DETERMINATION OF THE GEOGRAPHICAL ORIGIN OF RICE USING STABLE ISOTOPE RATIO AND MULTI-ELEMENT ANALYSES

ANGELA LI
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The University of York

Department of Chemistry

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Abstract

Twelve varieties of rice grown in various countries around the world (Australia, China, France, India, Italy, Japan, Korea, Malaysia, Myanmar, Pakistan, Spain, Taiwan, Thailand, USA and Vietnam) were analysed using IRMS and ICP-MS to assess the capacity for discrimination of their geographical origins using the stable isotope ratios of carbon, nitrogen and oxygen and the multi-elemental compositions. The data were processed by canonical discriminant analysis (CDA) enabling classification according to geographical origin. Fifteen key variables (δ^{13}C, δ^{15}N, δ^{18}O, Mg, Al, K, Mn, Fe, Co, Cu, Zn, As, Se, Mo and Cd) 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 & Pakistan, North America and Southeast Asia), enabling 90.7% correct classification for the model generated. Separate models were also constructed for the Aromatic (Basmati rice and Jasmine rice) and Japonica rice types with correct classifications of 95.7% and 77.3%, respectively. The study demonstrates that the methodology has good potential in identifying the geographical origin for different rice types.

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Acknowledgements

Declaration

I hereby declare that the work herein submitted for the MSc degree at The University of York, United Kingdom is the result of my own investigation, except where reference is made to the published literature. I also declare that the material submitted in this thesis has not been accepted for any other degree and is not being concurrently submitted for any other degree.

Chapter 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 highly subjected to debate (Maclean *et al.*, 2002). The domestication of rice is even considered to be one of the most significant advancement in human history (Maclean *et al.*, 2002).

The two rice species of importance to humans are *Oryza sativa*, which is grown globally; and *Oryza glaberrima*, which is grown in parts of West Africa (IRRI). Both of the species belong to the *Poaceae* (grass) family and there are probably more than 40,000 varieties of cultivated rice to date (Rice Association, 2004). 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.

The prevalence of rice mislabelling and adulteration threatens the honest livelihood of rice traders and undermines both the consumer confidence in food safety and the reliance on 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 & 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 ICP-HRMS (Kelly *et al.*,

2002; Yashui & Shindoh, 2000), SDS-PAGE of the seed proteins (Montalvan *et al.*, 1998), 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 (Yasui & Shindoh, 2000; Kawasaki *et al.*, 2002; Kelly *et al.*, 2002). Others have reported their findings based only on a single variety of rice (Yasui & 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.

The overall aims of this research were to expand 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 multi-element isotope and trace elements analysis by IRMS and ICP/MS, respectively. With the growing popularity of isotope ratio measurements in the area of food authenticity and traceability, an additional comparison study was performed between the carbon isotope measurements by conventional mass spectrometry versus spectroscopic techniques.

The objectives of the research presented in this thesis were:

i.   To examine the relationship between the isotopic compositions of C, N, H and O in rice and gross differences in the growth conditions and meteorological patterns experienced by the plant.

ii.   To investigate possible connections between the trace element contents in rice and the geology of the growing area.

iii. To evaluate the discrimination of the geographical origins of rice using combined data from multi-element isotope and trace element analysis and with processing by statistical approaches.

1.1 Overview of rice cultivation systems and introduction to rice varieties

Rice is cultivated in many different ways around the world and farming practices may even vary within the same locality. Although there are several species of both wild and cultivated rice, *Oryza* sativa is the species of cultivated rice that has been subjected to the most rigorous and far-reaching domestication and selection. It is this long history of domestication that enhanced the adaptation abilities of rice to a very wide range of ecological conditions (FAO, 2000).

1.1.1 Rice cultivation – from seed to market

Understanding the objectives behind each stage of the rice cultivation process is important knowledge for farmers in order for them to produce the desired rice quality and yield. Although the actual cultivation process may differ depending on the regulatory requirements or climatic variations within a particular country, the basic principles remain the same. In general, there are a number of steps involved in the production and processing of the rice grains before they can be released for sale in the market.

1.1.1.1 Growth stages of the rice plant

Using the information from IRRI (2009), the physical changes to a growing rice plant are generally categorised into three basic growth phases, namely: vegetative, reproductive and ripening. The vegetative phase involves seed germination, seedling and root development, tillering or stem production at the base of grass plants, and finally, stem elongation. The reproductive phase incorporates panicle initiation at the tip of the growing shoot which leads on to booting. Booting refers to the bulging of the flag leaf sheath resulting from the enlargement of the panicle. In addition, the heading stage, where the panicle tip emerges from the flag leaf sheath, is also included in the reproductive phase. Subsequently, the flowering phase occurs and fertilisation takes place. The ripening phase focuses on the growth of the grain itself and comprises (1) the milk grain stage where the grain starts to fill with a milky substance, (2) the dough grain stage where the milky component in the grain transforms into soft dough before turning into hard dough and the colour of the grain in the panicle changes from green to yellow, and lastly (3) the mature grain stage where the distinct grain takes up the fully developed hard texture and ripens to a yellow colour. To summarise, Figure 1.1 illustrates the individual growth stages of a rice plant.
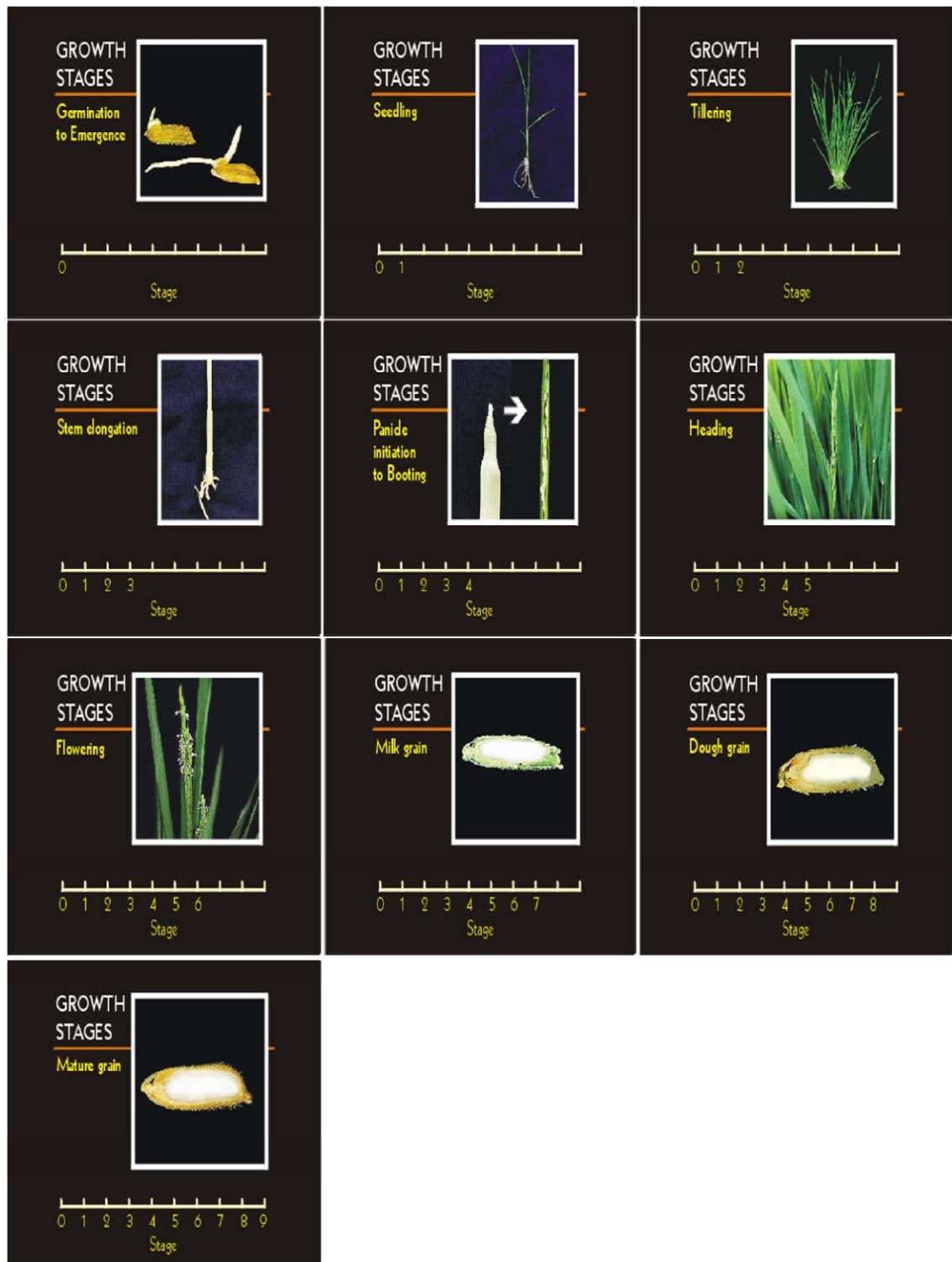
Figure 1.1 Summarised growth stages of a rice plant. Adapted from IRRI (2009).

The growth cycle of a rice plant may also be illustrated by the growth in height

of the rice plant and root extensions over its entire development period (Figure 1.2).
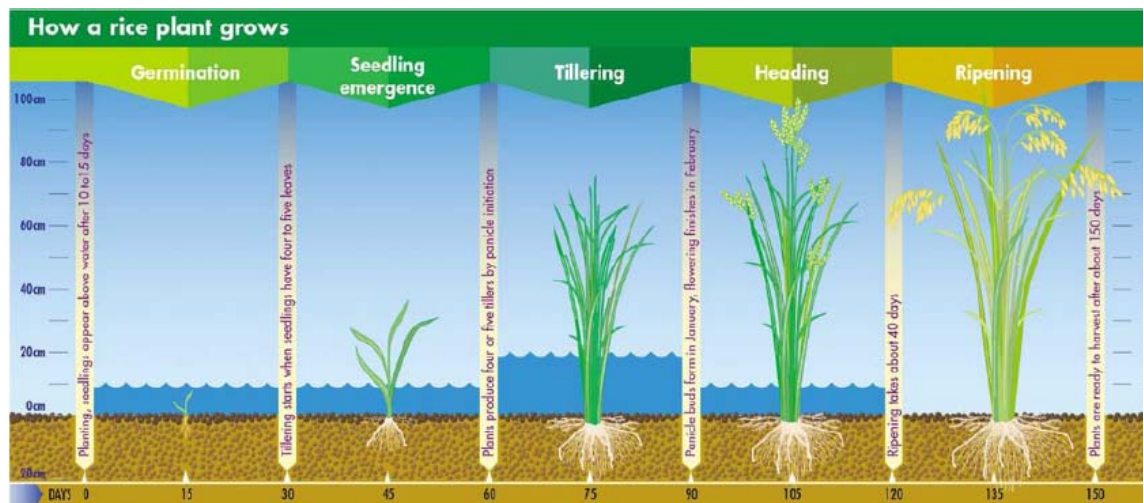
Figure 1.2 Growth cycle of a rice plant. Taken from from SunRice.

1.1.1.2 Harvesting

Rice harvesting typically consists of four main processes: cutting, hauling, threshing and cleaning (Figure 1.3). Traditional or small-scale rice growers may still rely on these individual manual tasks whereas modernised or large-scale rice growers use mechanical combine harvesters capable of handling these processes with one machine.

Figure 1.3 Four main operations of rice harvesting. From left to right: (1) Cutting the mature crop either at panicle height or above the ground, (2) Hauling: collecting the crop and transport to the threshing location, (3) Threshing: separating the paddy grain from the rest of the cut crop, (4) Cleaning: removing immature, unfilled and non-grain materials. Taken from IRRI (2009).

The application of a good harvesting method is critical to achieving maximum grain yield and reducing grain damage but without any compromise in quality. Some guidelines on the correct procedure for harvesting include harvesting rice with the desirable moisture content, as well as performing harvesting, threshing, proper grain cleaning and grain drying in sequential order and without unnecessary delays (IRRI, 2009).

1.1.2 Categorisation of rice grains

As discussed, the rice milling process itself gives rise to different fractions of rice which could be used for different marketing purposes. In fact, the commonly used term, "milled rice", is merely a generic expression for processed rice and does not provide any information on the specific rice variety and type. There are various ways to classify rice by varieties, types, grain quality, as well as the country of origin, and these differences bring about the price differentiation in the rice sold in the market.

1.1.2.1 Differentiation of rice by varieties and types

According to the Rice Association in the UK, rice varieties can be distinguished into two basic groups: long grain/all-purpose and specialty rice. All-purpose long grain rice is suitable for all kinds of cooking and the rice grain appears long and slim with an approximate length of 4 to 5 times its width (Rice Association, 2004). The types of long grain (Indica) rice include regular white rice, parboiled white rice and brown rice.

REGULAR LONG GRAIN WHITE RICE

The regular long grain white rice is considered as one of the most popular types due to its subtle flavour. Its use in the culinary area is very varied and it serves as a

pleasant complement to both rich and light flavoured dishes. This regular white rice is obtained by the removal of the husk and bran layer during the milling process (Rice Association, 2004). However, the removal of the bran and germ leads to in a reduction of vitamin B (thiamin) and some other B vitamins (Vaughan & Geissler, 1997).

PARBOILED LONG GRAIN RICE

Parboiled long grain white rice has a slightly richer flavour than its untreated counterpart due to the additional processing step after milling. The parboiling of rice involves steaming the milled rice under pressure, which helps to harden the grain, thus minimising the chances of the grain subsequently being over-cooked. This parboiling process has its ancient origins traceable to India but has since been commercialised for practical purposes. Parboiled rice is more nutritious than untreated white rice due to the migration of vitamins and minerals from the hulls and bran into the grain's endosperm during the parboiling process (Vaughan & Geissler, 1997). Furthermore, it must be noted that although the raw parboiled rice appears golden or yellowish in colour, it turns white upon cooking (Rice Association, 2004).

BROWN LONG GRAIN RICE

Brown long grain rice is fast becoming a favourite among health conscious rice lovers as it has higher vitamin, mineral and fibre contents than regular white rice. It is obtained by applying minimal milling where the bran layer is still retained on the grain after the husk has been removed. As a result, there is a distinctive nutty taste and an added chewy texture to this kind of rice (Rice Association, 2004).

On the contrary, the category of specialty rice includes far more varied rice types that have been developed due to the rice's selective adaptability to grow in a confined

geographical region, the diversified preference for certain rice variety from different populations around the world or even driven by consumers' health requirements or concerns. Aromatic rice is probably the most well-known type of rice in the specialty rice category. The presence of a potent aroma component, 2-acetyl-1-pyrroline, is responsible for the fragrance associated with aromatic rice varieties (Buttery *et al*., 1983). A study by Tanchotikul & Hsieh (1991) revealed that the 2-acetyl-1-pyrroline contents in Basmati and Jasmine rice were about 87.4 ppb and 156.1 ppb, respectively, giving an indication of the degree of the aroma of the rice.

BASMATI RICE

Basmati rice is one of the more popular rice varieties and is grown mainly at the foothills of the Himalayas in India and Pakistan. This type of rice has a very long, slender grain and possesses a fragrant flavour and aroma when cooked. The term "Basmati" means the "Prince of Fragrance" or "The Perfumed One" (Tilda) and was derived from two Sanskrit roots (*vas* = aroma) and (*mayup* = ingrained or present from the beginning) (Singh, 2000). As a result of the grain's aromatic properties and its cultivation locality, Basmati rice is closely associated with Indian dishes where the use of spices is capable of enhancing the rice's aroma. Similar to the long grain rice, Basmati rice is also available in the high fibre form, known as brown Basmati rice, which has a more intense aroma than the white Basmati rice (Rice Association, 2004). In addition, some Basmati rice that is sold in the market is labelled as aged rice. Ageing is considered as a mode of rice post-harvest processing where physiochemical and physiological changes take place in the rice upon storage, usually for periods of between 12 and 18 months. Aged rice, when cooked, becomes fluffy and harder in texture and

more resistant to swelling and disintegration. Hence, aged rice is normally higher in price than fresh rice (Butt *et al*., 2008).

JASMINE RICE

Jasmine rice, which is also known as Thai fragrant rice or Thai Hom Mali rice, has its origins traced to Thailand (Rice Association, 2004), hence the frequent association of the rice name with its country of origin. Jasmine rice also belongs to the class of aromatic rice but it has a slightly less distinctive flavour than Basmati rice. This type of rice is popular for use in Chinese and Southeast Asian dishes (Rice Association, 2004). With the same purpose for promoting a higher dietary fibre intake, the brown Jasmine rice is also available in the market.

JAPONICA RICE

Japonica rice refers to the short and medium sized grains that are more commonly cultivated in the temperate and mountainous regions of Japan and California. Due to its lower amylose content, this type of rice tends to soften rapidly after a definite period of cooking, giving rise to its unique stickiness and texture (California Rice Commission, 2010). Japonica rice is a popular choice of rice in Japanese and the Caribbean dishes and is also available as brown Japonica rice. Some examples of Japonica rice varieties include Calrose rice, Koshihikari rice and Akitakomachi rice.

GLUTINOUS RICE

Glutinous rice was originally developed to cater to the consumer tastes in Japan, Korea and certain parts of South East Asia but has since been subject to shrinking demand. This rice has a dull and soft grain appearance with the cut surface being

described as having a paraffin wax-like look. Glutinous rice may take up a coloured form depending on the variety but the colour is only restricted to the epidermal layer. Cooked glutinous rice is sweet and sticky hence it is generally preferred for use in desserts, beer and even some cultural ceremonies (Grist, 1975).

WILD RICE

Wild rice is an aquatic grass grain species that grows naturally in shallow lakes and river bed areas in the Great Lakes region of North America and in certain ecologically similar parts of Asia. This "wild" variety of rice remains an important crop especially for lake and river inhabitants and farmers (IWRA). The wild rice grain takes on an elongated and shiny black or brown appearance. The processing of this rice involves heating, which gives rise to its nutty flavour and chewy texture. Interestingly, wild rice has been referred to as the "caviar" of grains (Vaughan & Geissler, 1997). With coloured rice being seen as a beneficial source of antioxidants, other rice varieties with coloured kernel colours (black, red or purple) are gradually gaining popularity as a functional food due to the high anthocyanin contents (Sompong *et al.*, 2011).

LOW GLYCEMIC INDEX RICE

With rice being an integral staple food in some countries, the needs of diabetic individuals are also catered for by the market with the availability of rice which has low glycaemic index (GI). There is no definite classification of GI value for rice due to the wide variety and species of present globally. Research has, however, shown that the amylose content in rice relates to GI: low GI rice tends to have higher amylose content (Miller *et al.*, 1992).

ORGANIC RICE

Organic rice is simply a general term to describe any variety or type of rice that has been grown and processed in accordance to the guidelines for organic farming. Typically, organic farming involves the elimination of chemical pesticides and fertilisers, the application of composted manure and unwanted plant materials for soil fertilisation and the practice of crop rotation in the fields (IRRI, 2004).

NEW CROP RICE

New crop rice is a fairly new name that refers to rice that is packaged and released for sale in the market within a few months of rice harvest. In Japan, the agricultural regulatory standard requires all rice that are labelled as new crop to be processed and packaged for sale in the same year of harvest. Due to the higher moisture content in the new crop rice than in aged rice, the new crop rice provides a delicious fresh taste and is highly regarded in countries such as Japan (Itoh, 2010).

BROKEN RICE

During the rice milling process, a certain fraction of broken rice will inevitably be produced. This broken rice is generally separated from the intact whole rice grains but a certain proportion may be retained in the finished product depending on the standard to which the rice will conform (Grist, 1975). The broken rice kernels can be further separated into different categories by the percentage of the whole kernel remaining. The larger broken rice grains are typically sold for industrial use in the manufacture of pet foods, stock feed or breakfast cereals while the smaller broken rice grains can be ground into rice flour for other food applications (Ricegrowers' Association of Australia).

In summary, the various types of rice that have been discussed are summarised in Table 1.1 while Table 1.2 shows the classification of rice as long grain, medium grain or short grain by the Codex standard for rice (Codex Standard 198-1995).

Table 1.1 General summary of rice by their categories.

Long Grain / All Purpose Rice	Specialty Rice	Other Rice
▪ Long grain rice (white and brown) ▪ Parboiled rice	▪ Basmati rice (white and brown) ▪ Jasmine rice (white and brown) ▪ Japonica rice (white and brown) ▪ Glutinous rice ▪ Wild rice and coloured rice ▪ Rice for diabetics	▪ Organic rice ▪ New crop rice ▪ Broken rice

Table 1.2 Rice classifications by grain size (Codex Standard 198-1995).

OPTION 1: Kernel length/width ratio

Long grain rice	Medium grain rice	Short grain rice
ratio of 3.0 or more	ratio of 2.0–2.9	ratio of 1.9 or less

OPTION 2: Kernel length

Long grain rice	Medium grain rice	Short grain rice
length of 6.6 mm or more	length of 6.2 mm or more but less than 6.6 mm	length of less than 6.2 mm

OPTION 3: Combination of the kernel length and the length/width ratio

Long grain rice	Medium grain rice	Short grain rice
kernel length of more than 6.0 mm and with a length/width ratio of more than 2 but less than 3, or kernel length of more than 6.0 mm and with a length/width ratio of 3 or more	kernel length of more than 5.2 mm but not more than 6.0 mm and a length/width ratio of less than 3	kernel length of 5.2 mm or less and a length/width ratio of less than 2

1.1.2.2 Determination of rice grain qualities

The perception of rice grain quality is different for the various parties involved in the farm to fork chain. For the rice growers, grain quality denotes the quality of seed for planting and dry grains that have the least moisture and microbial deterioration for consumption. The miller or trader, on the other hand, focuses on rice with low moisture content, variety integrity and good total and head milled rice yield. Market quality for rice is primarily defined by physical properties and variety name while sensory quality is controlled by physico-chemical factors (Juliano, 1993).

A good understanding of the intrinsic and acquired qualities in each rice variety is beneficial to the rice farmer and miller so that they can target the attributes valued by the consumers, who drive the market. A list of these genetic and acquired qualities is given in Table 1.3.

Table 1.3 Distinction of genetic and acquired rice grain quality. Adapted from IRRI.

Genetic	Acquired
• chemical characteristics such as gelatinization temperature, gel consistency, and aroma • grain shape and size • bulk density • thermal conductivity • equilibrium moisture content	• moisture content • colour and chalkiness • purity • damage • cracked grains • immature grains • milling-related characteristics (head rice recoveries, whiteness and milling degree) are also included as relevant measures of quality because they are of concern to consumers

In addition, there are separate criteria for the physical characteristics in paddy rice and milled rice (Figure 1.4). Paddy rice quality is dependent on its moisture content, percentage of immature rice kernels, varietal purity, percentage of dockage, percentage of rice kernel discolouration, and percentage of cracks in individual rice kernels. Milled rice quality is determined by head rice recovery, percentage of Brewer's rice, percentage of damaged rice grains, chalkiness, presence of red or red streaked rice grains, and physical appearance of the rice grains (IRRI).

Paddy Rice   **Milled Rice**
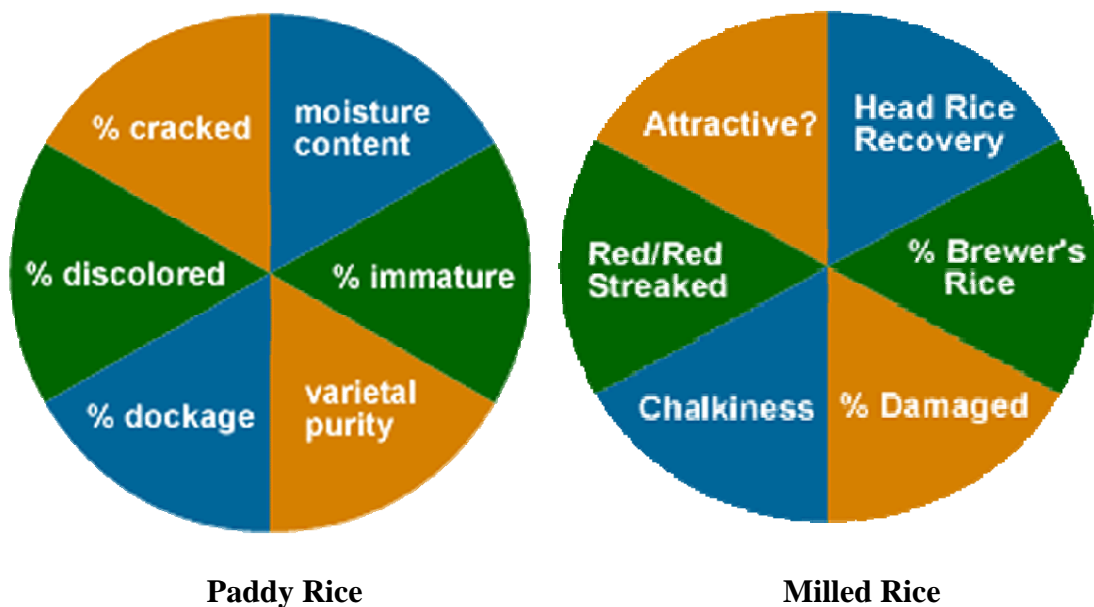
Figure 1.4 Physical characteristics of paddy rice and milled rice for quality determination. Taken from IRRI.

