grains through plant uptake. Although no single element has been identified to possess exclusive indicators for differentiating the rice geographical origin, it is the combination of several elemental compositions which contributes significantly to the overall discrimination of the rice samples by country of origin. In fact, the CDA treatment of the multi-elemental data alone was able to provide some differentiation of the geographical origin of rice, but with a lower percentage of the original grouped cases correctly classified. Nonetheless, the elemental composition of rice is comparatively resistant to seasonal variations and thus remains as a robust indicator in geographical origin studies.

Acknowledgements

The authors would like to gratefully acknowledge the technical assistance of the food authentication and metals teams from the Food and Environment Research Agency, York, UK.

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