PADDY RICE

The moisture content of paddy rice has substantial effects on every attribute of its quality. Milling at the desired moisture content will achieve the optimum milling yield. The presence of immature rice kernels can lead to excessive bran and broken

grains as these kernels are slender and chalky. Segregating the paddy varieties helps to facilitate the milling process and enhance the milling efficiency. Dockage or impurities should be reduced to a minimum for good quality paddy rice. Deterioration in the appearance of paddy rice is largely due to the exposure to water, pests and heat, triggering the biochemical changes which results in discolouration. Furthermore, the development of cracks in the paddy kernel can be attributed to the inconsistent temperature and moisture environments experienced by mature paddy rice (IRRI).

MILLED RICE

Alternatively for the qualification of good milled rice, a high head rice yield is one of the foremost criteria, as broken grain is only worth half the value of head rice. The term, Brewer's rice, refers to the small milled fragments of rice kernels that are frequently used in beer brewing, hence the greater the proportion of intact milled rice, the higher is its quality. Damaged or darkened rice kernels are usually the result of degradation by insects, mould, water or heat. The quality of the milled rice can become greatly reduced with the presence of even a few damaged grains. Physical attractiveness of milled rice is closely related to how well the rice has been milled. The presence of bran streaks in under-milled rice is not only physically unappealing but also affects the storage life of the rice due to the oil content from the residual bran. Moreover, the occurrence of red streaked kernels is also related to the degree of milling where parts of the bran layer are left on the rice. Chalkiness of rice denotes the presence of opaque spots on parts of the milled rice kernel. Although rice chalkiness has no effect on the sensory aspects after cooking, the milled rice quality still inevitably is downgraded (IRRI).

1.1.2.3 Rice as an important food commodity

With rice being a basic source of carbohydrate for almost half of the world's population, and a mainstay for some of the agricultural countries (FAO, 2011), it has become a food commodity that is deeply integrated into society. Global rice production has seen a steady growth in the past decade (Figure 1.5) with a positive forecast for the 2010 global rice production by FAO (FAO, 2011).
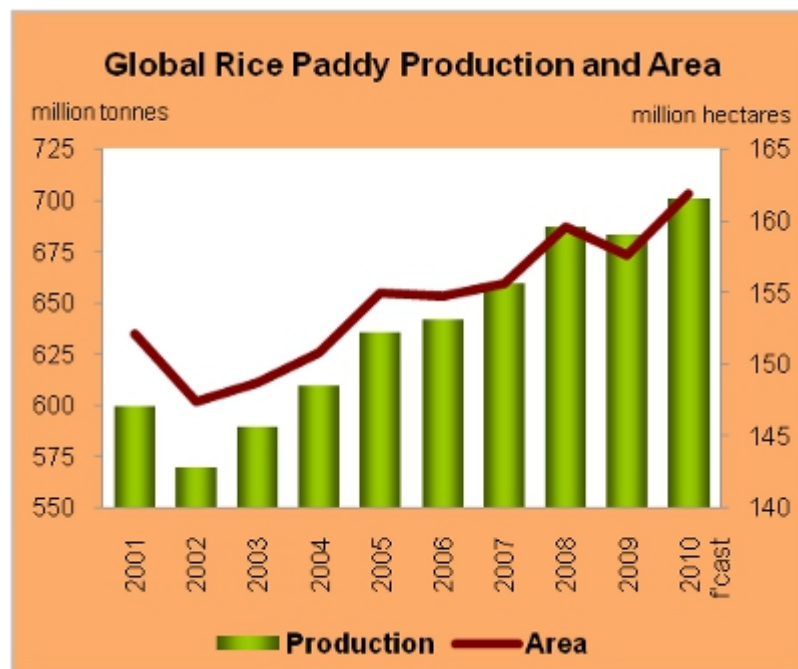
Figure 1.5 Global rice paddy production and area. Taken from FAO (2011).

Figure 1.6 Global rice trade and price index. Taken from FAO (2011).

As reflected in Figure 1.6, FAO raised its 2011 global rice trade forecast by nearly 900,000 tonnes to 31.4 million tonnes, only marginally below the forecast for 2010 (FAO, 2011). This implies that the demand for rice still has the potential to grow, but grew at a moderate rate in the past decade. In the same figure, the FAO rice export price index reflected a significant increase since 2002 with a major spike in the rice export price in 2008. In 2008, the rice export prices rose to an all-time high due to a very tight supply situation in key rice producing countries. International rice trade decreased, mainly due to restrictions in the main exporting countries. Since then, the world rice production in all the major Asian rice producing countries, especially Bangladesh, China, India, Indonesia, Myanmar, the Philippines and Thailand has increased to meet the demands. Governments in these countries have also played a significant role by introducing a series of incentives to raise production (FAO, 2008).

The distinction between the long grain (Indica) rice and specialty rice leads to their price differentiation as indicated in Table 1.4. Over the past five years, Japonica rice has been the higher valued rice variety by comparison with Indica and Aromatic rice varieties. Another interesting observation is that the rice price index for Aromatic rice has surpassed that of Indica rice over the last two years. Given that there was no significant change in the supply of such rice within the same period, this reflects increased demand for Aromatic rice arising from changes in consumer tastes and preferences. Nonetheless, the existence of a price differentiation for any food commodity is likely to prompt the emergence of food fraud; hence analytical approaches are necessary to curb such illegal proliferation.

Table 1.4 FAO rice price indices. Taken from FAO (2011).

FAO Rice Price Indices (2002-2004=100)					
	All	Indica		Japonica	Aromatic
		High quality	Low quality		
2006	137	135	129	153	117
2007	161	156	159	168	157
2008	295	296	289	315	251
2009	253	229	197	341	232
2010	229	211	213	264	231
2010 January	251	232	237	289	232
February	242	227	218	283	231
March	219	213	205	235	232
April	204	197	185	221	230
May	200	192	181	221	221
June	210	193	187	250	214
July	214	189	191	261	214
August	217	192	197	263	216
September	232	205	227	266	224
October	249	217	235	296	250
November	257	233	243	294	261
December	256	240	243	288	251
2011 January	253	237	239	288	240
2010 Jan.	251	232	237	289	232
2011 Jan.	253	237	239	288	240
% Change	0.9	1.8	0.7	-0.3	3.5

Source : FAO

N.B. - The FAO Rice Price Index is based on 16 rice export quotations. "Quality" is defined by the percentage of broken kernels, with high (low) quality referring to rice with less (equal to or more) than 20 percent brokens. The Sub-Index for Aromatic Rice follows movements in prices of Basmati and Fragrant rice.

1.1.2.4 Rice related food frauds

Two main issues are generally associated with the topic of food authenticity: (1) economic adulteration and (2) misbranding of products. Economic adulteration of food refers to the blending of a cheaper product with commodities of higher economic value, while the misbranding of products involves the deviation from production system, denomination of geographical origin, genetically modified food/food ingredients (Ulberth, 2004). Examples of rice related food frauds involving economic adulteration include a 2010 fake rice scandal in China where rice producers in Central China released counterfeit aromatic rice into the market. The imitation rice was created by the addition of artificial fragrance to regular rice and was sold as China's premium rice,

Wuchang rice. The Wuchang rice is cultivated only in the northern Heilongjiang province and is recognised for its exclusive aroma, thus justifying its high price (Tibet Business Daily, 2010). In the same year, the Chongqing Economic Times reported an estimate that more than 90% of the Thailand Jasmine rice that are sold in China are fake as the price of this aromatic rice can be double or triple that of the regular local grown rice (Global Times, 2010). In the United Kingdom, the increasing consumption of Basmati rice in the country has prompted the Food Standards Agency (FSA) to carry out a nation-wide survey on the authenticity of Basmati rice in 2004. A novel DNA test method was developed for this FSA survey to determine if there was any deliberate addition of non-Basmati rice to Basmati rice in view of the huge price difference between the two. The survey findings revealed that 54% of the 457 samples surveyed contained only Basmati rice, and 46% had non-Basmati rice detected at various levels (FSA, 2004).

Another example on the rice related food frauds involving the misbranding of products includes the most recent rice scandal, reported by The Korea Times in 2011, concerned fake rice being sold in Taiyuan, Shaanxi province in China. According to the report, the alleged fake rice was made from a mixture of potatoes, sweet potatoes and plastic which can be harmful to health when consumed (International Business Times, 2011).

Clearly, the determination of geographical authenticity and traceability is vital for important food commodities such as rice, where substantial volumes of rice are traded globally with more than half of the world's population depending on it as a staple food. The price disparity between regular rice and specialty rice provides considerable incentives for the persistent occurrence of counterfeit rice in the market. The prevalence

of rice related food frauds has far reaching consequences at the domestic, commercial, national and even international levels. Consumers lose confidence in the rice products sold in the market and become fearful for their health from the direct or indirect introduction of adulterated rice into their diets. Retailers suffer heavy economic losses from product recall while traders and/or producers endure the possible implications of their reputation and brand names. International relations could also be affected as global trading of counterfeit rice could impair the national pride associated with certain rice varieties. Government intervention by food regulators is required to have the affected rice product analysed, execute the appropriate legal actions and step up food surveillance programmes.

In view of the increasing prevalence of rice related food frauds, the development of an empirical model for the determination of rice geographical origin could help to identify the occurrence of economic adulteration of rice (based on the database for the isotopic ratio and elemental profile of rice from various countries of origins) and the misbranding of rice (based on the typical isotopic ratio and elemental profile of rice).

1.2 Introduction to stable isotopes and stable isotope ratio analysis

DEFINITION OF STABLE ISOTOPES

The term isotope was introduced by Professor Frederick Soddy at the University of Glasgow and originated from two Greek words *isos* meaning 'equal in quantity or quality' and *topos* meaning 'place or position', hence isotope has the combined meaning of 'in an equal position' (of the periodic table of chemical elements) (Meier-Augenstein, 2010). Isotopes are defined as atoms possessing the same number of protons but different numbers of neutrons in their respective nuclei. These isotopes exist in both the

stable and unstable (radioactive) forms (Hoefs, 2009). The natural abundances of stable isotopes of light elements that occur commonly in organic matter studies are given in Table 1.5.

Table 1.5 Stable isotopes of light elements and their typical natural abundance. Adapted from Meier-Augenstein (2010).

Chemical Element	Isotope	Abundance (atom %)
Hydrogen	^{1}H	99.985
	^{2}H	0.015
Carbon	^{12}C	98.89
	^{13}C	1.11
Nitrogen	^{14}N	99.63
	^{15}N	0.37
Oxygen	^{16}O	99.76
	^{17}O	0.04
	^{18}O	0.20
Sulfur	^{32}S	95.02
	^{33}S	0.76
	^{34}S	4.22

1.2.1 Measurement of stable isotope ratios

Stable isotope ratio (or the ratio of the heavy to light stable isotope) measurement by IRMS is considered as a specialised area of analytical sciences with its special notations and methods of operation. The concepts of calibration, result calculation and unit expression are uniquely different from the conventional mass spectrometric techniques for quantitative analysis.

1.2.1.1 General notations used in IRMS

THE DELTA VALUE (δ)

The delta value is a special notation associated with the quantification of the isotopic ratio in a sample, relative to a reference material of nominal isotope ratio. This differential comparison between the sample and reference material is achieved through the application of equation (1.1).

$$\delta_{ref} = \left(\frac{R_{samp} - R_{ref}}{R_{ref}}\right) \cdot 1000 \qquad (1.1)$$

The above equation can be further simplified to obtain equation (1.2).

$$\delta_{ref} = \left(\frac{R_{samp}}{R_{ref}} - 1\right) \cdot 1000 \qquad (1.2)$$

Where δ_{ref} is the isotope ratio of the sample relative to a reference material and expressed in delta units. R_{samp} and R_{ref} are the absolute isotope ratios of the sample and reference material respectively. The final multiplication by 1000 converts the value to parts per thousand (‰ or per mil). Per mil is derived from the Latin word, *mille*, which stands for one thousand, in the same way that per cent or % is derived from *centum*. The rationale for the delta value to be calculated in per mil instead of the commonly used per cent is to amplify the very small magnitude of changes to the isotopic ratio at natural abundance levels.

Using the example from Kelly (2003), the natural flavour chemical vanillin has a typical $^{13}C/^{12}C$ isotope ratio of 0.010989786 while that of the vanillin that has been artificially synthesised from petrochemical precursors may be 0.01089989. This difference from the fourth decimal place onwards may appear insignificant, yet it is critical to the differentiation of the vanillin sources. By applying the equations (1.1) or (1.2) using the Pee Dee Belemnite reference material, which has the $^{13}C/^{12}C$ isotope ratio of 0.011237, the isotope ratios for the natural and synthetic vanillin can be

expressed into the delta notation to become -22.0‰ and -30.0‰, respectively. In this way, the difference in ^{13}C abundance has been amplified for easy distinction between the two sources of vanillin.

ISOTOPE REFERENCE MATERIALS

The primary reference materials used to express the natural variations of isotopic composition for the light bio-elements are listed in Table 1.6. Since its definition by Craig (1961a), standard mean ocean water (SMOW) was adopted as a reference to express the relative variations of $^{2}H/^{1}H$ and $^{18}O/^{16}O$ ratios in natural waters. Craig's definition of SMOW was based on a National Institute for Standards and Technology (previously the National Bureau of Standards), water standard known as NBS-1. Unfortunately, a major shortcoming of the defined SMOW was that it never existed as a real sample to be used as a measurement calibrant. This was solved with the preparation of Vienna standard mean ocean water (VSMOW) by the International Atomic Energy Agency (IAEA), which possesses almost identical isotopic composition as the defined SMOW, to be the *de facto* primary reference material (Gonfiantini *et al.*, 1993). The Pee Dee Belemnite (PDB) standard comprised calcium carbonate from the rostrum of a Cretaceous belemnite, and is regarded as the primary reference for carbon isotopes in the same way that SMOW was for hydrogen and oxygen isotopes. The depletion of PDB and some concerns that the original material was not homogeneous prompted the emergence of a new Vienna Pee Dee belemnite (VPDB) using a limestone standard known as NBS-19 (Brand *et al.*, 2009). Atmospheric nitrogen is an ideal choice for nitrogen isotope primary reference material because of its homogenous nature and its involvement in some important botanical processes. Lastly, Cañon Diabolo troilite (CDT) possessed the meteoritic sulfur which was used as the primary reference material

for sulfur isotopes. Like PDB, however, CDT has been exhausted and a new Vienna Cañon Diabolo troilite (VCDT) scale was constructed using the silver sulfide standard, IAEA-S-1, as the calibration material.

Table 1.6 Isotopic compositions of the primary reference materials. Adapted from Kelly (2003).

Primary reference material	Isotope ratio	Accepted value (x 10^6, ppm) (with 95% Confidence interval)
Standard Mean Ocean Water (SMOW)	$^2H/^1H$	155.76 ± 0.10
	$^{18}O/^{16}O$	2005.20 ± 0.43
	$^{17}O/^{16}O$	373 ± 15
Pee Dee Belemnite (PDB)	$^{13}C/^{12}C$	11237 ± 9.0
	$^{18}O/^{16}O$	2067.1 ± 2.1
	$^{17}O/^{16}O$	379 ± 15
Air	$^{15}N/^{14}N$	3676.5 ± 8.1
Cañon Diabolo Troilite (CDT)	$^{34}S/^{32}S$	45004.5
	$^{33}S/^{32}S$	8100.0

Following the identification of the primary reference materials for isotopic measurements, reference materials were prepared, quantified for their isotopic compositions and distributed globally for method calibration and/or validation work. Table 1.7 shows a representative list of these international reference materials. However, for practical reasons, laboratories often prefer to have their own in-house working or laboratory standards that have been calibrated against a suitable reference material (Table 1.7), to ensure a constant supply of such reference materials.

Table 1.7 Representative list of international reference materials for stable isotope ratio mass spectrometry (IRMS) administered and distributed by the IAEA (Vienna, Austria). Adapted from Meier-Augenstein (2010).

International reference material	Code	$\delta^{13}C_{VPDB}$ (‰)[a]	$\delta^{15}N_{AIR}$ (‰)	$\delta^{2}H_{VSMOW}$ (‰)	$\delta^{18}O_{VSMOW}$ (‰)
TS-limestone	NBS-19	+1.95			-2.20
Lithium carbonate	LSVEC	-46.6			-26.6
Oil	NBS-22	-30.031[a]		-118.5	
Sucrose	IAEA-CH-6	-10.449[a]			
Polyethylene foil	IAEA-CH-7	-32.151[a]		-100.3	
Wood	IAEA-C4	-24.0			
Wood	IAEA-C5	-25.5			
Wood	IAEA-C9	-23.9			
Sucrose	IAEA-C6	-10.8			
Oxalic acid	IAEA-C7	-14.5			
Oxalic acid	IAEA-C8	-18.3			
Caffeine	IAEA-600	-27.771[a]	(+1.0)[b]		
L-Glutamic acid	USGS 40	-26.389[a]	-4.5		
L-Glutamic acid	USGS 41	+37.626[a]	+47.6		
Cellulose	IAEA-CH-3	-24.724[a]			
Ammonium sulphate	IAEA-N1		+0.4		
Ammonium sulphate	IAEA-N2		+20.3		
Potassium nitrate	IAEA-NO-3		+4.7		
Water	VSMOW			0	0
Water	GISP			-189.5	-24.8
Water	SLAP			-428	-55.5
Benzoic acid	IAEA-601				+23.3
Benzoic acid	IAEA-602				+71.4

[a]Note the $\delta^{13}C$ values given in this table include the latest values published by the IAEA as of 30 November 2006.
[b]This $\delta^{15}N$ value is based on data from one laboratory only.

1.2.1.2 Principles of isotope ratio mass spectrometry (IRMS)

The detection of natural isotope variations in a sample requires a very high measurement precision in the IRMS instrumentation. The mass spectrometer for isotopic measurements makes use of specialised static magnetic sector fields as well as the simultaneous collection and quantification of multiple isotopes with Faraday cups (Gremaud & Hilkert, 2008). Figure 1.7 shows a simplified diagram of an IRMS design

layout. Samples are typically converted into the gases: CO_2, N_2, CO, H_2 and SO_2 before being ionised and focused for collection and detection. A variety of IRMS interfaces have been developed to cater to the different analytical approaches for converting organic and inorganic matter into gases. Apart from using the elemental analyser (EA) for bulk stable isotope analysis with the IRMS, the mass spectrometer can also be coupled to conventional gas chromatography (GC) and liquid chromatography (LC) technologies for compound-specific isotope analysis (Gremaud & Hilkert, 2008).
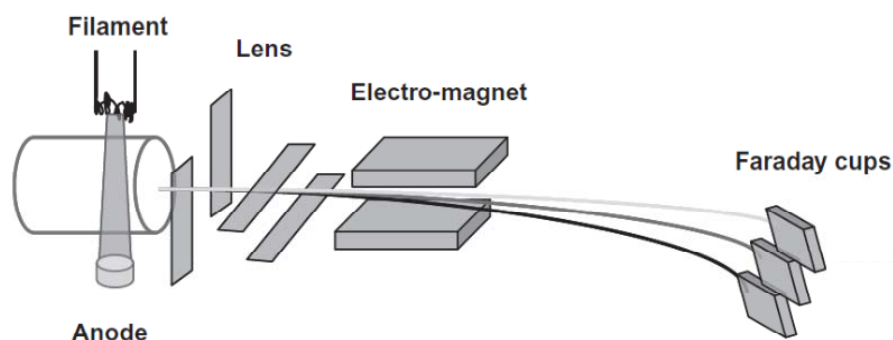
Figure 1.7 Schematic of an isotope ratio mass spectrometer. Taken from Fay & Kussmann, 2010.

IONISATION

The simple gases that are produced from the samples enter the ion source where they undergo ionisation through the bombardment by a stream of electrons. These electrons are released from a heated filament typically made from tungsten. The resulting interaction between the gas molecule and electron induces the loss of an electron to produce a positively charged ion as illustrated in equation (1.3).

$$M \xrightarrow{e^-} M^+ + 2e^- \qquad (1.3)$$

The newly formed positively charged ion possesses excess energy which can result in bond dissociation, similar to the process of fragmentation or cracking. This is important in the understanding of the occurrence of isobaric interference in some isotope ratio measurements (Kelly, 2003). A classic example is the interference to N_2 measurements by CO as both share the same mass. This CO is formed during the ionisation of CO_2, thus special care should be taken to ensure that traces of CO_2 be removed for nitrogen isotope analysis (Kelly, 2003).

MASS SEPARATION

Following ionisation, the positively charged ions are accelerated into the magnetic sector of the flight tube. The role of the magnetic field is to resolve the ion beam according to mass to charge (*m/z*) ratio. In other words, heavier isotopologues are deflected less than the lighter isotopologues although the ions have the same kinetic energy and charge.

ION COLLECTION

The resolved ion beams are eventually channelled to the collector assembly which is made up of dedicated Faraday cups, each having its own separate amplifier electronics in order to cancel out ion beam fluctuations from temperature drifts or electron bean variations (Gremaud & Hilkert, 2008). The typical alignment of the Faraday cups is shown in Figure 1.8.
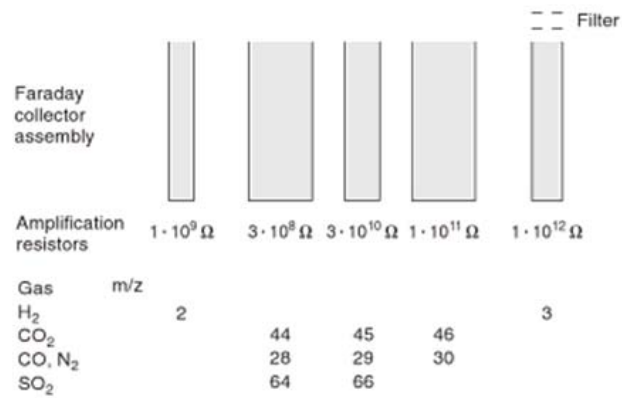
Figure 1.8 Typical Faraday cup arrangements for measurement of the isotope ratios of the most common gas species. Taken from Gremaud & Hilkert (2008).

The universal triple collector (which is represented by the three middle Faraday cups) allows for the measurement of all isotope ratios in C, N, O and S isotope analysis while the remaining two bracketing Faraday cups form the H_2 collector. An additional energy discrimination filter at the entrance of the m/z 3 Faraday cup helps to overcome the interference by the low energy m/z 4 ($He^{+\bullet}$) generated from the helium carrier gas used in continuous flow IRMS systems (Brand, 2007).

1.2.1.3 Bulk stable isotope analysis (BSIA)

The elemental analyser is the most widely used application that can be coupled with the IRMS. In this approach, the whole (bulk) sample is first converted into gases which are separated by chromatography to allow for multi-element isotope ratio analysis. Modern commercially available elemental analysers offer an automated approach to on-line high precision BSIA. Only a small amount of sample is required inside a tin or silver capsule which is loaded into an autosampler carousel for the analysis. Depending on the stable isotope of interest, two basic conversion techniques,

the combustion or high-temperature pyrolysis mode, can be selected (Gremaud &
Hilkert, 2008).

FLASH COMBUSTION ELEMENTAL ANALYSER

The flash combustion technique used in the elemental analyser applies to the
determination of C, N and S isotope ratios in all organic and most inorganic samples
(Gremaud & Hilkert, 2008). The tin capsule which contains the sample is introduced
from the autosampler into the combustion reactor maintained at 1020°C where an
instantaneous and complete oxidation process takes place. This instantaneous
combustion is achieved by the optimum timing for the introduction of a pulse of oxygen
gas when the tin capsule reaches the hot zone in the combustion reactor. The exothermic
nature of this reaction can lead to a momentary spike in temperature of up to 1800°C
that lasts for a few seconds, hence the term flash combustion is used (Gremaud &
Hilkert, 2008). The carbon and nitrogen contained in the sample is converted to CO_2
and NO_X respectively as shown in equation (1.4). This reaction is facilitated by the
presence of the catalyst, Cr_2O_3, which is used as a packing material in the combustion
reactor.

$$2C_N H_X O_Y N_Z \xrightarrow[\text{in excess } O_2]{\text{1020°C combustion}} 2NCO_2 + 2ZNO_x + 2O_2 + XH_2O \quad (1.4)$$
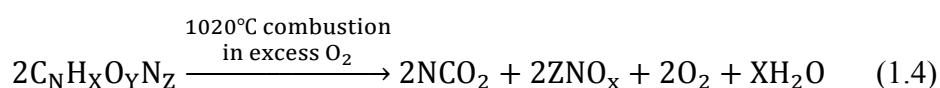
Following combustion, the resulting gaseous products pass through a reduction furnace
maintained at 650°C. The reduction column contains copper where the excess oxygen is
scavenged by the reduced copper and NO_X is reduced to N_2, as illustrated in equation
(1.5).

$$2NCO_2 + 2ZNO_x + 2O_2 + XH_2O \xrightarrow{\text{650°C reduction}} 2NCO_2 + ZN_2 + XH_2O \quad (1.5)$$
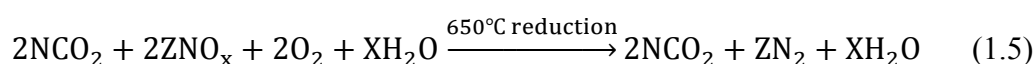
An additional water trap is set up to remove the water produced from the combustion process before the final dry gaseous products enter the chromatographic column with the help of helium as the carrier gas. The separated gases are eluted at different retention times and, if required, are diluted before their introduction into the IRMS. A summarised pictorial representation of the flash combustion EA-IRMS is shown in Figure 1.9.
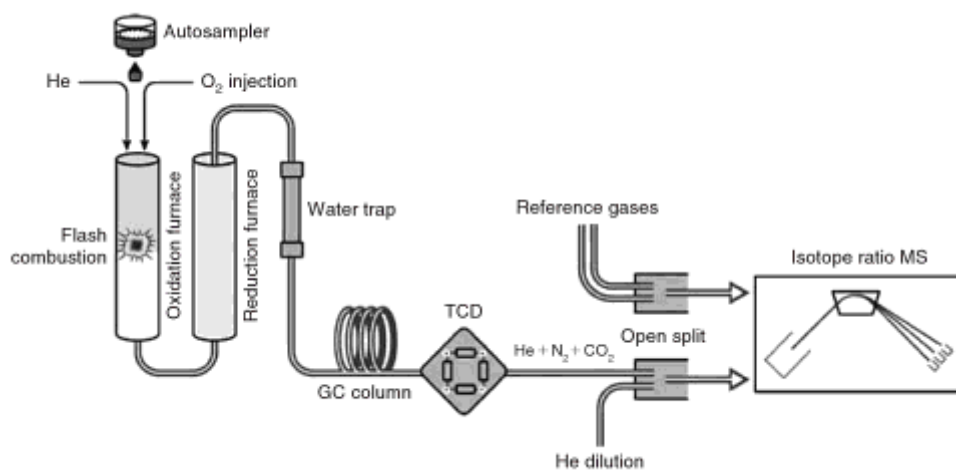
Figure 1.9 Configuration of the elemental analyser IRMS system in flash combustion mode. Taken from Gremaud & Hilkert (2008).

HIGH TEMPERATURE PYROLYSIS ELEMENTAL ANALYSER

The high temperature pyrolysis technique used in the elemental analyser for determining hydrogen and oxygen isotope ratios is a relatively new approach by comparison with the flash combustion technique (Gremaud & Hilkert, 2008). The hydrogen and oxygen contained in the sample are converted into H_2 and CO respectively in the presence of a high temperature reducing environment, typically exceeding 1400°C (Gremaud & Hilkert, 2008). Figure 1.10 shows the high temperature

conversion process during pyrolysis. The excess carbon from this reaction is deposited as glassy carbon.
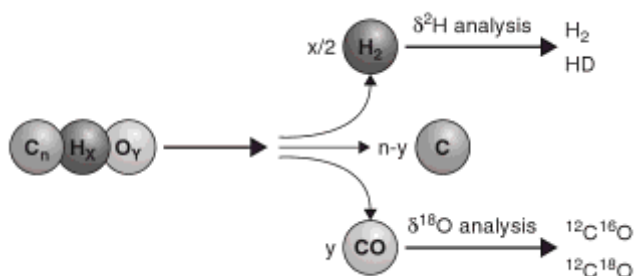
Figure 1.10 High temperature conversion during pyrolysis. Adapted from Gremaud & Hilkert (2008).

The use of a glassy carbon tube and glassy carbon packing material in the high temperature pyrolysis reactor (Figure 1.11) ensures that the conversion process is free from interference by oxygen, and prevents the occurrence of memory effect on the subsequent conversion reactions. The gaseous products are separated in the same manner as described above.
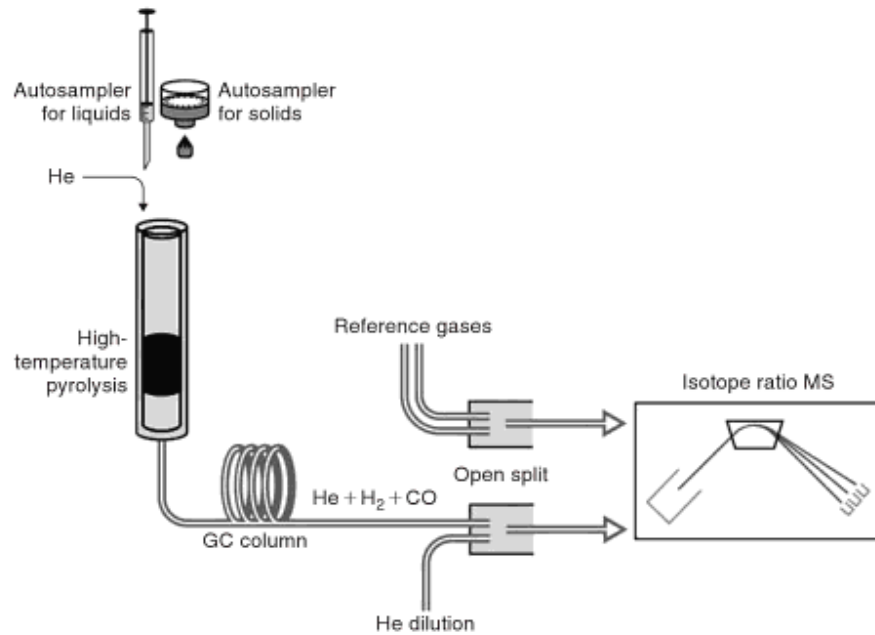
Figure 1.11 Configuration of the elemental analyser IRMS system in high temperature pyrolysis mode. Taken from Gremaud & Hilkert (2008).

1.2.1.4 Recent advances in stable isotope measurement

Apart from the IRMS instrumentation, other alternative techniques to stable isotope measurements exist including multiple collector inductively coupled plasma mass spectrometry (ICP-MS), infrared spectrometry, and nuclear magnetic resonance (NMR) spectroscopy which focuses on site-specific isotope ratio determinations. Despite the IRMS being the traditional choice for isotope ratio measurements, the increased interest in traceability and/or authenticity, ranging across various fields and sectors in society, has led to a changing market demand for alternative low cost, high measurement precision and high sample throughput solutions. An example of such alternative approach is the cavity ring down spectroscopy technique.

In recent years the commercial availability of CRDS has been a significant drive for its use in isotope ratio analysis as an alternative to conventional IRMS. This technique employs the manipulation of a laser beam which is used to detect the distinctive rotational-vibrational transitions of different isotopic species in the mid and near infrared spectrum (Hofstetter & Berg, 2011). The sensitivity of the CRDS technique is attributed to the long absorption path length, usually several kilometres, which is created by a series of reflective mirrors fitted inside the optical cavity. In this way, the laser beam can be circulated inside the cavity for a substantial number of reflections. A schematic of the CRDS analyser is shown in Figure 1.12.
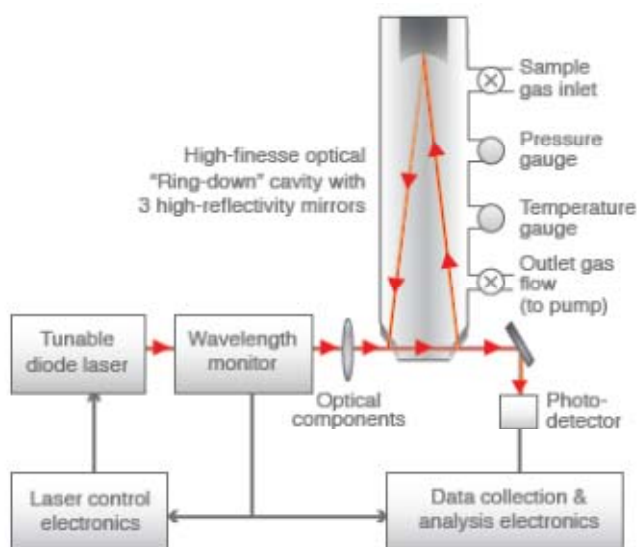
Figure 1.12 Schematic of CRDS analyser. Taken from Saad *et al.* (2009).

Upon the abrupt cessation of the laser beam, the light energy undergoes an exponential decay resulting from the scattering and absorption losses inside the cavity

as well as loss of light during each reflection by the mirrors (Kerstel, 2007). This exponential energy decay in time is referred to as "ring down" and is measured by recording the intensity of the light leaking out of the cavity as a function of time.

Although it has been reported that the CRDS technique has the capability of analysing isotopes from water (^{2}H/^{1}H, ^{18}O/^{16}O), CO_2 and CH_4 (^{13}C/^{12}C), or N_2O (^{15}N/^{14}N, ^{18}O/^{16}O), the CRDS technique still lacks the versatility of IRMS: once the CRDS instrument has been configured for the analysis of a selected gas, it cannot be modified to analyse another. Nevertheless, this is compensated by its affordability and more compact nature (Hofstetter & Berg, 2011). However, the accuracy and precision of the CRDS technique do not yet match the GC/IRMS for compound-specific analysis of isotope ratios and detection limits are orders of magnitude higher (Hofstetter & Berg, 2011).

1.2.2 Carbon stable isotopes

As discussed earlier, carbon has two naturally occurring stable isotopes: ^{12}C and ^{13}C, with the former having a natural abundance level of 98.89%. The ratio of the heavier isotope, ^{13}C, to the light isotope, ^{12}C, can be determined by the IRMS and can be further translated into information for food authenticity or traceability studies. The original source of carbon in plants comes from the CO_2 in air. The process of photosynthesis is known to result in the depletion of ^{13}C in plant tissue with respect to ambient air (Körner *et al.*, 1988). It must, however, be noted that this difference in the isotopic distribution is dependent on the various CO_2 fixation pathways undertaken by the plants, as distinguished by the three categories of photosynthetic plants: C_3, C_4 and CAM (Gremaud & Hilkert, 2008). Many plants, including rice, are C_3 plants while the classic examples of C_4 plants include corn, sorghum and sugar cane. Figure 1.13 shows

the typical $\delta^{13}C$ ranges within these three categories of plant together with other carbon sources.
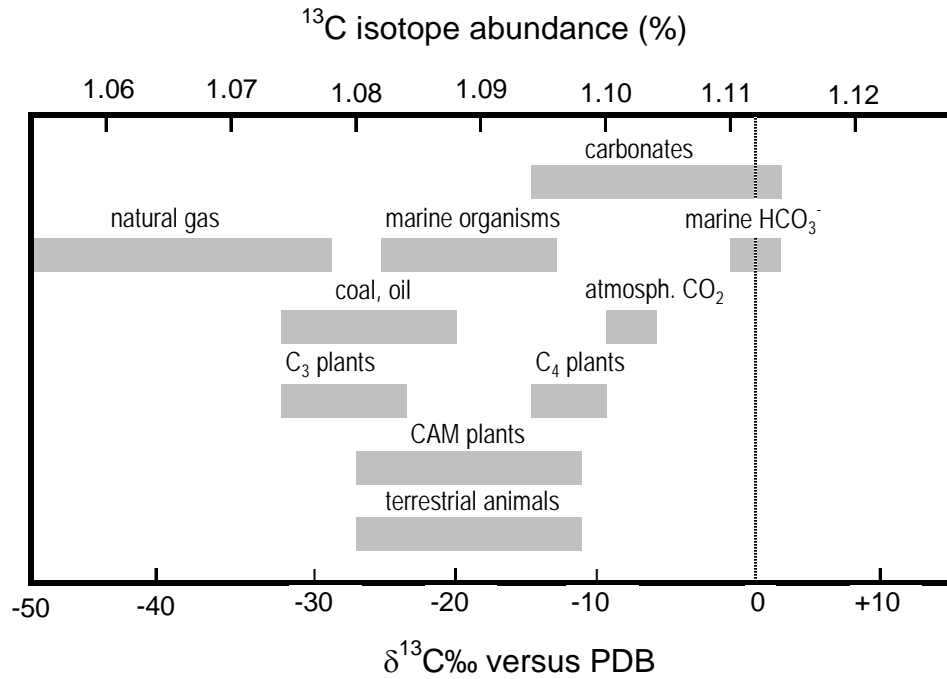
Figure 1.13 ^{13}C isotope variation ranges of carbon pools. Adapted from Winkler (1984).

1.2.2.1 Carbon dioxide fixation in C₃ plants

The differences in the isotope fractionation associated with the different pathways for carbon assimilation and fixation in plants enable the characteristic ranges of $\delta^{13}C$ value to be used to differentiate between C_3 and C_4 plants. In rice, CO_2 fixation leads to the formation of a three-carbon compound, 3-phosphoglycerate (3-PGA), hence plants using the 3-PGA pathway are commonly known as C_3 plants (Meier-Augenstein, 2010).

Figure 1.14 illustrates the dark reactions of photosynthesis in C_3 plants. In this process, two molecules of 3-PGA are formed when CO_2 combines with ribulose 1,5-bisphosphate, ribulose-bisphosphate carboxylase being the catalyst. The 3-PGA is

usually reduced by nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) and adenosine triphosphate (ATP) to form glyceraldehyde 3-phosphate, which feeds a complex series of reactions known as the Calvin cycle (or reductive pentose phosphate cycle), from which hexoses like glucose and fructose are formed (O'Brien, 1992).
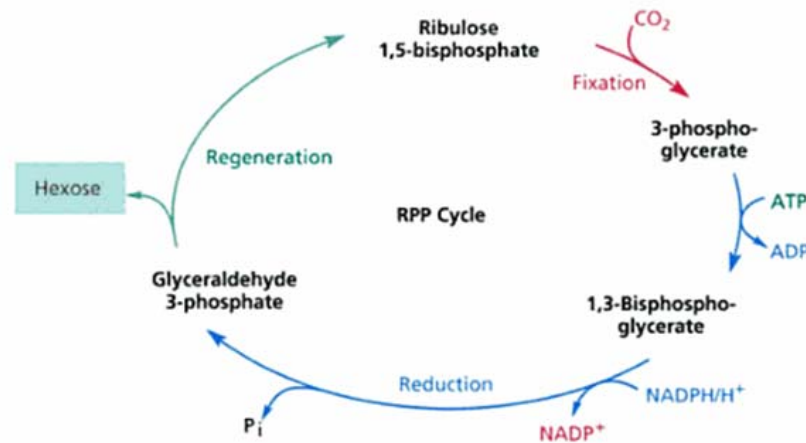
Figure 1.14 Carbohydrate formation in the photosynthetic dark reactions through the reductive pentose phosphate (RPP) cycle or Calvin cycle. Taken from McMurry & Begley (2005).

1.2.2.2 Applications of carbon stable isotope ratio analysis

A broad range of applications for carbon stable isotope ratio determination in the area of food authenticity and traceability have been reported in the literature. Although the use of the $\delta^{13}C$ value may not be the sole determining factor in some analyses, it can be used as complementary data for multivariate statistics applications. Some of the reported applications for $\delta^{13}C$ analysis include: the characterisation of food authenticity; the detection of adulteration in vinegar to characterise the botanical origin of acetic acid and to detect adulterations of vinegar using synthetic acetic acid by SNIF-NMR and IRMS (Thomas & Jamin, 2009); wine and fruit juices through the determination of the carbon isotope ratios of sugars and malic acid in apple juices, and the assessment of

watering in beverages from the stable isotope analyses of hydrogen, carbon and oxygen (Jamin *et al*., 1997; Calderone & Guillou, 2008); honey from the measurement of $\delta^{13}C$ in the bulk honey, its protein fraction, fructose, glucose, di- and trisaccharides using the IRMS coupled to elemental analyser and liquid chromatograph (Elflein & Raezke, 2008); flavouring agents in tea (Mar Caja *et al*., 2009); and vanillin from ice cream and yoghurt by $\delta^{13}C$ analysis (Lamprecht & Blochberger, 2009); egg classification to differentiate between inorganic and organic eggs from the stable carbon and nitrogen isotope values of whole yolk, delipidised yolk, albumen and egg membrane (Rogers, 2009) and identification of corn-fed chicken through the $\delta^{13}C$ analysis of the fat and protein component of the chicken meat (Rhodes *et al*., 2010). Other applications involve the identification of geographical origins for food items such as: wheat based on the elemental analysis of cadmium, lead, selenium and strontium as well as the stable isotope analysis of carbon and nitrogen (Branch *et al*., 2003); mozzarella cheese to fulfil the EC Regulation on the Protected Designation of Origin by carrying out physical-chemical determinations, ^{1}H nuclear magnetic resonance for the semi quantitative determination of some amino acids, organic acids, alcohols and sugars, and IRMS determination of $\delta^{13}C$ and $\delta^{15}N$ (Brescia *et al*., 2005); honey by the analysis of some physico-chemical parameters, elemental content using total reflection X-ray fluorescence spectrometry and stable carbon and nitrogen isotope ratios using IRMS, or by the analysis of hydrogen, carbon, nitrogen and sulphur stable isotope ratios (Kropf *et al*., 2010; Schellenberg *et al*., 2010); cow's milk through the determination of stable carbon, nitrogen, oxygen, sulphur and strontium isotope ratios (Crittenden *et al*., 2007); coffee beans from the analysis of $\delta^{13}C$, $\delta^{2}H$ and $\delta^{18}O$ in the extracted caffeine or the analysis of bean composition based on $\delta^{13}C$, $\delta^{2}H$ and $\delta^{18}O$, as well as the carbon and nitrogen percentage (Weckerle *et al*., 2002; Rodrigues *et al*., 2009); beer using the

carbon isotope ratios (Brooks *et al*., 2002); beef through the determination of $\delta^{13}C$, $\delta^{14}N$

and $\delta^{34}S$ in defatted muscle or the $\delta^{13}C$ and $\delta^{14}N$ analysis in defatted meat, crude fat and

tail hair (Bahar *et al*., 2008; Guo *et al*., 2010) and alcoholic beverages by the $\delta^{13}C$ and

$\delta^{2}H$ determinations in the ethanol (Hattori *et al*., 2008).

1.2.3 Nitrogen stable isotopes

The atmosphere is the principal reservoir for nitrogen in nature and has a

consistent $^{15}N/^{14}N$ ratio of 0.366%, making it suitable as the primary standard for

reporting $\delta^{15}N$ values. The nitrogen available in the atmosphere is not directly utilised

by most organisms except for a few nitrogen-fixating soil bacteria. The nitrogen cycle in

the biosphere encompasses five main processes: nitrogen fixation, nitrogen uptake

(through growing organisms), nitrogen mineralisation (decay), nitrification and

denitrification (Meier-Augenstein, 2010).

The fixation of atmospheric nitrogen, through the conversion of nitrogen into

ammonium, is carried out by symbiotic bacteria which populate the roots of some

plants. The nitrogen fixation process generally gives rise to a small fractionation effect

which translates into a shift of around 0-2‰ (Gremaud & Hilkert, 2008). Further

assimilation of ammonium into nitrogen metabolism can result in a greater impact, with

a significant shift in $\delta^{15}N$ from 0-27‰. In addition, bacterial nitrification and

denitrification processes can lead to the depletion of ^{15}N, giving a typical $\delta^{15}N$ range

from -10 to -40‰ (Gremaud & Hilkert, 2008). On the other hand, nitrogen fractionation

during mineralisation is insignificant or none (Gremaud & Hilkert, 2008). Figure 1.15

shows a graphical depiction of the ^{15}N isotope variation ranges of nitrogen pools.
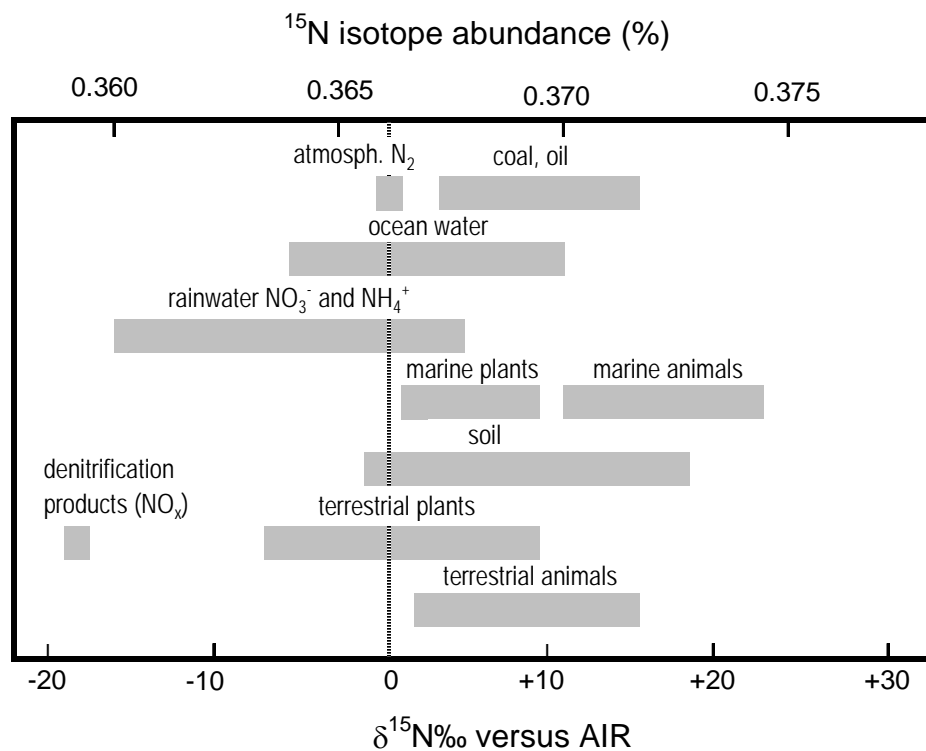
Figure 1.15 ^{15}N isotope variation ranges of nitrogen pools. Adapted from Winkler, 1984; Cornell *et al*. (1995).

In the context of plant materials, the enrichment or depletion of ^{15}N is influenced by plant species (clover is known to be depleted in ^{15}N), the soil in which the plant is grown (as this has an effect on the mineralisation, nitrification and denitrification processes), and the type of fertiliser (synthetic or organic N) applied for agricultural purposes (Gremaud & Hilkert, 2008).

1.2.3.1 Applications of nitrogen stable isotope ratio analysis

Similar to the applications of carbon stable isotope ratio analysis, nitrogen stable isotope ratio analysis is useful in food authenticity and traceability testing. The nitrogen stable isotope ratio is also often used together with other stable isotope ratios, and sometimes with multi-elemental data, to identify the geographical origin of food. Some examples of these applications include studies the geographical origins of wheat

(Branch *et al.*, 2003), mozzarella cheese (Brescia *et al.*, 2005), cow's milk (Crittenden *et al.*, 2007), honey (Kropf *et al.*, 2010; Schellenberg *et al.*, 2010), beef (Bahar *et al.*, 2008; Guo *et al.*, 2010), and coffee beans (Rodrigues *et al.*, 2009), as well as food authenticity studies on organic versus conventional tomatoes and lettuces from the trace element (manganese, calcium, copper and zinc) and nitrogen isotope data (Kelly & Bateman, 2010), and identification of corn-fed chicken (Rhodes *et al.*, 2010).

1.2.4 Oxygen stable isotopes

Three stable isotopes exist in nature, namely ^{16}O, ^{17}O and ^{18}O, having the relative abundances of 99.76%, 0.04% and 0.20%. Of the minor isotopes, only ^{18}O has been employed in isotopic studies by IRMS and the range of natural variation in $\delta^{18}O$ is shown in Figure 1.16.
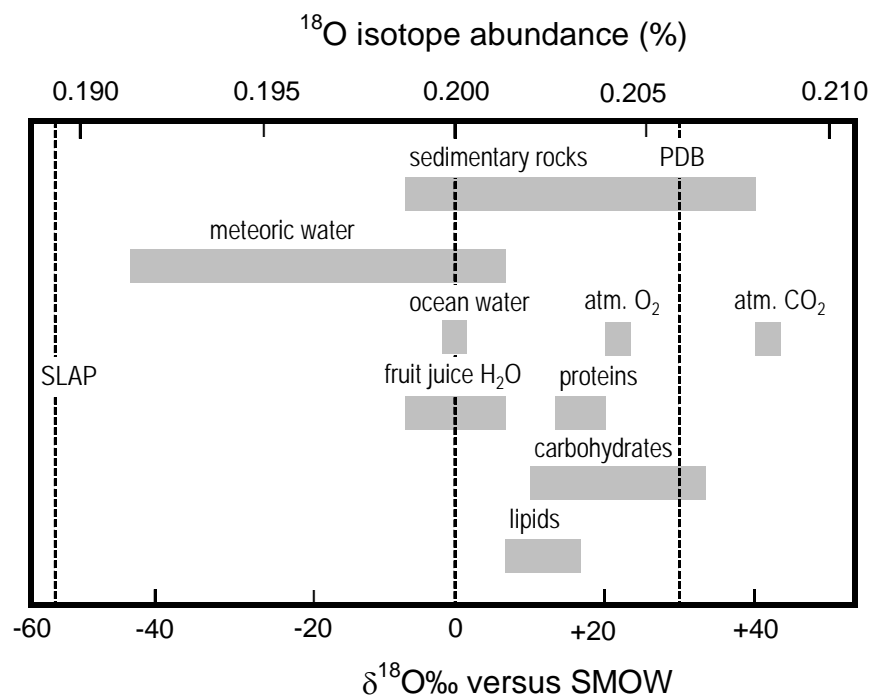
Figure 1.16 $\delta^{18}O$ isotope ranges of oxygen pools. Adapted from Winkler (1984).

The hydrological cycle is the main mechanism for the transfer of oxygen and hydrogen and its subsequent incorporation into organic and inorganic materials (Meier-

Augenstein, 2010). Thus, similar isotopic fractionation patterns of O to H can be observed from evaporation, condensation and precipitation processes resulting in an analogous geographical distribution of the heavier and light oxygen isotopes to the hydrogen isotopes.

The hydrological cycle follows the movement of water and energy between several water reserves and the Earth's major compartments: the lithosphere, atmosphere, biosphere and hydrosphere (Meier-Augenstein, 2010). The ocean body can be viewed as the starting point of this cycle with evaporation forming clouds and precipitation affecting the transfer of water from the oceans to the continents. Isotopic fractionation in meteoric water occurs during the processes of evaporation, condensation and precipitation which in turn, results in water from different geographical locations having dissimilar isotopic compositions. Subject to latitude, altitude, temperature and distance to the open waters, hydrogen isotopes are typically enriched in water from coastal or near equatorial regions while the reverse is observed in water from inland or high altitude and/or latitude regions (Meier-Augenstein, 2010).

Study of the isotopic composition of precipitation from different parts of the world first established this relationship between the δ^2H and δ^{18}O (Craig, 1961b). Equation (1.6) below describes this relationship, a correlation also referred to as the meteoric water line (MWL).

$$\delta^2\text{H} = 8\delta^{18}\text{O} + 10 \qquad\qquad (1.6)$$

A schematic model of the evaporation/condensation processes from the hydrological cycle (Figure 1.17) shows that convective mixing between the evaporative water and residual depleted vapour from the cloud layer occurs over the oceans. The δ^2H and $\delta^{18}O$ values of the evaporation flow, the marine vapour and precipitation all fit into the MWL. However, deviations from MWL have been observed in precipitation over island and coastal areas and in condensation near the sea surface (Yurtsever & Gat, 1981).
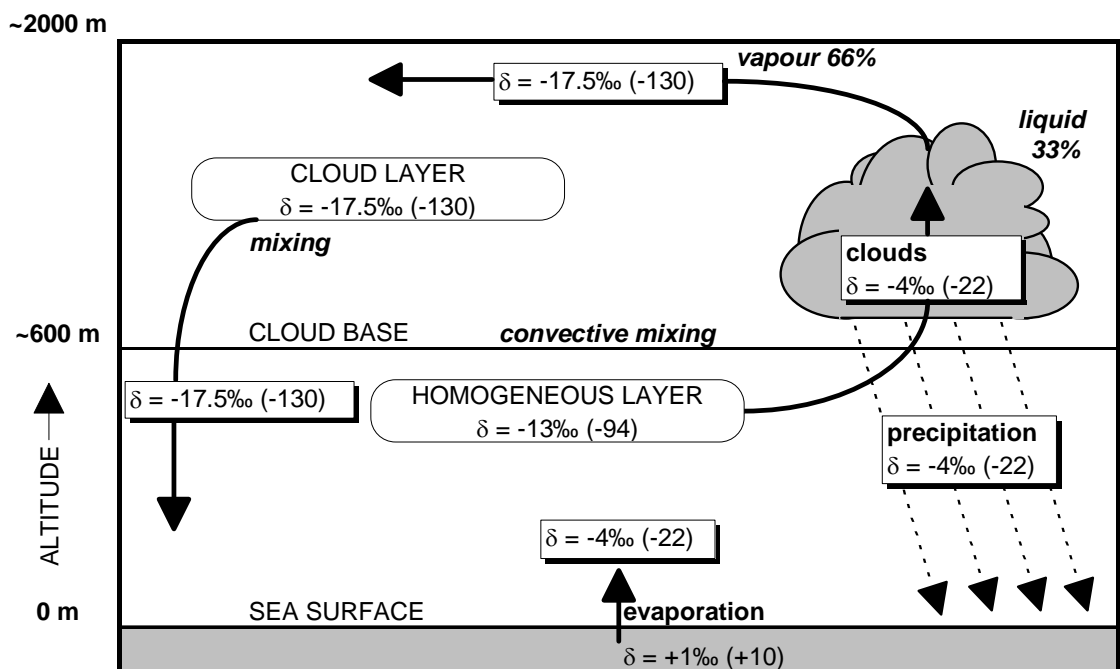
Figure 1.17 Evaporation/condensation model with typical delta values for atmospheric water masses. The first δ-value refers to $\delta^{18}O\text{‰}$ and the second (in brackets) refers to $\delta^2H\text{‰}$. Adapted from Yurtsever & Gat (1981).

1.2.4.1 Applications of oxygen stable isotope ratio analysis

The relationship of the oxygen stable isotope ratio to geographical location makes it a popular choice for food traceability studies, like the hydrogen stable isotope ratio analysis. Examples of the application of oxygen stable isotope ratios include the

identification of geographical origins: ethanol from beverages (Hattori *et al.*, 2008), cow's milk (Chesson *et al.*, 2010), honey (Schellenberg *et al.*, 2010), flavouring agents in tea (Mar Caja *et al.*, 2009), coffee beans (Weckerle *et al.*, 2002), meat (Horacek *et al.*, 2010) including beef (Heaton *et al.*, 2008) and vegetable oils (Breas *et al.*, 1998; Angerosa *et al.*, 1999).

1.2.5 Hydrogen stable isotopes

The primary source of hydrogen in nature is found in the hydrosphere. Ocean water is the main reservoir for hydrogen since 96.54% of the total volume of water on Earth exists as ocean water (Meier-Augenstein, 2010). Hydrogen has two stable isotopes, ^{1}H and ^{2}H, with natural abundances of 99.985% and 0.015%, respectively. The doubling of the mass for the heavier isotope results in significantly different physico-chemical properties, in particular the rate of reaction. This means that physical processes such as evaporation and condensation, as well as biological processes such as enzyme catalysed reactions, can lead to highly diverse changes from the natural abundance levels of ^{2}H (White, 1988). Examples of typical ^{2}H isotope ranges in various materials are shown in Figure 1.18.
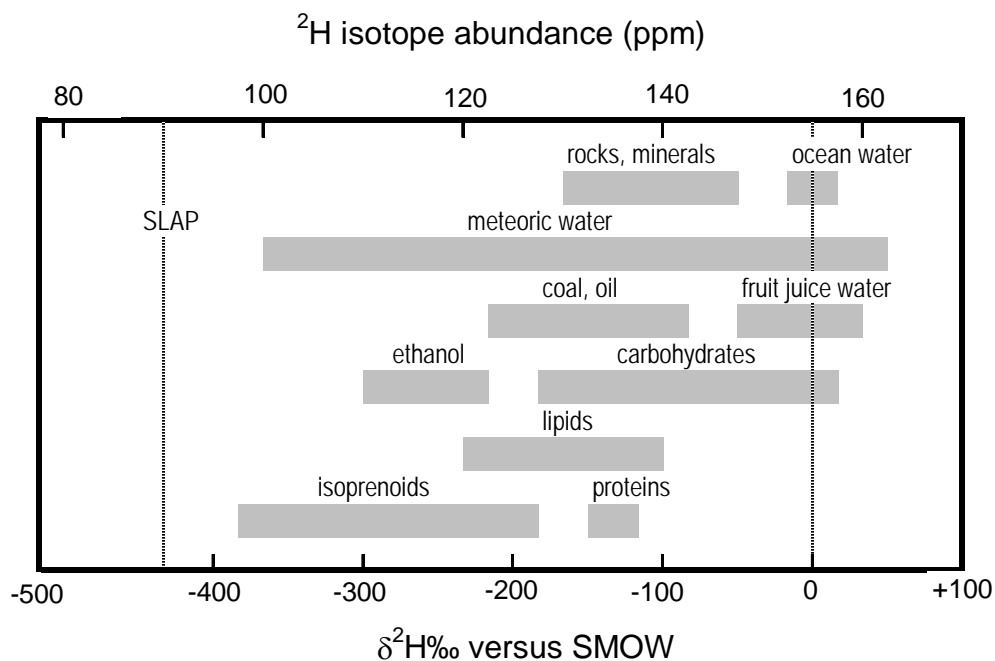
Figure 1.18 ^{2}H isotope variation ranges of hydrogen pools. Adapted from Winkler (1984).

1.2.5.1 Applications of hydrogen stable isotope ratio analysis

Analysis of hydrogen stable isotope ratios is frequently associated with food traceability studies or geographical origin determination, as the δ^2H values have a close association to the geo-location. Literature reports involving hydrogen stable isotope ratio analysis include the identification of geographical origins of ethanol from beverages (Hattori *et al*., 2008), cow's milk (Chesson *et al*., 2010), honey (Schellenberg *et al*., 2010), flavouring agents in tea (Mar Caja *et al*., 2009), coffee beans (Weckerle *et al*., 2002) and meat (Horacek *et al*., 2010) including beef (Heaton *et al*., 2008). In addition, food authenticity investigations involving hydrogen stable isotope ratio measurement have been reported in the detection of illegal watering in beverages (Calderone & Guillou, 2008) and the authenticity of phenolic essential oils (Nhu-Trang *et al*., 2006).

1.3 An introduction to plant mineral nutrients and multi-element analysis

Mineral nutrients have definite and crucial roles in plant metabolism. Based on the extent of the growth requirement for a given nutrient, the nutrient can be classified as either a macronutrient or a micronutrient (Marschner, 1995). The soil is the source of mineral nutrients for plants and the availability of these nutrients to plants depends both on the intrinsic soil composition and the presence of fertilisers. Multi-element analysis is also applicable to the determination of geographical origin, complementing the stable isotope ratio analysis approach. The trace elemental composition of plants reflect, to a certain extent, that of the soil in which they were grown. The level of trace elements depends on the topography and soil characteristics, hence for the application of this to geographic origin studies, there needs to be a unique soil map for every country (Kelly *et al.*, 2002).

1.3.1 Mineral nutrition in plants

Mineral nutrition is important to the metabolism and growth of plants. The elements or their ions, which are selectively absorbed from the soil, are made available from either the weathering of rocks or from the decomposition of organic materials. Macro-elements and trace elements are a necessity for plant survival. Macro-elements participate directly in, or indirectly support, plant metabolism. Trace elements, or micronutrients, are often heavy metals which have direct involvements in enzyme reactions (Schulze *et al.*, 2005).

1.3.1.1 Uptake of minerals by plants

Mineral nutrient concentrations in soils are subjected to a huge variation with soil moisture, soil depth, pH, cation-exchange capacity, redox potential, amount of soil

organic matter present and microbial activity, season of the year and application of fertiliser (Asher, 1978). All these factors will in turn affect the uptake of minerals by plants.

A close relationship exists between nutrient mobility in soil and nutrient availability to plants, and can be summarised by the concept of 'bioavailability of nutrients' (Barber, 1995). Ions are taken into the roots primarily from the root tip, and the components involved in the concept are root interception, mass flow and diffusion, as illustrated in Figure 1.19. The uptake of nutrients along the root tip has an unequal distribution as the transport relies on ion radius and chemical composition (Schulze *et al*., 2005). Nutrient transport in plants and their distribution to various parts of the plant for uptake, storage and utilisation occurs via the xylem and phloem.
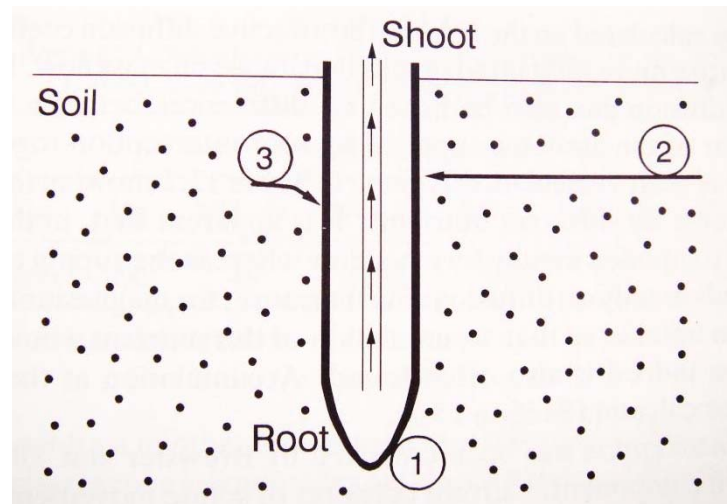
Figure 1.19 Schematic presentation of the movement of mineral elements to the root surface of soil-grown plants. ① Root interception: soil volume displaced by root volume. ② Mass flow: transport of bulk soil solution along the water potential gradient (driven by transpiration). ③ Diffusion: nutrient transport along the concentration gradient. • = Available nutrients. Taken from Marschner (1995).

1.3.1.2 Geological influences from soil

The environment exhibits a vast range of soil types, arising from seemingly countless in geology, climate, vegetation and other organisms, and topography, as well as the time for these factors to combine and influence soil formation (White, 2006). The significant influence of the soil mineral nutrients on plants has led to many studies of the geographical origins of foods using multi-element ICP-MS analysis. Luykx & van Ruth (2008) cited several examples of the successful application of this approach on onions (Ariyama *et al.*, 2006), nuts (Gómez-Ariza *et al.*, 2006), tea (Moreda-Piñeiro *et al.*, 2003), and wine (Coetzee *et al.*, 2005). Ariyama *et al.* (2006) reported that despite elemental concentration differences in onion samples subjected to different fertilisation conditions, crop year and soil type, variations were more pronounced between onion samples from different geographical locations. Onions of the same variety were grown at seven different fields and the mineral concentrations (Li, Na, Mg, P, Co, Ni, Cu, Zn, Rb, Mo, Cd, Cs, Ba and Tl) were found to be largely different between onions from different provenances.

Understanding the main factors affecting soil formation would provide insights into the geographical elemental characteristics of the soil as well as the plants on which they were grown. The rate of weathering of rocks depends on temperature, rate of water percolation, oxidation status of the weathering zone, surface area of rock exposed and the types of mineral present. Thus, the climate plays an important role in soil formation. These factors, in turn, affect the composition of the soil ecosystem which comprises plants, micro-organisms, animals and human activities. The colonisation of plants, the activities of animals and micro-organisms and the agricultural manipulations by man brings about a complex matrix of effects on soil. In addition, relief also has a recognised

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effect on the local climate, as changes in land elevation affect the temperature, the amount and form of the precipitation and the intensity of storm activities. Lastly, the rate of soil formation is another determining factor on the complex climatic, geological and geomorphological changes to soil (White, 2006).

1.3.2 Measurement of multi-element levels

Sample preparations for total metal analysis typically rely on one of two common acid digestion methods: classical hotplate digestion procedure and microwave-assisted acid digestion (Zhang, 2007). Both methodologies use mineral/oxidising acid(s) and the application of an external heat source to break down the sample matrix and liberate the metals in an analysable form. As microwave-assisted acid digestion system offers faster and more reproducible results, it has become the more popular choice over the conventional hotplate approach (Zhang, 2007). Several analytical techniques exist for trace element analysis and the selection of technique is largely dependent on the detection limit requirements for the analysis. Inductively coupled plasma mass spectrometry (ICP-MS) generally plays a more prominent role in the determination of elemental composition and stable isotope tracers in nutritional studies (Stürup, 2004).

1.3.2.1 Microwave acid digestion technique

The first report on the use of a microwave oven for acid digestion for metal analysis dates from 1975 (Abu-Samra *et al*., 1975). Since then, two types of commercially available microwave heating systems (the open-vessel system which operates under atmospheric pressure and the closed-vessel that employs elevated pressure) have been used for laboratory preparation. Although the open-vessel system provides a greater homogeneity of the radiation than the closed-vessel unit, where microwaves are dispersed in the multi-vessel cavity, it can only operate with one sample

at a time (Kou & Mitra, 2003). The greater sample throughput capability and elevated pressure offered in the closed-vessel system makes it the preferred technique for a robust digestion in trace element work.

Figure 1.20 shows a typical closed-vessel system or cavity type microwave digestion unit. The magnetron generates the microwaves which are radiated from its antenna into the waveguide. This waveguide is made of a microwave reflective metal that directs the waves into the microwave cavity. The role of the mode stirrer inside the cavity is to reflect the incoming microwaves and create a homogenous microwave field within. Samples are rotated by the rotor during the digestion process to further improve the homogeneity of the microwave field in the cavity (Kingston & Walter, 1998).
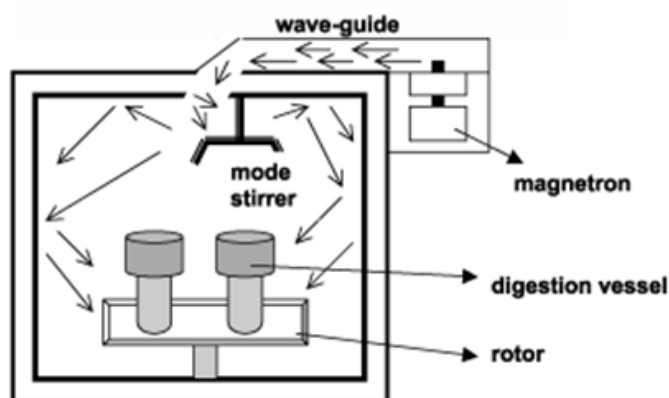
Figure 1.20 Cavity type microwave digestion system. Taken from Oliveira (2003).

Several stages of a digestion process are involved in a typical chemical reaction profile inside the closed vessels, as illustrated in Figure 1.21. At the start of digestion, the microwave energy heats the acid and the vessel containing the acid and sample. Once the temperature reaches the atmospheric pressure boiling point of the acid, the acid converts into the gaseous phase and condenses against the cooler vessel surface.

The energy from the acid vapour is thus released to the vessel walls and thereby is transferred to the microwave cavity. The final stage of heating, also known as "sustained dynamic thermal non-equilibrium", maintains the reaction temperature during the digestion. This is achieved by balancing the amount of microwave energy absorbed by the acid and the energy released by the vessel. In general, the microwave acid digestion system attains a pressure much lower than is obtained under a normal thermodynamic equilibrium while maintaining a sufficiently high temperature for the acid solution (Kingston & Walter, 1998). This behaviour is the reason behind the efficient acid dissolution reaction in such a system.
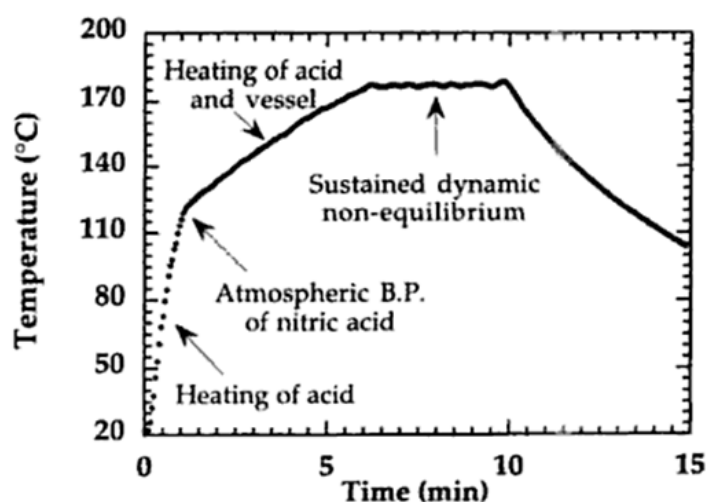
Figure 1.21 Stages involved in a microwave heating process based on the EPA method 3051. Taken from Kingston & Walter (1998).

1.3.2.2 Principles of inductively coupled plasma mass spectrometry (ICP-MS)

Like all other mass spectrometers, the ICP-MS comprises an ion source, a mass analyser and an ion detector. The plasma (or heated argon gas) is the selected ion source

in the ICP-MS technique. A typical schematic of the quadrupole ICP-MS is shown in Figure 1.22.
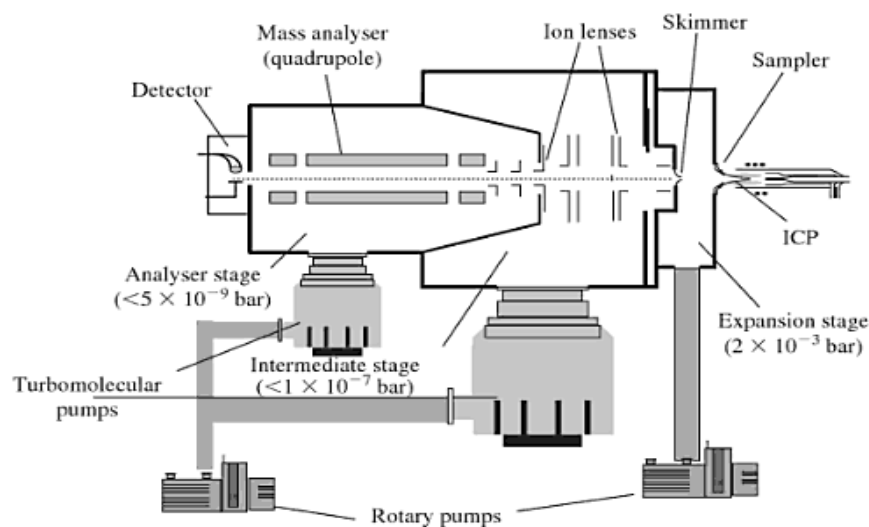
Figure 1.22 Schematic diagram of an ICP-MS. Taken from O'Connor & Evans (1999).

The acid digested samples are introduced into the ICP-MS in the liquid state via a nebuliser which produces a fine mist with high velocity argon. The aerosol then enters a spray chamber where small droplets are formed and subsequently vapourised in the plasma torch. The plasma is generated by heating argon gas to an operating temperature between 6,000 and 10,000K (Fay & Kussmann, 2010) and is sustained by the intense radio frequency (rf) field. Instantaneous thermal decomposition and atomisation of the sample is followed by simultaneous ionisation of the analyte atoms. An interface, comprising differentially pumped chambers, and separated by the sampling and skimmer cones, is important to segregate the high temperature ICP source operating at atmospheric pressure and the mass spectrometer operating at high vacuum (Vela *et al.*, 1993). The hot plasma passes through the sampling cone and undergoes rapid expansion at the interface. A fraction of this gas containing the ions enters through the skimmer

cone and the ion lens assembly before being accelerated into the mass analyser to the detector (Dass, 2007). Electron multipliers are the most commonly used detectors in ICP-MS (Aras & Ataman, 2006).

1.4 Review of existing publications for the determination of the geographical origins for food

The increasing consumer interest in the geographical origins of food can be attributed to a variety of reasons, from the unique organoleptic qualities associated with regional products to health concerns, sense of patriotism, influence from media, decreased confidence in the quality and safety of products, animal welfare and environmental awareness on production method. The latter can come from belief that smaller regional producers have more animal and environmental friendly production cyapproaches (Gilg & Battershill, 1998; Ilbery & Kneafsey, 1998; Kelly, 2003). The EU has recognised the importance of food traceability, and supported through the introduction of regulations, to address this issue (Table 1.8).

Table 1.8 European Union systems for the promotion and protection of food products of recognised quality and origin. Taken from Reid, O'Donnell & Downey (2006).

System	Legislation	Types of food protected	Selected examples of protected foods
Protected designation of origin (PDO)	European Council Regulation (EC) No. 2081/92	Foodstuffs which are produced, processed and prepared in a given geographical area using recognised know-how	Buerre d'Ardenne (Belgium); Kalamata olives (Greece); Roquefort cheese (France); Imokilly Regato cheese (Ireland); Miel de la Acarria honey (Spain)
Protected geographical indication (PGI)	European Council Regulation (EC) No. 2081/92	Foodstuffs in which a common geographical link occurs in at least one of the stages of production, processing or preparation	Timoleague brown pudding (Ireland); Claire island Salmon (Ireland); Riz de Carmague (France); Newcastle brown ale (UK)
Traditional specialty guaranteed (TSG)	European Council Regulation (EC) No. 2082/92	Foodstuffs possessing a traditional character, either in the composition or means of production	Mozzarella cheese (Italy); Sahti beer (Finland); Jamón Serrano (Spain)

Reports on analytical approaches for the determination of geographical origin in agricultural products have been on the rise since the 1980s and their scope covers both processed products and fresh produce (Luykx & van Ruth, 2008). Publications on the identification of geographical origins for food showed that a broad variety of instrumental techniques can be employed.

1.4.1 Publications relating to the geographical origins of rice

Studies on the geographical origins of rice can be dated back to the early 2000s. Yasui & Shindoh (2000) examined thirty four kinds of brown rice cultivated in Japan based on their trace-element composition determination by inductively coupled plasma – optical emission spectrometry (ICP-OES) and inductively coupled plasma – high resolution mass spectrometry (ICP-HRMS). Chemometric discrimination by cluster analysis and principal component analysis (PCA) of the elements manganese, zinc, iron, copper, rubidium, molybdenum, barium, strontium and nickel allowed the distinction of

the brown rice samples cultivated in the Tohoku/Kanto area from those in Hokuriku, and those in Tohoku from those in Kanto. Kawasaki *et al.* (2002) focused on the strontium isotope ratio ($^{87}Sr/^{86}Sr$) of brown rice to estimate their provenance. Multiple collector ICP-MS was used to determine the $^{87}Sr/^{86}Sr$ values in brown rice samples from Japan, China, Australia, Vietnam and California. The results obtained showed that Australian rice had the highest Sr isotope ratio, those of the Chinese and Vietnamese rice samples were slightly higher than most of the Japanese ones, and Californian rice had lower Sr isotope ratios than almost all the Japanese samples in the study. In the same year, Kelly *et al.* (2002) reported the application of IRMS and ICP-MS techniques to determine the geographical origins of premium long grain rice from USA, Spain, Italy, France, Pakistan and India. Nine key components ($\delta^{13}C$, $\delta^{18}O$, boron, holmium, gadolinium, magnesium, rubidium, selenium and tungsten) were identified by canonical discriminant analysis as giving the most prominent distinction between rice samples from these regions. American rice samples were found to contain high levels of boron while European rice had high levels of magnesium. Indian/Pakistani rice samples were found to have characteristic low ^{18}O abundances. This study also showed that the concentration of specific elements like boron and magnesium could be used exclusively as reasonable indicators for the rice geographical origin, while the role of the other elements in the empirical model is more intricate but yet makes a significant contribution to the overall geographical discrimination.

An alternative technique, near-infrared reflectance spectroscopy, was employed by Kim *et al.* (2003) to identify the geographical origins of short and medium grain rice from Korea, USA, Australia and China. The spectral regions of interest were 500-600nm, 700-900nm, and 980-2,498nm. The spectral regions <500nm, 600–700 nm, and 900–980 nm were not used for this study because of large variation of the spectrum

within group (domestic vs. foreign) of rice. A prediction model was successfully developed using a modified partial least square method, with more than 98% of the rice in the study being correctly identified.

In 2008, Suzuki *et al.* investigated the geographical origins of a single rice cultivar, the Koshihikari rice, from Australia, Japan and USA. The carbon and nitrogen contents as well as the $\delta^{13}C$, $\delta^{15}N$ and $\delta^{18}O$ values were determined by IRMS and data interpretation utilised pentagonal radar plots to distinguish the elemental and isotopic compositions of the cultivated areas. In the study, rice from Australia and USA were clearly distinguished from Japanese rice by high $\delta^{15}N$ or high $\delta^{18}O$ values. The $\delta^{13}C$, $\delta^{15}N$ and $\delta^{18}O$ differences in Japanese rice samples were once again explored by Suzuki *et al.* (2009). These short grain rice samples were obtained from Japan's nine main growing locations: Hokkaido, Yamagata, Niigata, Nagano, Ibaraki, Tokyo, Mie, Hiroshima and Okinawa. The results obtained suggested that the $\delta^{18}O$ and $\delta^{13}C$ values in the rice reflected differences in growth conditions including latitude and water stress while the $\delta^{15}N$ value gave a strong discrimination between organic and conventional rice. In addition to the elemental contents and stable isotopic compositions of C, N and O, $\delta^{2}H$ determination was included by Korenaga *et al.* (2010) on both polished and unpolished rice from Japan, California, Australia, Thailand, Vietnam and China. However, it was pointed out that the uncertainty for the $\delta^{2}H$ measurement was too large and thus was excluded from the statistical analysis of the principal components on $\delta^{13}C$, $\delta^{15}N$ and $\delta^{18}O$. Clear distinctions were observed between the Japanese rice samples and those from Australia and USA.

The most recent geographical traceability study of rice was carried out by Gonzálvez *et al.* (2011) on the "Arròs de Valencia" rice grain using mineral element composition. This rice growing tradition is protected under the "Arròs de Valencia"

PDO, which comprises the "Senia", "Bahia" and "Bomba" rice grain varieties grown in the wetlands from the Provinces of Alicante, Castellon and Valencia, and uses specific methods of cultivation. From the study, lanthanides, cadmium and cobalt have been identified as the most influential indicators of the geographical origin of rice samples due to their different concentrations in soils and their effective uptake by plants.

In summary, there is a slight bias in the study of rice origins towards the Asian countries, possibly due to the rice being a staple food there. Chemometric approaches proved to be essential in the study of geographical origins.

1.4.2 Publications relating to the geographical origins of other foodstuff

A summary of literature publications relating to the geographical origins of food commodities by various instrumental approaches is shown in Table 1.9. It should be noted that some of these publications may involve the use of more than one analytical technique.

Table 1.9 Summary of literature relating to the determination of the geographical commodities by various analytical techniques. Modified summary from Luykx & van Ruth (2008).

Instrumental Technique	Parameters Measured	Food Commodity	Reference
Mass Spectrometry			
IRMS	$\delta^{18}O$	Cow's milk	Renou et al., 2004
	$\delta^{13}C, \delta^{15}N, \delta^{34}S$	Beef	Schmidt et al., 2005
	$\delta^{13}C, \delta^{15}N, \delta^{18}O, \delta^{34}S$	Butter	Rossmann et al., 2000
	$\delta^{13}C, \delta^{15}N, \delta^{18}O, \delta^{34}S$	Cabbage, onion, lettuce	Georgi et al., 2005
	$\delta^{13}C$	Wine	Martinell et al., 2003
	$\delta^{13}C, \delta^{18}O, \delta^{2}H$		Ogrinc et al., 2001
	$\delta^{13}C, \delta^{15}N$	Coffee	Serra et al., 2005
	$\delta^{13}C, \delta^{15}N$	Pear	Perez et al., 2006
ICP-MS	Co, Ni, Cu, Rb, Mo, Cd, Cs	Onion	Ariyama et al., 2007
	Multi-element		Ariyama et al., 2006
	Cu, Mn, Ni, Zn	Pine nut	Gómez-Ariza et al., 2006
	Co, Cr, Cs, Cu, Ni, Pb, Rb, Ti, V	Tea	Moreda-Piñeiro et al., 2003
	Multi-element	Wine	Coetzee et al., 2005
PTR-MS	Dimethyl sulfide, dimethyl sulfoxide, dimethyl disulfide, p-cymene, 2-acetyl-5-methylfuran, benzothiazole, bis(methylthio)methane, tris(methylthio) methane	Truffles	Aprea et al., 2007
	60 most predominant ions from the volatile compounds	Cheese	Galle et al., 2011
	Protonated ethanol on m/z 47[+]	Wine	Spitaler et al., 2007
GC-MS	Multiple volatile compounds	Cheese	Pillonel et al., 2003 Povolo et al., 2007
	(E,E)-3,7,11-tri-methyl-2,4,10-dodecatriene	Cheese, milk	
	Neutral volatile compounds	Whey culture (used in Mozzarella cheese)	Mauriello et al., 2003
	Multiple volatile compounds	Honey	Radovic et al., 2001

(Continued)

Table 1.9 Summary of literature relating to the determination of the geographical commodities by various analytical techniques. Modified summary from Luykx & van Ruth (2008). (continued)

Instrumental Technique	Parameters Measured	Food Commodity	Reference
Spectroscopy			
NMR	^{13}C NMR spectra	Olive oil	Shaw et al., 1997
	^{1}H NMR spectra		Mannina et al., 2001
			Rezzi et al., 2005
	^{1}H NMR spectra		
	^{1}H NMR spectra	Wine	Brescia et al., 2003
			Ogrinc et al., 2001
	^{2}H NMR spectra		
	^{1}H NMR spectra	Propolis	Watson et al., 2006
	^{2}H NMR spectra	Mustard oil	Remaud et al., 1997
IR	Spectral range of 800–1800 cm^{-1}	Wine	Picque et al., 2005
	NIR region of 400–2500 nm		Liu et al., 2006
	Spectral range of 4000 cm^{-1} to 900 cm^{-1} (except for between 2918 cm^{-1} and 2849 cm^{-1})	Cheese	Karoui et al., 2004
	Spectral range of 800-4000 cm^{-1}	Olive oil	Tapp et al., 2003
	Spectral range of 400-2498 nm		Downey et al., 2003
	Spectral range of 631 and 3718 cm^{-1}	Honey	Ruoff et al., 2006
	NIR region of 800-500 nm	Grapes	Arana et al., 2005
	Spectral range of 650 to 4600 cm^{-1}	Soy sauce	Iizuka & Aishima, 1999
Fluorescence	Excitation wavelength at 250 and 290 nm; emission wavelength at 410 nm	Cheese	Karoui et al., 2005
	Excitation wavelength at 290 nm, 250 nm and 380 nm; emission wavelength at 410 nm	Milk	Karoui et al., 2005
	Excitation and emission wavelength at 250-700 nm	Olive oil	Dupuy et al., 2005
Atomic	Se	Beef	Hintze et al., 2001
	17 mineral elements	Honey	Paramás et al., 2000
	Na, K, Ca, Mg, Fe, Cu	Wine	Frías et al., 2001
	K, Rb	Orange juice, macadamia nut, pistachio nut	Schwartz & Hecking, 1991
	Na, K, Rb, Ca, Mg, Fe, Cu, Zn, Mn	Potatoes	Rivero et al., 2003
	Multi-element	Coffee	Anderson & Smith, 2002
	Multi-element	Strawberry, blueberry, pear	Perez et al., 2006

(Continued)

Table 1.9 Summary of literature relating to the determination of the geographical commodities by various analytical techniques. Modified summary from Luykx & van Ruth (2008). (continued)

Instrumental Technique	Parameters Measured	Food Commodity	Reference
Separation			
HPLC	Polyphenol	Wine	Rodriquez-Delgado et al., 2002
			Héberger et al., 2003
	Free amino acids and biogenic amines		Bellí et al., 2002
	Ochratoxin A		
	Flavonoids	Honey	Tomás-Barberán et al., 1993
	Triglyceride	Olive oil	Stefanoudaki et al., 1997
	Ethanol-soluble and ethanol-insoluble fractions of the pH 4.6-soluble nitrogen	Cheese	Di Cagno et al., 2003
GC	Various acid contents	Wine	Etièvant et al., 1989
			Shimoda et al., 1993
	Headspace wine constituents		
	Fatty acids	Milk	Collomb et al., 2002
	Fatty acids	Olive oil	Olliver et al., 2003
	Headspace cocoa mass constituents	Cocoa mass	Hernández & Rutledge, 1994
	Chiral terpenes	Orange juice	Ruiz del Castillo et al., 2003
CE	Flavonoid	Chinese fruit (*Frucus aurantii)* extracts	Peng et al., 2006
	Na, K, Ca, Mg, Mn, Li	Wine	Núñez et al., 2000
	Profile fingerprinting	Herb	Wang et al., 2005
Others			
Sensor technology	Organoleptic characterisation	Olive oil	Guadarrama et al., 2001
	Volatile compound fingerprinting	Wine	Penza & Cassano, 2004
	Volatile organic fractions	Orange juice	Steine et al., 2001
DNA technology	Isolated microbial DNA from cheese	Cheese	Bonizzi et al., 2006
	Microbial diversity in whey culture	Whey culture	Mauriello et al., 2003
	Yeast species on fruit surfaces	Grapes	Raspor et al., 2006
	Microsatellite	Olive oil	Sefc et al., 2000
Sensory analysis	Wormwood taste, louche effect, and bitterness	Absinthe	Lachenmeier, 2007
	Adhesivity, friability, elasticity, firmness, aroma, odour, bitterness, saltiness, acidity and sweetness	Cheese	Pillonel et al., 2002
	16 sensory attributes	Olive oil	Stefanoudaki et al., 2000

In the context of this study, the mass spectrometric techniques of IRMS and ICP-MS have been selected for the bulk analysis of rice for the isotopic signatures and elemental profiles. These two approaches are the frequently adopted methodologies for the geographical traceability of rice (refer to section 1.4.1). With previous rice studies being carried out using the IRMS and/or ICP-MS techniques, a comparison could be made with the published rice data. Chromatographic separation techniques for compound-specific analysis of rice would not be suitable as well as time-consuming for a high throughput study of such a large sample size.

Chapter 2 – Stable isotope ratio measurement of rice samples by IRMS analysis

Rice samples obtained for this study were subjected to the carbon, nitrogen, oxygen and hydrogen stable isotope ratio analysis by IRMS to determine the isotopic signature of rice from different geographical origins.

2.1 Stable isotopes of carbon ($^{13}C/^{12}C$ ratio)

A total of 214 rice samples were analysed in duplicate for carbon isotope ratio analysis. The raw $\delta^{13}C$ values obtained from the instrument data were corrected for drift before being compared (see section 6.3.4.1).

2.1.1 Quality controls for carbon stable isotope ratio analysis

Quality controls for the $^{13}C/^{12}C$ analysis comprised two reference materials, fructose from the TRACE project and National Institute for Standards and Technology (NIST) 1568 (rice flour) measured in batches on seven different days. The between-batch precision for the $\delta^{13}C$ measurements of the fructose reference material (Table 2.1; Figure 2.1) shows standard deviations < 0.08‰ for all analysis batches, indicating an excellent consistency in the quality control results. Most of the $\delta^{13}C$ results for the fructose reference material fall within 2σ of the mean of means value (Figure 2.1).

Table 2.1 Compilation of the fructose quality control data from batches subjected to $\delta^{13}C$ analysis.

Date of Analysis	Average (‰)	Standard Deviation (‰)	n
23-Nov-10	-24.55	N.A.*	2
24-Nov-10	-24.44	0.07	4
25-Nov-10	-24.55	0.02	4
29-Nov-10	-24.61	0.03	3
30-Nov-10	-24.52	0.02	4
13-Dec-10	-24.51	0.03	4
14-Dec-10	-24.54	0.03	4

* Note that the standard deviation is not applied when n < 3.
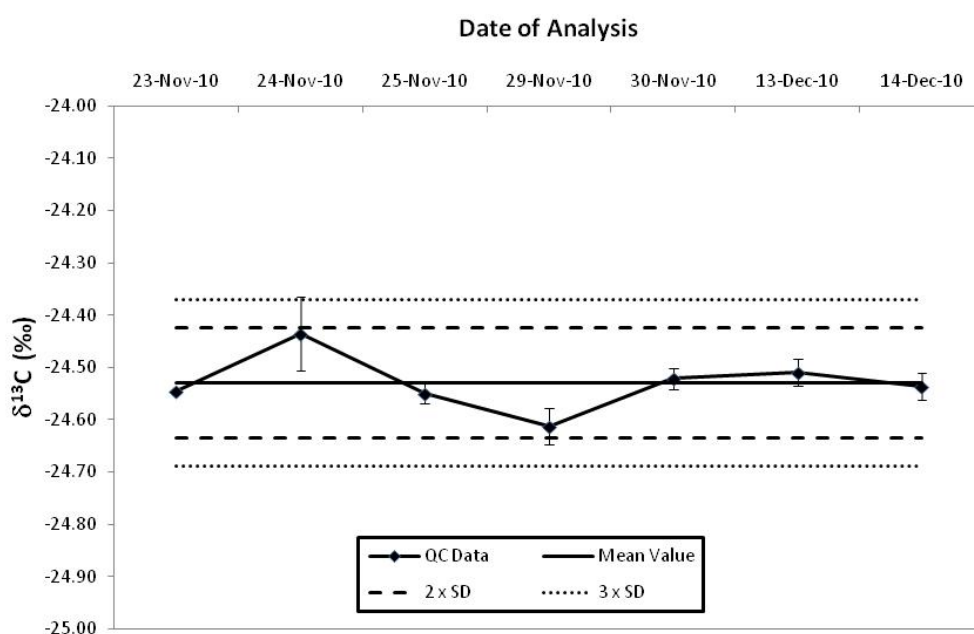
Figure 2.1 Mean $\delta^{13}C$ values for fructose across different batches (assigned value for fructose from inter-laboratory comparison was -24.59‰).

The NIST 1568 rice flour reference material, measured in batches on seven different days, gave standard deviations < 0.07‰ (Table 2.2; Figure 2.2), indicating an excellent consistency in the quality control results. Most of the $\delta^{13}C$ results for the rice flour reference material fall within 2σ of the mean of means value (Figure 2.2).

Table 2.2 Compilation of the NIST 1568 rice flour quality control data from batches subjected to $\delta^{13}C$ analysis.

Date of Analysis	Average (‰)	Standard Deviation (‰)	n
23-Nov-10	-25.84	N.A.*	2
24-Nov-10	-25.63	0.06	4
25-Nov-10	-25.75	0.01	4
29-Nov-10	-25.82	0.04	3
30-Nov-10	-25.75	0.01	4
13-Dec-10	-25.80	0.04	4
14-Dec-10	-25.87	0.01	4

* Note that the standard deviation is not applied when n < 3.
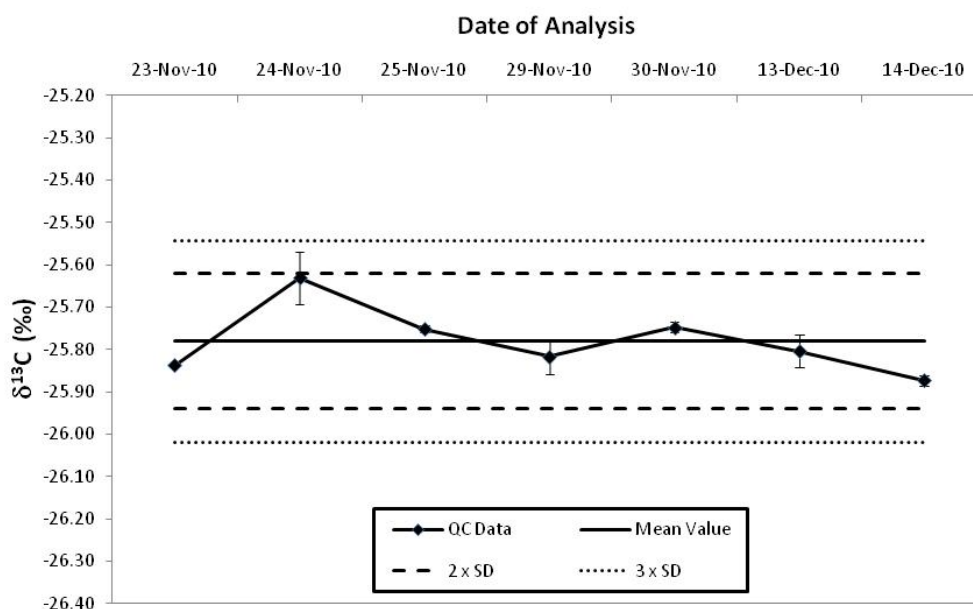
Figure 2.2 Mean $\delta^{13}C$ values for NIST 1568 rice flour across different batches.

Adopting the quality control procedure for measurements suggested by Jamin *et al*. (2004), the deviation between the replicate measurements for each rice sample was set at < 0.3‰. Measurement was repeated when the set deviation limit was exceeded, which involved about 10% of the total samples.

2.1.2 Carbon isotope compositions

The carbon isotopic data obtained from all the rice samples in the study were expressed relative to the international PDB reference. The measured $\delta^{13}C$ values were found to be consistent with the range for C_3 plant materials as illustrated in Figure 1.17. The $\delta^{13}C$ variation of the rice samples ranged from -28.85‰ to -25.52‰ (Table 2.3). Using a boxplot (Figure 2.3) it was observed that the rice samples are not significantly distinguishable by their countries of origin based on the carbon isotopic data alone. The one possible exception is for the Malaysian rice samples which gave noticeably depleted $\delta^{13}C$ values ranging from -28.17‰ to -28.85‰. However, it should be noted that despite the maximum and minimum values are represented by the whiskers in the boxplot, these are not the actual maximum and minimum values in the results dataset due to the identification of outliers by the software. Outliers (represented by the stars and dots) are points lying beyond 1.5 times the interquartile range from the 25th and 75th percentiles of the data.

Table 2.3 Mean, maximum and minimum $\delta^{13}C$ values of rice samples for each country represented in the study.

Country of Origin	Mean $\delta^{13}C$ Value (‰)	Max $\delta^{13}C$ Value (‰)	Min $\delta^{13}C$ Value (‰)	No. of Samples
Australia	-26.87	-26.33	-27.47	4
China	-26.92	-26.51	-27.41	4
France	-26.86	-25.95	-27.59	3
India	-27.49	-26.90	-27.99	30
Italy	-26.78	-25.95	-27.51	5
Japan	-26.98	-26.41	-28.25	27
Korea	-25.92	-25.70	-26.15	2
Malaysia	-28.42	-28.17	-28.85	6
Myanmar	-27.26	-26.94	-27.58	2
Pakistan	-27.37	-26.62	-28.67	18
Spain	-26.14	-26.00	-26.41	3
Taiwan	-27.26	-26.75	-28.80	10
Thailand	-27.33	-26.29	-28.50	64
USA	-26.62	-25.52	-28.09	20
Vietnam	-27.69	-26.51	-28.45	16

Key: – Median ☐ 25%-75% I Min-Max
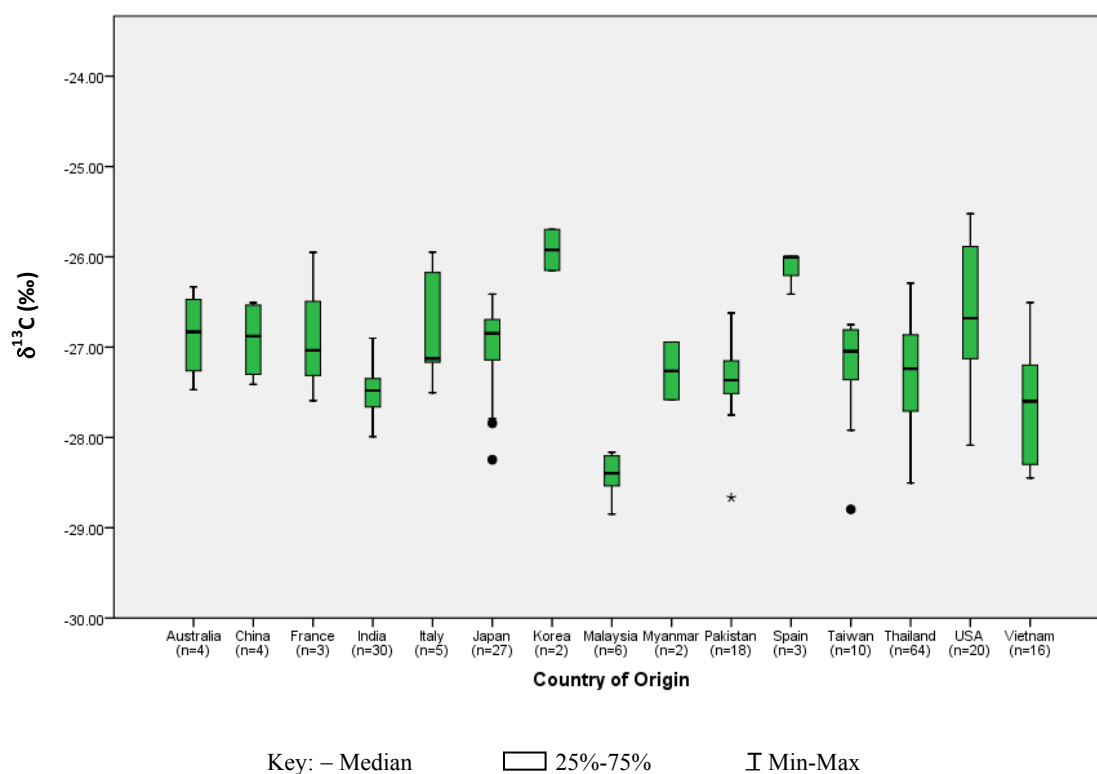
Figure 2.3 Box and whisker plot showing $\delta^{13}C$ variations for all rice types according to country of origin.

As the carbon atoms of the carbohydrate component of the rice samples originate from atmospheric carbon dioxide, the deviation in the $\delta^{13}C$ value of plant carbohydrate from that of carbon dioxide in the atmosphere can be attributed to the natural process of fractionation (Kelly *et al*., 2002). The processes giving rise to fractionation include the diffusion of carbon dioxide, via the leaf stomata, to the site of assimilation and the fixation of carbon dioxide via enzymatic carboxylation reactions (Francey & Farquhar, 1982).

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.

Although the actual conditions for the paddy cultivation of the rice samples in this study are unknown, a comparison between annual average temperature and the mean $\delta^{13}C$ value of the rice from each respective country may be able to provide some insight into the farming environment. The role of temperature was chosen for discussion in this study as the temperature data was more easily available from various sources. Global annual average temperatures for individual countries (Figure 2.4) were obtained from the International Institute for Applied Systems Analysis (IIASA). Typically, the data were collected between the years 1961 and 1990, and averaged (FAO, 2007).
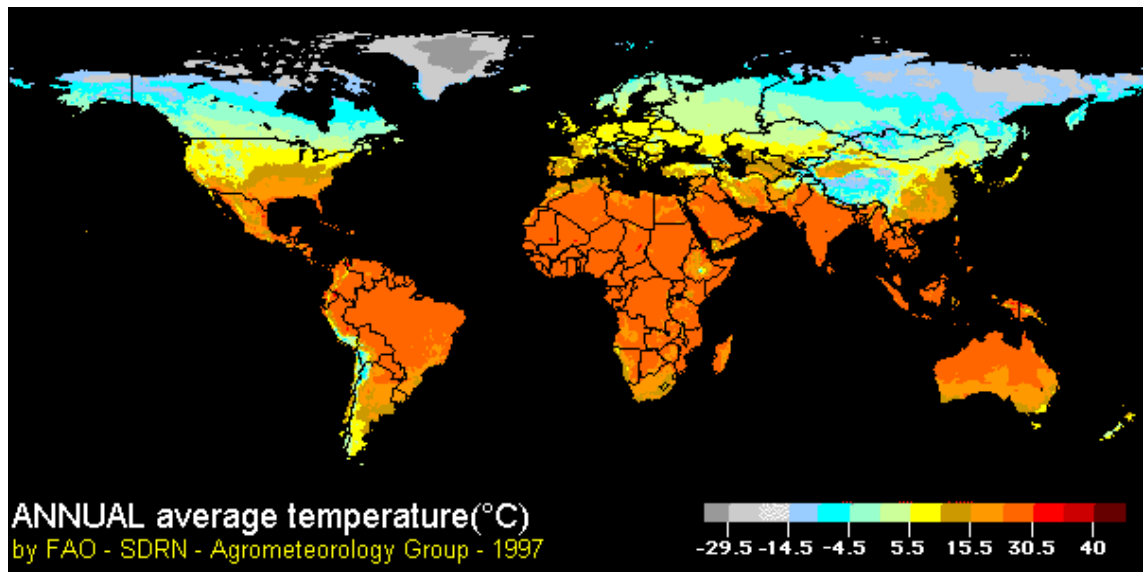
Figure 2.4 Global annual average temperature. Taken from FAO, 2007.

Given that annual average temperature varies within the same country, it would be most effective to compare the temperature data for the rice cultivation regions of the various countries. Since the specific rice cultivation location is unavailable for most of the rice samples in this study, the rice cultivation regions of the countries in the study were obtained from various sources (Table 2.4). The temperature information for these specific rice cultivation regions was obtained from an online climate estimator (FAO, 2002) using the longitude and latitude of the location.

Table 2.4 Rice cultivation regions of the countries in this study.

Country	Rice Cultivation Regions
Australia [1]	New South Wales, Victoria
China [2]	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
France [3]	Camargue
India [2]	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
Italy [4]	Po Valley
Japan [2]	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
Korea [2]	Busan, Chungcheongbuk-do, Chungcheongnam-do, Daegu, Daejeon, Gangwon-do, Gwangju, Gyeonggi-do, Gyeongsangbuk-do, Gyeongsangnam-do, Incheon, Jeju, Jeollabuk-do, Jeollanam-do, Seoul, Ulsan
Malaysia [2]	Johor, Kedah, Kelantan, Melaka, Negeri Sembilan, Pahang, Perak, Perlis, Pulau Pinang, Sabah, Sarawak, Selangor, Trengganu
Myanmar [2]	Ayeyarwady, Bago, Chin, Kachin, Kayah, Kayin, Magway, Mandalay, Mon, Rakhine, Sagaing, Shan, Tanintharyi, Yangon
Pakistan [2]	Baluchistan, N.W.F.P., Punjab, Sind
Spain [5]	Extremadura, Seville, Tarragona, Valencia
Taiwan [6]	Changhua, Chiayi, Hsinchu, Hualien, Kaohsiung, Keelung, Miaoli, Nantou, Penghu, Pingtung, Taichung, Tainan, Taipei, Taitung, Taoyuan, Yilan, Yunlin
Thailand [2]	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 Ayutthaya, 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
USA [7]	Arkansas, California, Louisiana, Missisipi, Missouri, Texas
Vietnam [2]	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

[1] Ricegrowers' Association of Australia.
[2] World Rice Statistics (IRRI).
[3] Notteghem, Roux-Cuvelier, André & Roumen.
[4] Russo & Callegarin.
[5] Herruzo & Morote.
[6] Council of Agriculture Executive Yuan R.O.C. (Taiwan).
[7] USA Rice Federation.

The mean $\delta^{13}C$ value of rice samples from each country is arranged in increasing order (Figure 2.5), the colour scale matching that from Figure 2.4. The values of the mean $\delta^{13}C$ for rice and the mean annual temperature of the cultivation regions for the respective countries used in Figure 2.5 are given in Table 2.5. An interesting observation is that the rice samples from countries with higher annual average temperatures typically have more negative $\delta^{13}C$ values, though it is evident that there is not an exact correlation. Elevated temperatures are generally associated with a greater exposure to sunshine. This may, therefore, suggest that the rice grown in these countries experience higher irradiance levels which would stimulate photosynthesis. Assuming that all other environmental factors that may affect carbon fractionation processes are constant, and since the photosynthetic pathway is the principal factor controlling ^{13}C depletion (Smith *et al.*, 1976), more depleted carbon isotopic ratios would be expected to be observed. Notably, a study on the effect of irradiance on carbon isotope ratios in selected plant tissues reported a slight decrease in their ^{13}C content with greater light intensity or increased exposure to light conditions (Smith *et al.*, 1976). Other similar reports on the ^{13}C depletion in plants from elevated temperatures were made by Smith *et al.* (1973) and Bender & Berge (1979). In a review of literature on the carbon isotope fractionation in plants, O'Leary (1981) noted that reports on the effect of elevated temperature on the carbon isotopic ratio conflict. Thus, if the temperature effect is real, it must be very small.
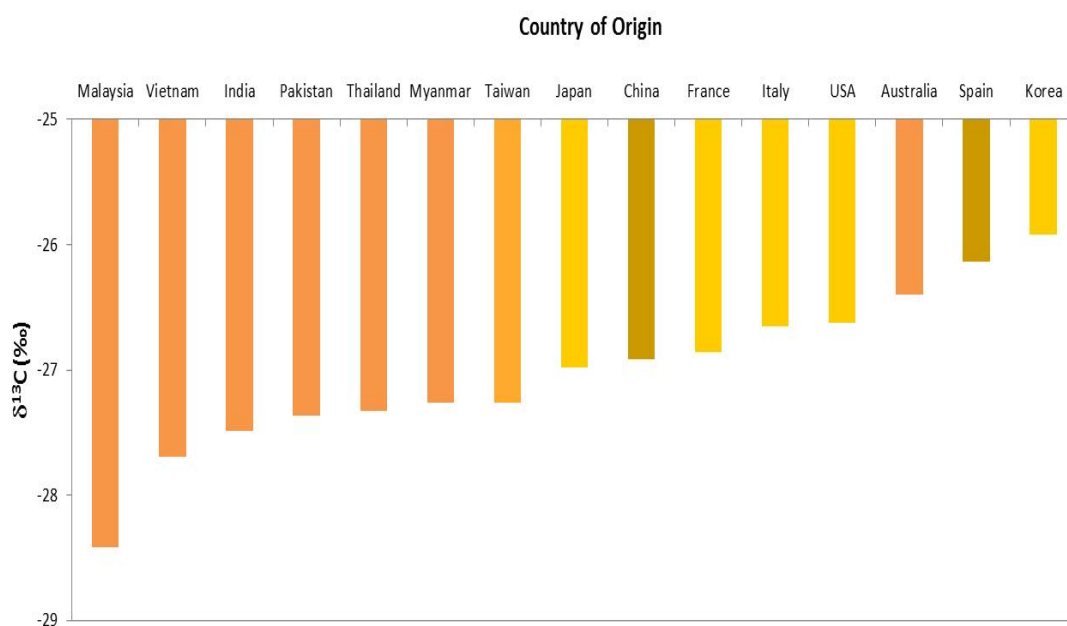
Figure 2.5 Mean δ^{13}C value of the rice samples from each country.

Table 2.5 Mean δ^{13}C value in rice and the mean annual temperature of the rice cultivation regions for the respective countries.

Country of Origin	Mean δ^{13}C of Rice (‰)	Mean Annual Temperature of Rice Cultivation Regions (°C) (FAO, 2002)
Malaysia	-28.42	26.5
Vietnam	-27.69	24.6
India	-27.49	23.8
Pakistan	-27.37	22.8
Thailand	-27.33	27.1
Myanmar	-27.26	25.5
Taiwan	-27.26	20.6
Japan	-26.98	14.3
China	-26.92	12.6
France	-26.86	11.8
Italy	-26.65	12.7
USA	-26.62	16.0
Australia	-26.40	15.6
Spain	-26.14	16.8
Korea	-25.92	12.5

The annual temperatures for the rice cultivation regions (listed in Table 2.4) were obtained from the online climate estimator (FAO, 2002).

It should be noted that, due to the changes in the seasons, rice is not grown throughout the year in countries that experience harsh climate conditions. This is especially true for temperate and sub-polar countries. Thus, an approximate crop calendar for rice was collated to identify the three main stages of rice cultivation (Figure 2.6). In general, the countries represented in the study follow a similar calendar cycle for rice cultivation. An interesting observation is that some countries display a much longer rice cultivation cycle than others. This may imply that more than one rice cultivation cycle could be taking place and could result in some overlapping of the different stages. This situation is typically associated with countries that have a suitable climate for rice cultivation for most part of the year.
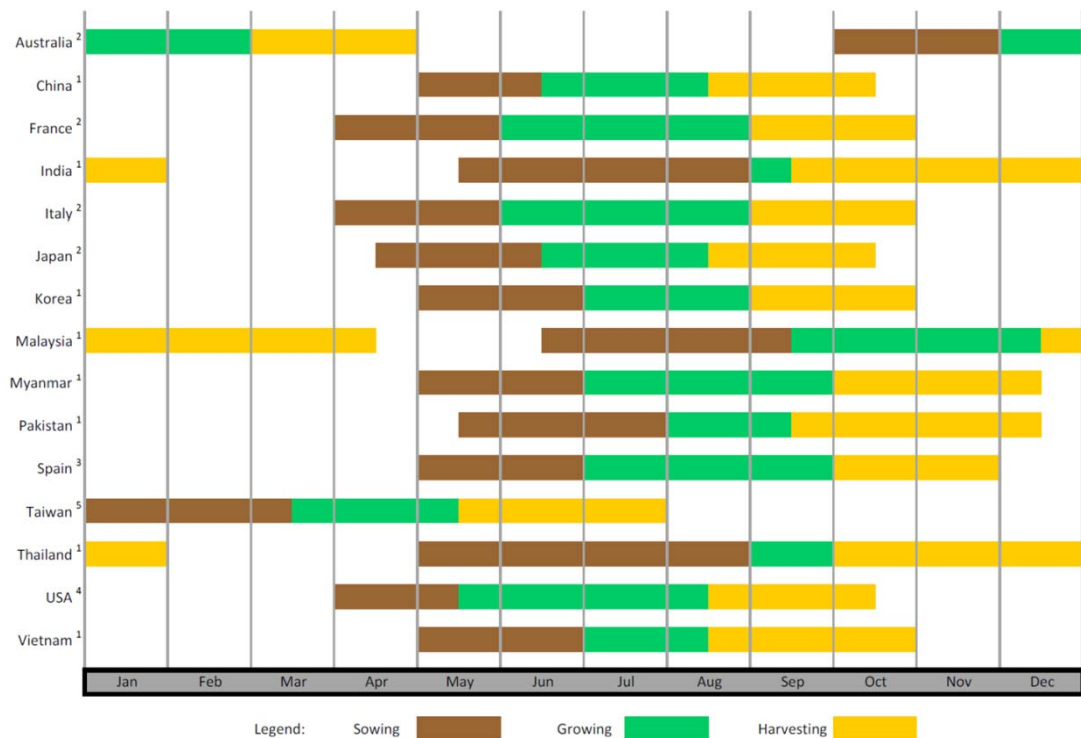
[1] Global Information and Early Warning System (GIEWS) on food and agriculture.
[2] FAO, 2004.
[3] USDA, 2010a.
[4] USDA, 2010b.
[5] IRRI, 1987.

Figure 2.6 Crop calendar for rice.

With the additional information on the rice crop calendar, a further analysis was carried out into the relationship between the mean $\delta^{13}C$ value of rice and the mean temperature of the rice cultivation regions of the respective countries during the rice growing months (Figure 2.7). The rice growing stage was selected for comparison because carbon fractionation processes associated with photosynthesis take place during this period, hence the rice grain will possess a carbon isotopic profile relating to the climatic conditions during its development. Two groups of countries were distinguished: group A comprising countries from temperate and/or subtropical regions and group B comprising countries from tropical or subtropical regions. It should be noted that countries like Australia and USA display both temperate and subtropical characteristics in various parts of the country, thus they are not as distinctly classified as the other countries in this study. Nonetheless, the ^{13}C depletion in rice once again appears to be associated with higher mean temperatures as experienced in the group B countries.
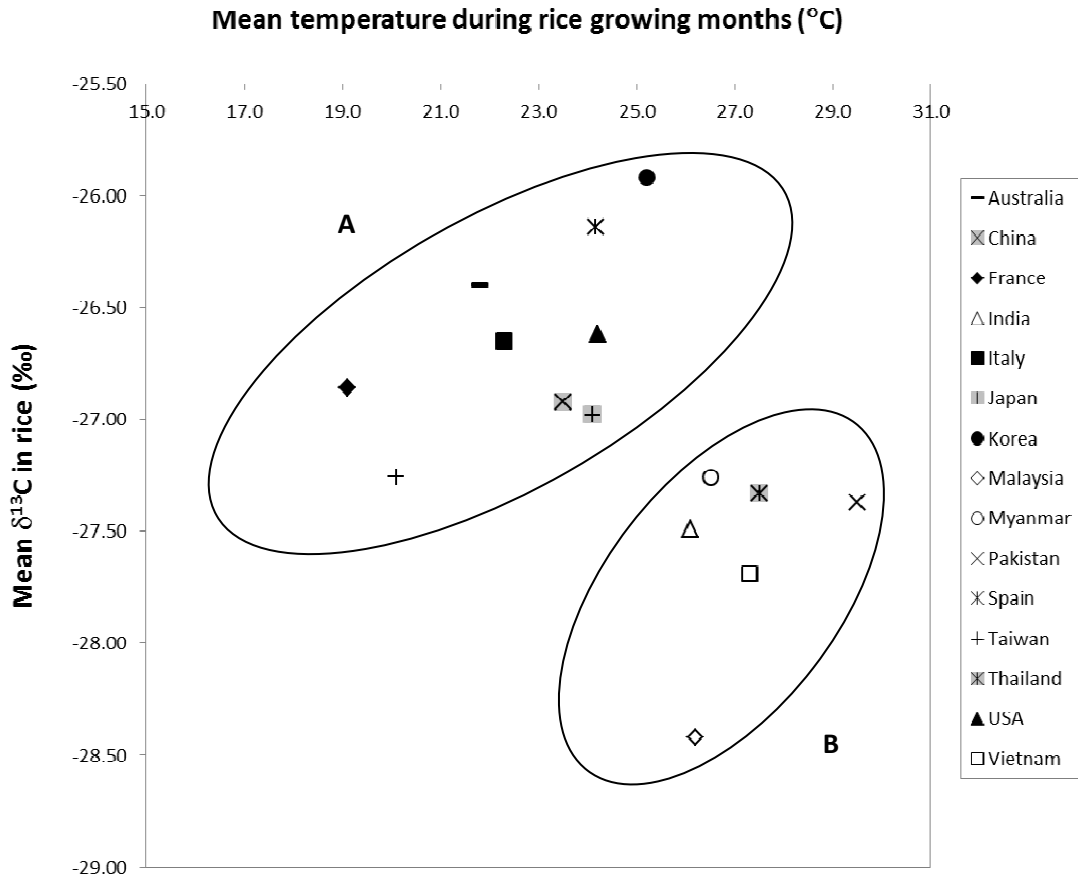
Figure 2.7 Comparison between the mean $\delta^{13}C$ value in rice and the mean temperature of the rice cultivation regions of the respective countries during the rice growing months. (A – temperate and/or subtropical countries and B – tropical and/or subtropical countries).

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 & Sharkey, 1982). The consequence would be decreased discrimination against ^{13}C. 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 during the rice growing months was performed to test for a possible

relationship (Figure 2.8). 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 (Australian Government Bureau of Meteorology, 2011). 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.

The general trend observed is that the greater the water vapour pressure, the more negative the $\delta^{13}C$ value of the rice samples (Figure 2.8). 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 behaviour 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 trend.
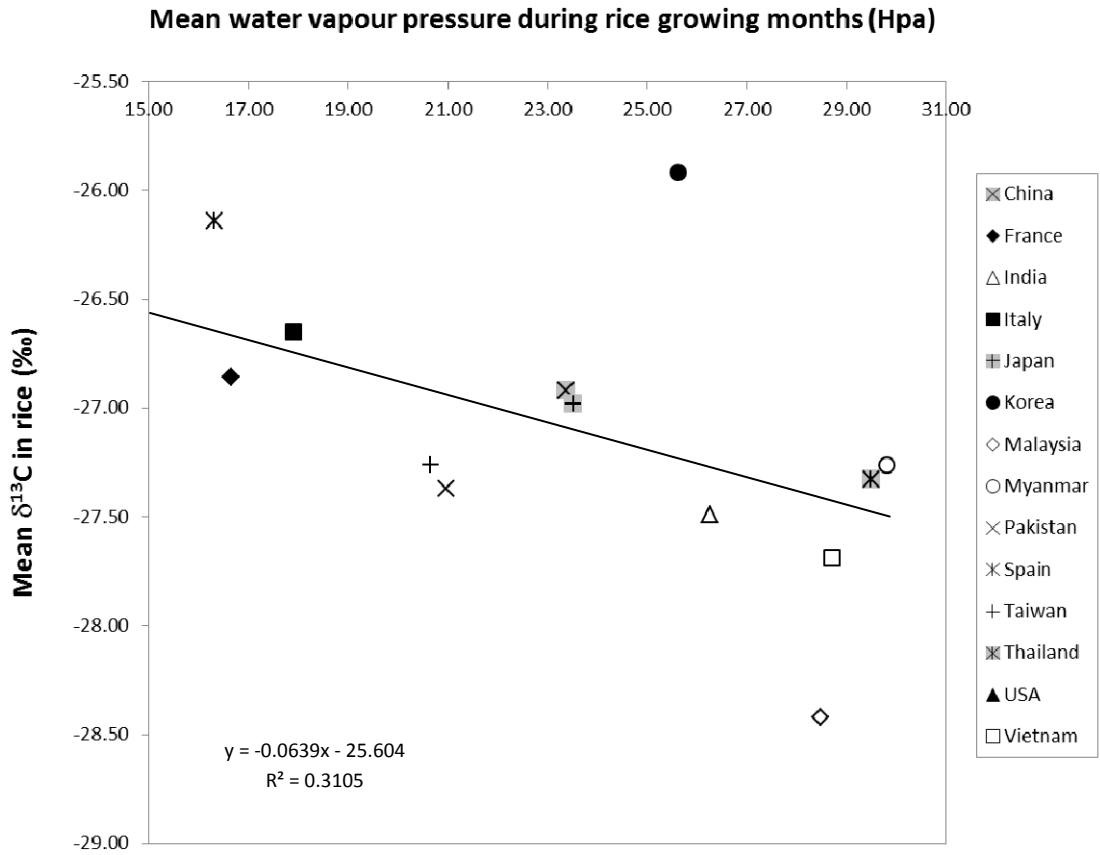
Figure 2.8 Comparison between the mean $\delta^{13}C$ value in rice and the mean water vapour pressure of the rice cultivation regions of the respective countries during the rice growing months. The water vapour pressures for the rice cultivation regions (listed in Table 2.4) were obtained from the online climate estimator (FAO, 2002).

2.2 Stable isotopes of nitrogen ($^{15}N/^{14}N$ ratio)

Nitrogen isotope ratio measurements of the 214 rice samples were analysed in triplicate. The raw $\delta^{15}N$ values obtained from the instrument data were adjusted for linearity followed by drift correction before being compared (see sections 6.3.4.1 and 6.3.4.2).

2.2.1 Quality controls for nitrogen stable isotope ratio analysis

Quality controls for the $^{15}N/^{14}N$ analysis comprised two reference materials, wheat flour from the TRACE project and NIST 1568 (rice flour) measured in batches on fourteen different days. The between-batch precision for the $\delta^{15}N$ measurements of the wheat flour reference material (Table 2.6; Figure 2.9) shows standard deviations < 0.3‰ for all analysis batches, indicating a good consistency in the quality control results. Most of the $\delta^{15}N$ results for the wheat flour reference material fall within 2σ of the mean of means value (Figure 2.9).

Table 2.6 Compilation of the wheat flour quality control data from batches subjected to $\delta^{15}N$ analysis.

Date of Analysis	Average (‰)	Standard Deviation (‰)	n
10-Jan-11	2.28	0.10	6
12-Jan-11	2.16	0.07	9
13-Jan-11	2.15	0.08	9
17-Jan-11	2.27	0.11	9
18-Jan-11	2.14	0.11	9
19-Jan-11	2.13	0.07	9
20-Jan-11	2.08	0.14	9
31-Jan-11	1.96	0.09	5
15-Feb-11	2.34	0.21	9
16-Feb-11	2.15	0.18	9
17-Feb-11	2.17	0.21	9
22-Feb-11	2.42	0.18	9
01-Mar-11	2.58	0.05	9
02-Mar-11	2.32	0.15	6
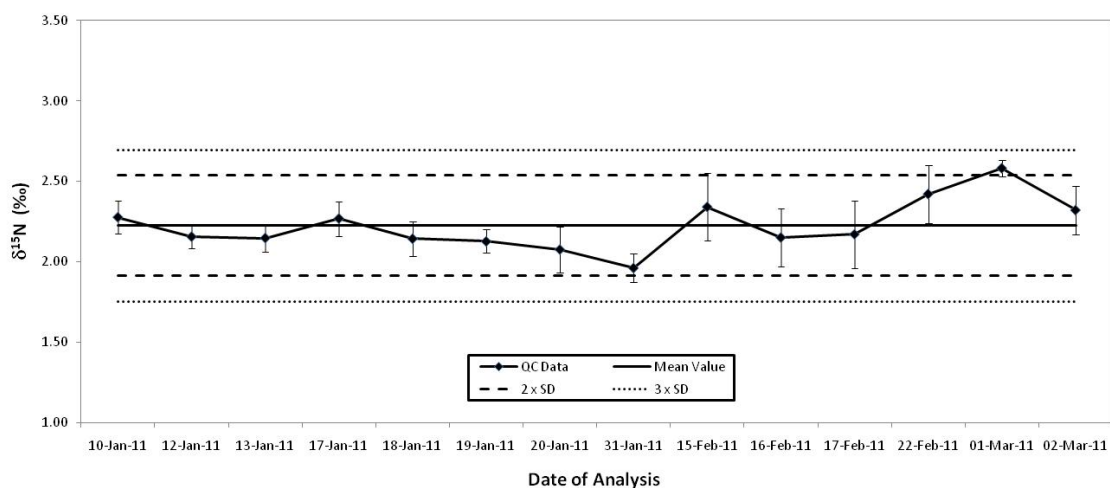
Figure 2.9 Mean $\delta^{15}N$ values for wheat flour across different batches (assigned value for wheat flour from inter-laboratory comparison was 2.31‰).

The NIST 1568 rice flour reference material, measured in batches on fourteen different days, gave standard deviations $\leq$ 0.3‰ (Table 2.7; Figure 2.10) indicating a good consistency in the quality control results. Most of the $\delta^{15}N$ results for the NIST 1568 rice flour reference material fall within 2σ of the mean of means.

Table 2.7 Compilation of the NIST 1568 rice flour quality control data from batches subjected to $\delta^{15}N$ analysis.

Date of Analysis	Average (‰)	Standard Deviation (‰)	n
10-Jan-11	6.49	0.08	6
12-Jan-11	6.27	0.08	9
13-Jan-11	6.28	0.12	9
17-Jan-11	6.26	0.19	9
18-Jan-11	6.15	0.11	9
19-Jan-11	6.07	0.14	9
20-Jan-11	6.22	0.14	9
31-Jan-11	6.07	0.14	6
15-Feb-11	6.41	0.24	9
16-Feb-11	5.99	0.06	9
17-Feb-11	6.32	0.30	9
22-Feb-11	6.69	0.14	9
01-Mar-11	6.79	0.10	9
02-Mar-11	6.66	0.13	6
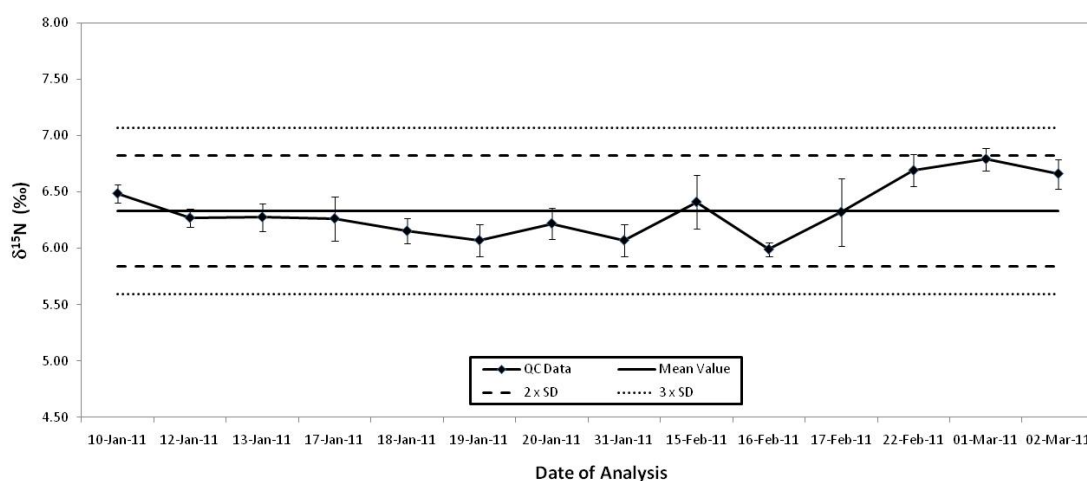
Figure 2.10 Mean $\delta^{15}N$ values for NIST 1568 rice flour across different batches.

The deviation between the triplicate measurements for each rice sample was set at < 0.4‰, which was slightly higher than that set for the carbon isotope ratio analysis. Since the nitrogen content is much lower than the carbon content in rice, the variations for $\delta^{15}N$ measurements were expected to be higher as explained by the Horwitz Curve

(Horwitz, 1982). Measurement was repeated when the set deviation limit was exceeded, which involved about 10% of the total samples.

2.2.2 Nitrogen isotope compositions

The nitrogen isotopic data obtained from the rice samples were expressed relative to air. The δ^{15}N variation of these rice samples ranged from 0.24‰ to 12.01‰ (Table 2.8). Using a boxplot (Figure 2.11) it was observed that the variation in the δ^{15}N values is larger than that of the δ^{13}C values in general, and one striking observation is that the rice samples from Spain had the highest δ^{15}N values.

Table 2.8 Mean, maximum and minimum δ^{15}N values of rice samples for each country represented in the study.

Country of Origin	Mean δ^{15}N Value (‰)	Max δ^{15}N Value (‰)	Min δ^{15}N Value (‰)	No. of Samples
Australia	5.61	6.36	4.91	4
China	5.98	6.60	5.26	4
France	4.18	6.15	3.15	3
India	3.23	4.34	1.89	30
Italy	4.90	5.73	3.11	5
Japan	3.25	5.89	0.65	27
Korea	4.14	4.85	4.14	2
Malaysia	1.29	2.07	0.92	6
Myanmar	1.84	1.91	1.78	2
Pakistan	2.83	3.69	0.98	18
Spain	8.76	12.01	6.55	3
Taiwan	3.56	5.99	2.12	10
Thailand	2.81	6.00	0.50	64
USA	2.61	6.89	0.76	20
Vietnam	1.80	5.74	0.24	16

Key: – Median   ☐ 25%-75%   I Min-Max
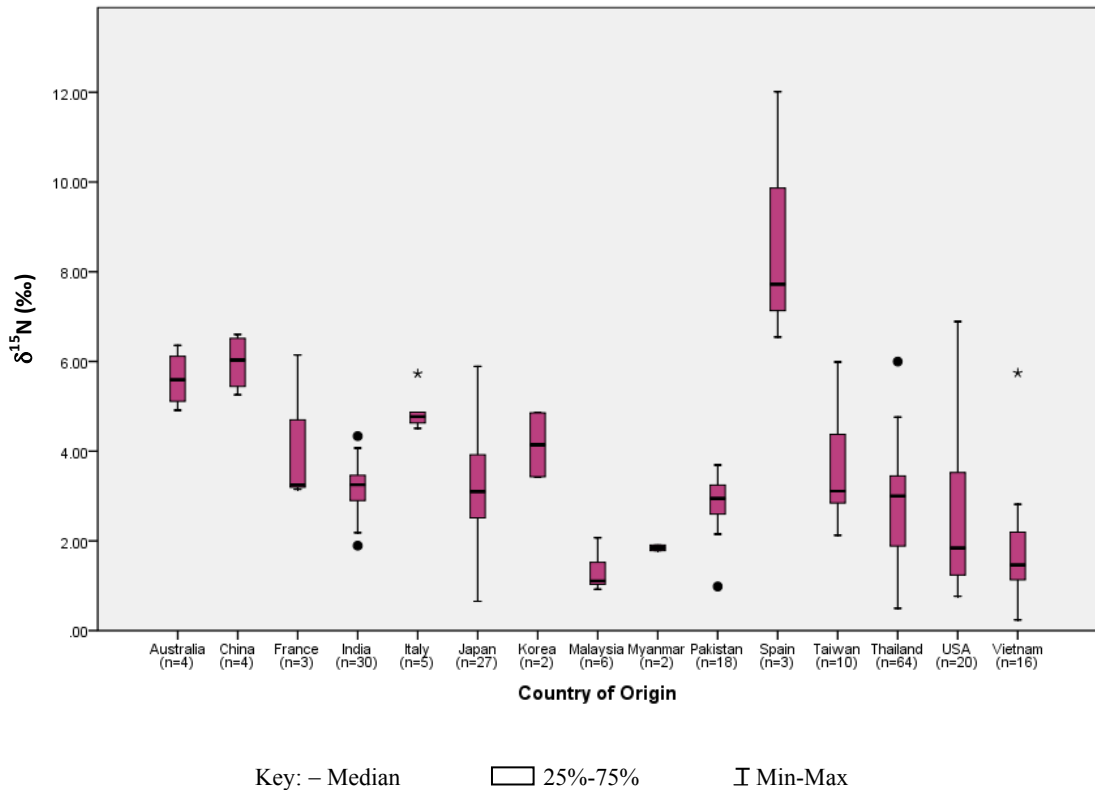
Figure 2.11 Box and whisker plot showing $\delta^{15}N$ variations for all rice types according to country of origin.

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 volatilization, 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 general 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 δ^{15}N values lower than the mean whereas those from Australia and China typically had δ^{15}N values higher than the mean (refer to Figure 2.11) 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 δ^{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 δ^{15}N value of the soil increases, which is consistent with the higher δ^{15}N values found in the Australian rice samples in this study.

Organic farming practice follows recommendations including very strict limits on the use of chemical synthetic pesticides and synthetic fertilisers (Directorate General for Agriculture and Rural Development of the European Commission). Bateman and Kelly (2007) compared the nitrogen isotope signatures of synthetic fertilisers and fertilisers that may be permitted in organic agriculture systems. Their findings were consistent with other published literature (Kendall, 1998) where synthetic nitrogen fertilisers are typically more depleted in ^{15}N compared to the fertilisers used in organic systems. Kendall (1998) reported that the organic fertilisers including green manures, composts and liquid/solid animal waste, typically have higher δ^{15}N values and a much wider range of compositions (2-30‰) than synthetic fertilisers, thus reflecting their

more diverse origins. Comparison of the $\delta^{15}N$ values of organic and conventional rice samples in this study (Figure 2.12) shows that a strong differentiation could not be observed, though the organic rice generally has a higher median $\delta^{15}N$ value than the conventional rice. Moreover, some conventional rice samples possess higher $\delta^{15}N$ values than the organic rice in this study, possibly suggesting that agricultural practices that mimic organic farming may have been used in the production of these samples. An increase in the sample size for organic rice may be able to provide a more indicative insight into its $\delta^{15}N$ distribution.

Key: – Median   ☐ 25%-75%   ⊥ Min-Max
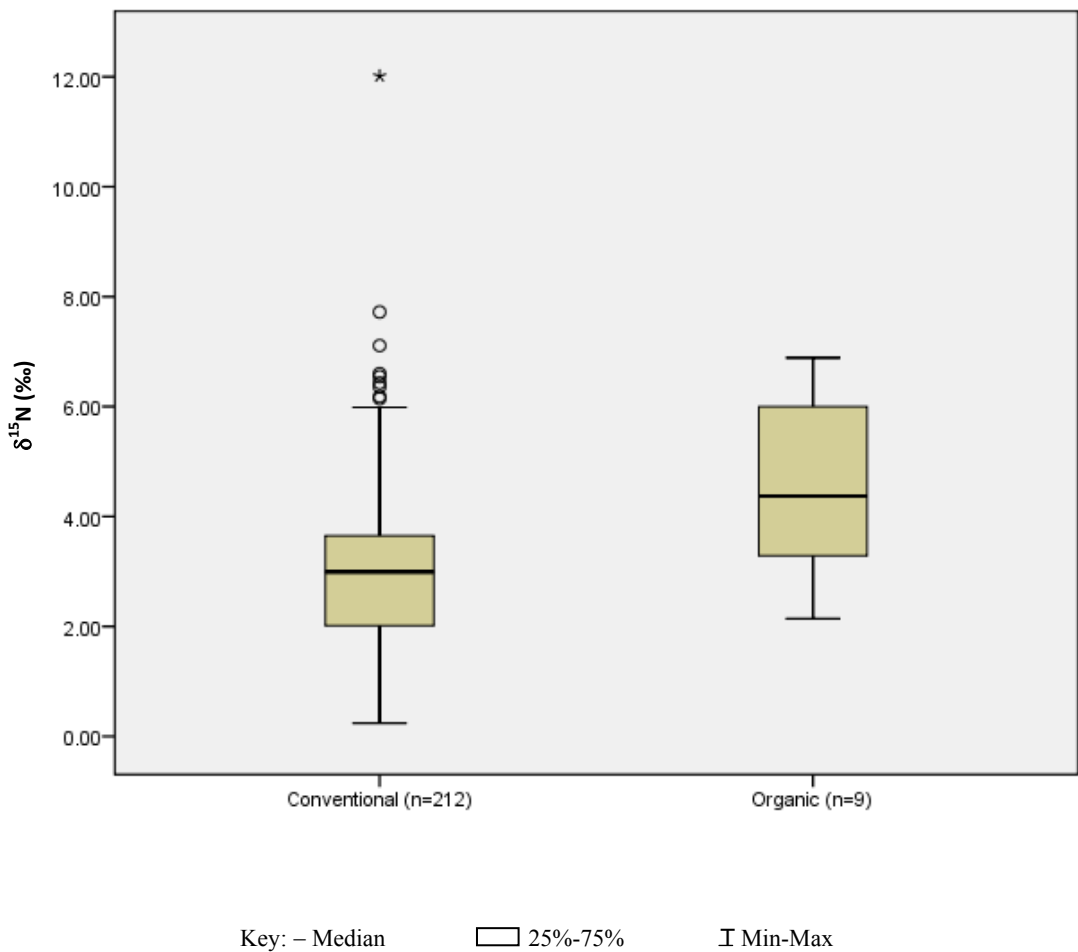
Figure 2.12 Comparison between the conventional and organic rice samples analysed for $\delta^{15}N$.

2.3 Stable isotopes of oxygen ($^{18}O/^{16}O$)

Oxygen isotope ratio measurements of the 214 rice samples were analysed in triplicate. The raw $\delta^{18}O$ values obtained from the instrument data were first corrected for drift followed by linearity before being compared (see sections 6.3.4.1 and 6.3.4.2).

2.3.1 Quality controls for oxygen stable isotope ratio analysis

Quality controls for the $^{18}O/^{16}O$ analysis comprised two reference materials, IAEA-CH-6 (sucrose) and NIST 1568 (rice flour) measured in batches on twelve different days. The between-batch precision for the $\delta^{18}O$ measurements of the IAEA-CH-6 reference material (Table 2.9; Figure 2.13) shows standard deviations < 0.8‰ for all analysis batches, indicating a fair consistency in the quality control results. Most of the $\delta^{18}O$ results for the IAEA-CH-6 reference material fall within 3σ of the mean of means value (figure 2.13).

Table 2.9 Compilation of the IAEA-CH-6 quality control data from batches subjected to $\delta^{18}O$ analysis.

Date of Analysis	Average (‰)	Standard Deviation (‰)	n
24-Jun-11	36.81	0.45	5
25-Jun-11	36.61	0.33	6
26-Jun-11	36.35	0.20	6
28-Jun-11	36.27	0.52	6
29-Jun-11	36.43	0.42	6
02-Jul-11	37.01	0.40	6
03-Jul-11	36.53	0.70	4
04-Jul-11	37.01	0.37	6
05-Jul-11	36.59	0.52	6
09-Jul-11	36.00	0.35	3
10-Jul-11	36.42	0.68	3
13-Jul-11	36.37	0.38	5
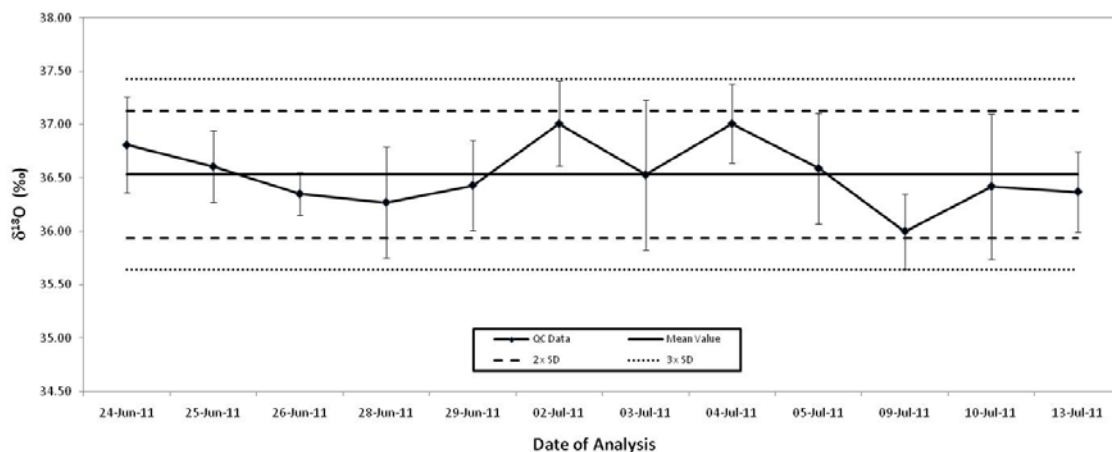
Figure 2.13 Mean $\delta^{18}O$ values for the IAEA-CH-6 across different batches (adopted value for IAEA-CH-6 from Kornexl *et al*. (1999) was 36.4‰).

The NIST 1568 rice flour reference material, measured in batches on twelve different days, gave standard deviations ≤ 0.9‰ (Table 2.10; Figure 2.14), indicating a fair consistency in the quality control results. Most of the $\delta^{18}O$ results for the NIST 1568 rice flour reference material fall within 3σ of the mean of means value.

Table 2.10 Compilation of the NIST 1568 rice flour quality control data from batches subjected to $\delta^{18}O$ analysis.

Date of Analysis	Average (‰)	Standard Deviation (‰)	n
24-Jun-11	21.69	0.41	5
25-Jun-11	22.67	0.57	6
26-Jun-11	22.07	0.88	5
28-Jun-11	22.15	0.34	5
29-Jun-11	22.59	0.18	3
02-Jul-11	22.30	0.86	6
03-Jul-11	23.06	0.39	5
04-Jul-11	21.96	0.87	6
05-Jul-11	21.19	0.76	6
09-Jul-11	21.90	0.26	6
10-Jul-11	21.39	0.88	6
13-Jul-11	20.80	0.60	3
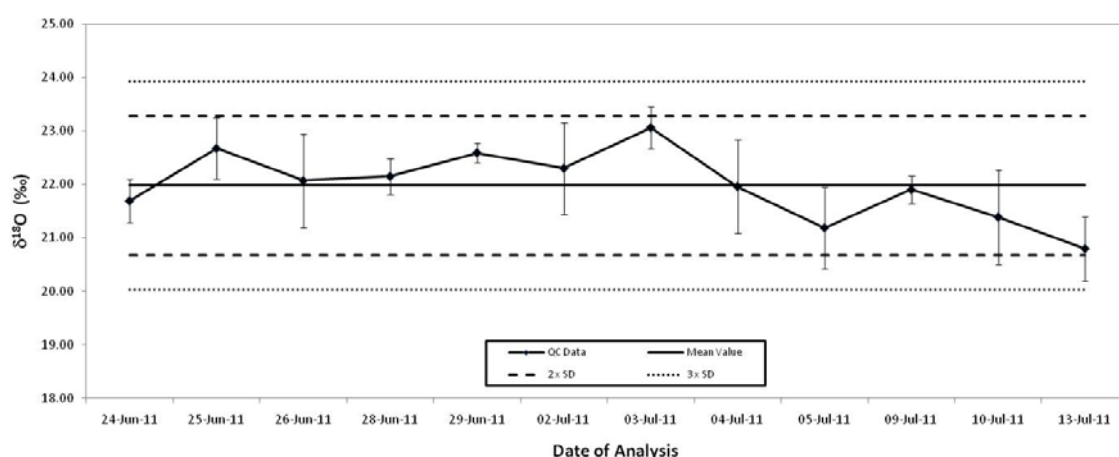
Figure 2.14 Mean $\delta^{18}O$ values for NIST 1568 rice flour across different batches.

2.3.2 Oxygen isotope compositions

The oxygen isotopic data obtained from all the rice samples in the study were expressed relative to the international SMOW reference. The $\delta^{18}O$ variation of the rice samples ranged from 15.17‰ to 29.79‰ (Table 2.11). In general, there was a significant variation in the $\delta^{18}O$ values found in the rice samples. With reference to Figure 2.15, 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).

Table 2.11 Mean, maximum and minimum $\delta^{18}O$ values of rice samples for each country represented in the study.

Country of Origin	Mean $\delta^{18}O$ Value (‰)	Max $\delta^{18}O$ Value (‰)	Min $\delta^{18}O$ Value (‰)	No. of Samples
Australia	26.70	28.33	24.85	4
China	17.36	20.16	15.74	4
France	19.84	21.91	18.44	3
India	21.72	29.79	18.62	30
Italy	20.86	22.13	19.88	5
Japan	19.58	21.88	16.99	27
Korea	21.27	21.78	20.76	2
Malaysia	19.89	21.36	16.52	6
Myanmar	19.07	20.53	17.61	2
Pakistan	22.32	26.29	18.01	18
Spain	21.90	22.41	21.43	3
Taiwan	20.66	22.31	19.04	10
Thailand	20.23	23.48	15.17	64
USA	21.90	25.84	18.21	20
Vietnam	19.35	22.43	15.47	16

Key: – Median   ☐ 25%-75%   Ⅰ Min-Max
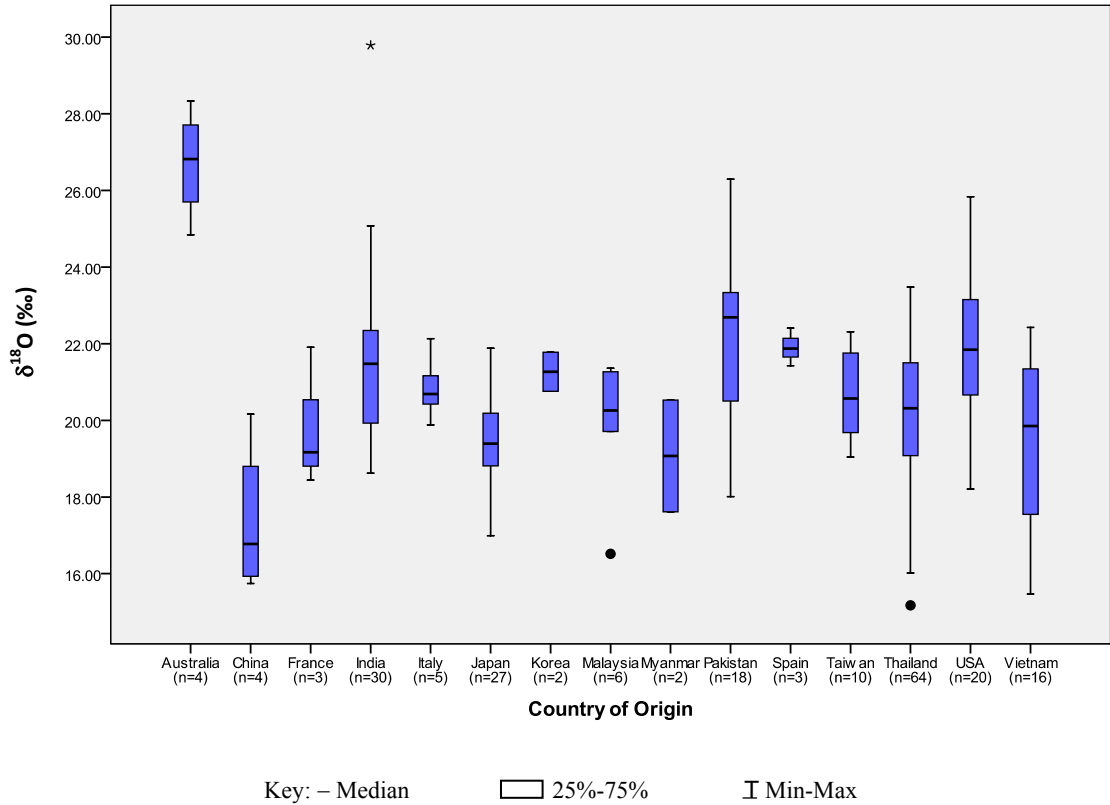
Figure 2.15 Box and whisker plot showing $\delta^{18}O$ variations for all rice types according to country of origin.

Water is not isotopically fractionated when taken up by plants (Dawson & Ehleringer, 1993). Thus, the water in plant tissues typically possesses a very 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 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 2.16). It is possible that a better linear correlation may have been achieved if the specific rice cultivation location and growing conditions were available.
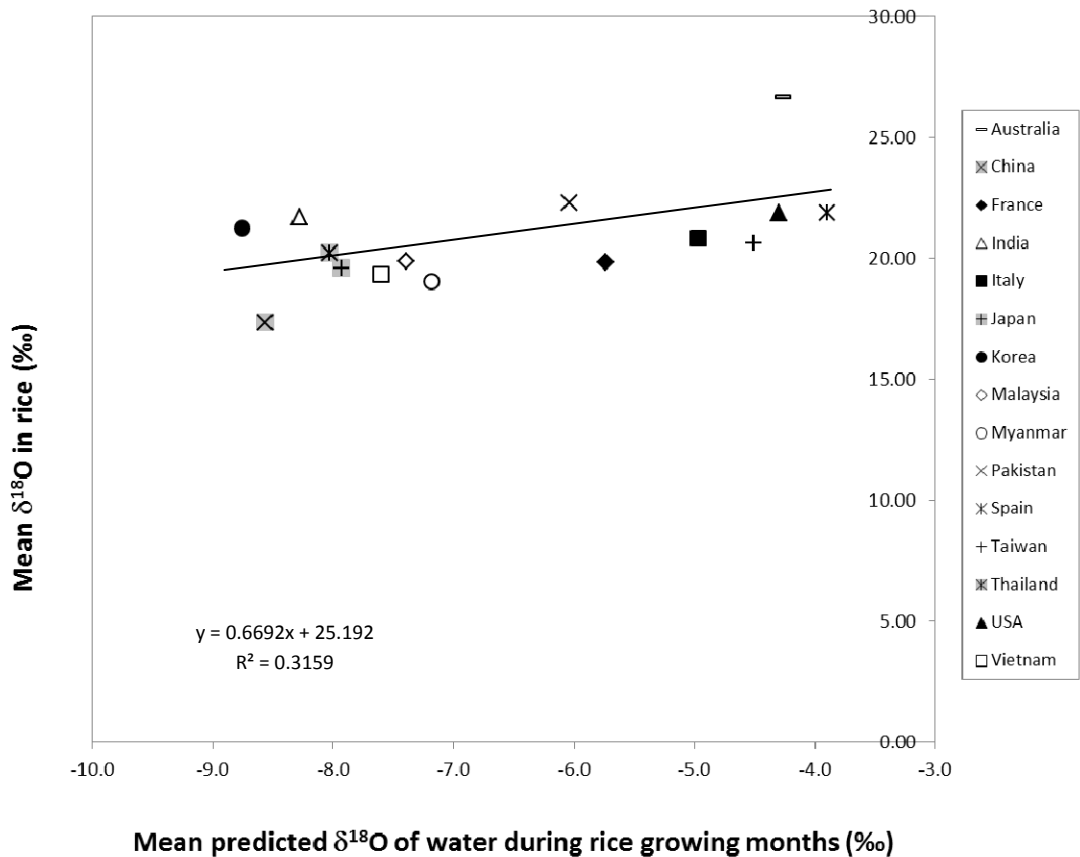
Figure 2.16 Comparison of the mean $\delta^{18}O$ value in rice and the mean predicted $\delta^{18}O$ value of precipitation at the rice cultivation regions of the respective countries during the rice growing months.

Since the water source for plant growth generally originates from meteoric water or snowmelt water (Korenaga *et al*., 2010), one of the main sources of isotopic variation among the rice samples analysed is related to the disparity in the amount of precipitation inputs received at the rice cultivation area. However, it should be mentioned that the main control of isotopic composition of oxygen (and hydrogen) in precipitation is temperature, which can affect evaporation from the soil surface and

evaporation from the leaf surface during transpiration, and in turn results in the isotopic variation in the plant source water (Marshall *et al*., 2007). The relationship between the mean $\delta^{18}O$ values in rice and the mean precipitation levels of the rice cultivation regions of the respective countries during the rice growing months shows an overall negative correlation (Figure 2.17). With clouds and precipitation forming under equilibrium conditions, the heavier ^{18}O, which has a greater preference to accumulate in the liquid phase, is enriched in the initial precipitation. As a result, rain clouds gradually become isotopically lighter over time (Marshall *et al*., 2007). In other words, the observation of ^{18}O depletion in the rainwater is associated to the precipitations from these isotopically lighter rain clouds over time. A possible explanation for the anomalies in the $\delta^{18}O$ values of rice with changing precipitation level, most pronounced for Australia and China, is that the mean precipitation level used for the comparison does not accurately represent the actual precipitation level as the exact rice cultivation location is unknown.
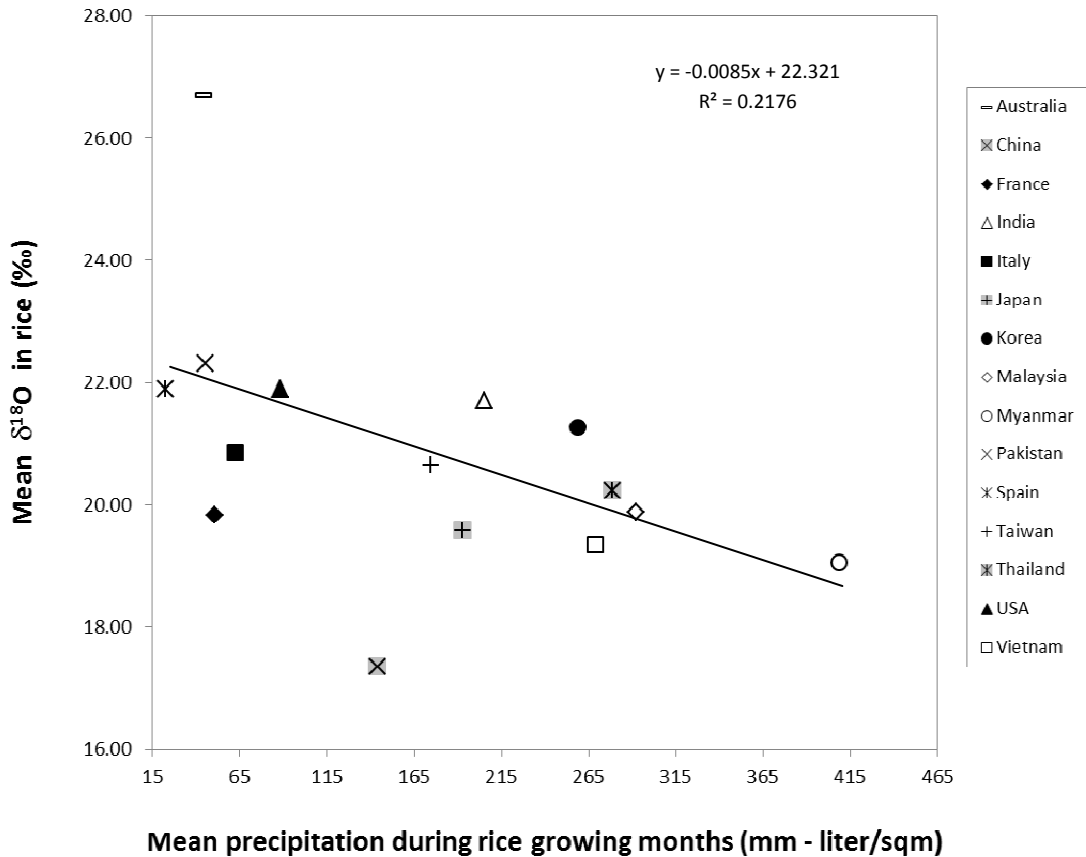
Figure 2.17 Comparison of the mean δ^{18}O value in rice and the mean precipitation of the rice cultivation regions of the respective countries during the rice growing months. The precipitation data for the rice cultivation regions (listed in Table 2.4) were obtained from the online climate estimator (FAO, 2002).

Several interesting points were made by Korenaga *et al.* (2010) on the δ^{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 δ^{18}O value than the usual precipitate. Therefore, the δ^{18}O value in rice is a reflection of the regional irrigation water used for cultivation. The same trend in δ^{18}O was found in this study where

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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 exerts 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 (listed in Table 2.4) 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 Figure 2.18 shows that 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. Notably, Nakashita *et al*. (2006) reported a similar finding where the amount of $\delta^{18}O$ in plants in the northern Japanese Alps region had a tendency to increase with increasing altitude.
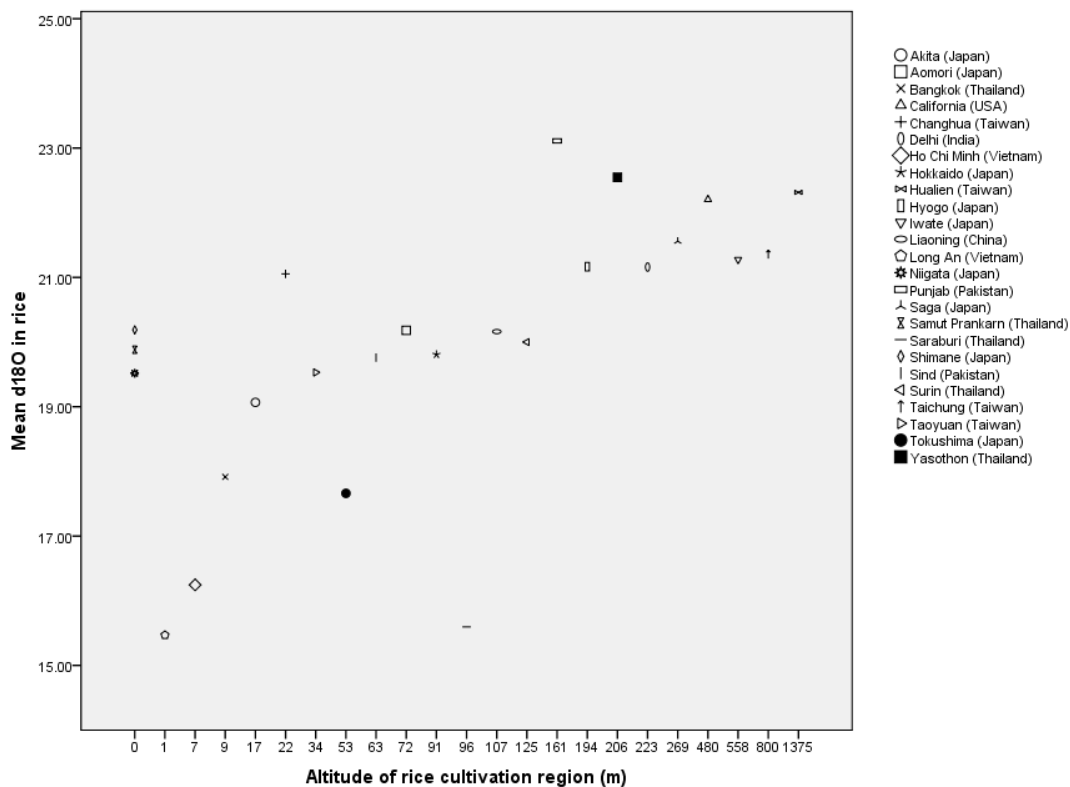
Figure 2.18 Comparison of the mean $\delta^{18}O$ value in rice and the altitude of the rice cultivation regions of the respective countries.

2.4 Stable isotopes of hydrogen (^{2}H/^{1}H ratio)

Hydrogen isotope ratio measurements of the 214 rice samples were analysed in triplicate. The raw δ^2H values obtained from the instrument were first corrected for H_3^+ followed by drift correction before being compared (see section 6.3.4.1).

2.4.1 Quality controls for hydrogen stable isotope ratio analysis

Quality controls for the ^{2}H/^{1}H analysis comprised two reference materials, rice from the FIT-PTS (Food analysis using Isotopic Techniques – Proficiency Testing Scheme) and NIST 1568 (rice flour) measured in batches on four different days. The between-batch precision for the δ^2H measurements of the FIT-PTS rice reference

material (Table 2.12; Figure 2.19) shows standard deviations $\leq$ 3‰ for all analysis batches, indicating an acceptable consistency in the quality control results. Most of the δ^2H results for the FIT-PTS rice reference material fall within 2σ of the mean of means value (Figure 2.19).

Table 2.12 Compilation of the FIT-PTS rice reference material quality control data from batches subjected to δ^2H analysis.

Date of Analysis	Average (‰)	Standard Deviation (‰)	n
30-Aug-11	-38.47	2.28	6
07-Sep-11	-42.67	0.89	6
08-Sep-11	-43.74	3.08	4
09-Sep-11	-40.89	2.61	9
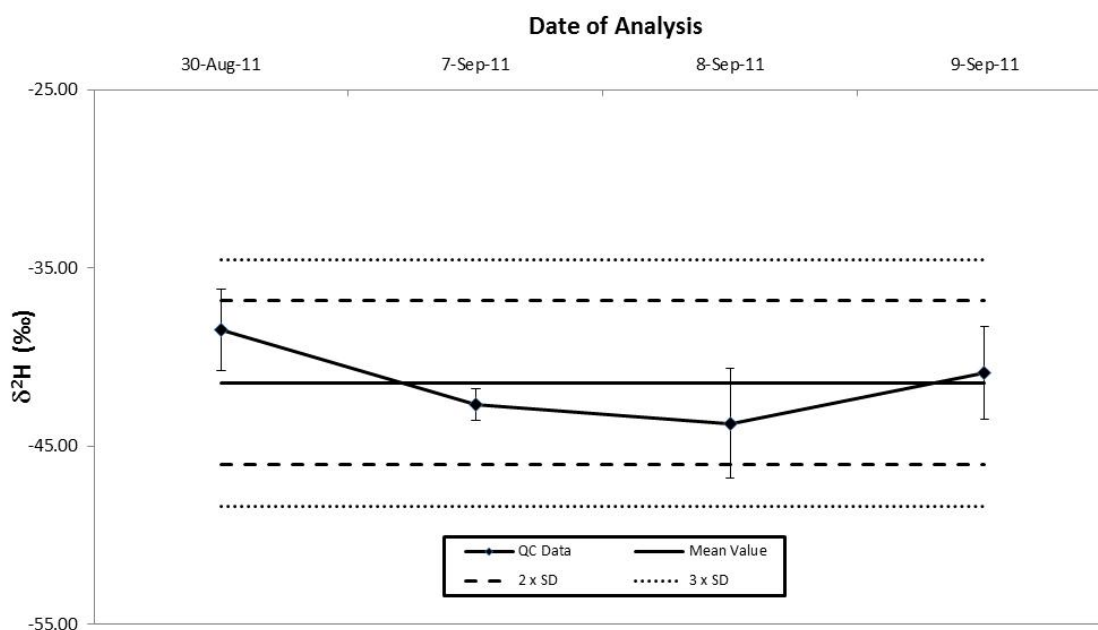
Figure 2.19 Mean δ^2H values for FIT-PTS rice reference material across different batches. (Assigned value for FIT-PTS rice reference material was -42.07‰.)

The NIST 1568 rice flour reference material, measured in batches on four different days, gave standard deviations $\leq$ 3‰ (Table 2.13; Figure 2.20), indicating an

acceptable consistency in the quality control results. Most of the δ^2H results for the

NIST 1568 rice flour reference material fall within 2σ of the mean of means value.

Table 2.13 Compilation of the NIST 1568 rice flour quality control data from batches subjected to δ^2H analysis.

Date of Analysis	Average (‰)	Standard Deviation (‰)	n
30-Aug-11	-41.22	2.75	11
07-Sep-11	-46.28	1.73	12
08-Sep-11	-45.52	3.04	12
09-Sep-11	-43.85	2.33	15
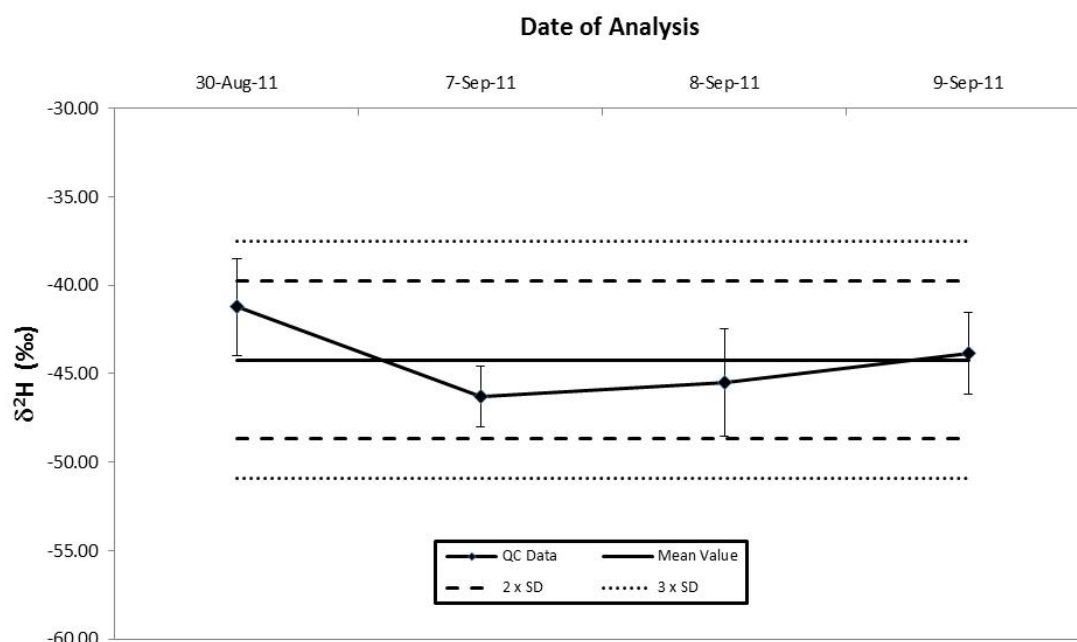
Figure 2.20 Mean δ^2H values for NIST 1568 rice flour across different batches.

2.4.2 Hydrogen isotope compositions

The hydrogen isotopic data obtained from all the rice samples in the study were expressed relative to the international SMOW reference. The δ^2H variation of these rice samples ranged from -70.95‰ to -26.81‰, as shown in Table 2.14. In general, there

was a noticeable variation in the δ^2H values among the rice samples and this variation is similar to the δ^{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 δ^{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.

Table 2.14 Mean, maximum and minimum δ^2H values of rice samples for each country represented in the study.

Country of Origin	Mean δ^2H Value (‰)	Max δ^2H Value (‰)	Min δ^2H Value (‰)	No. of Samples
Australia	-33.75	-28.24	-42.04	4
China	-60.92	-47.76	-70.95	4
France	-48.75	-44.02	-54.51	3
India	-46.94	-26.81	-58.56	30
Italy	-57.42	-47.32	-62.73	5
Japan	-59.89	-32.82	-68.71	27
Korea	-54.16	-51.82	-56.51	2
Malaysia	-53.08	-47.20	-59.62	6
Myanmar	-44.71	-43.11	-46.31	2
Pakistan	-46.98	-40.05	-65.72	18
Spain	-43.62	-38.59	-49.36	3
Taiwan	-44.33	-37.52	-52.55	10
Thailand	-52.25	-32.82	-62.68	64
USA	-53.03	-46.47	-62.19	20
Vietnam	-50.80	-46.24	-62.74	16
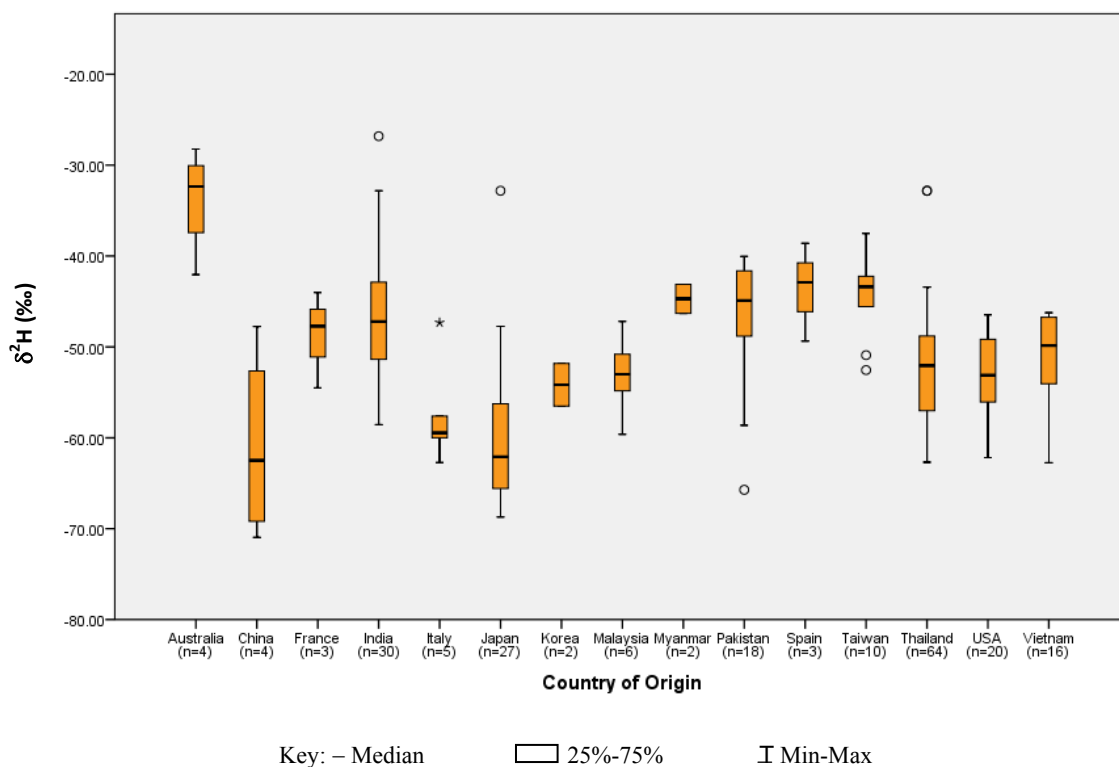
Figure 2.21 Box and whisker plot showing δ^2H variations for all rice types according to country of origin.

In fact, the relationship between the hydrogen and oxygen isotopic ratios in precipitation has long been established by the global meteoric water line (MWL) where $\delta^2H = 8\ \delta^{18}O + 10$ (Craig, 1961b). The specific slope of this linear correlation results because the condensation in rain clouds occurs under equilibrium conditions, and is simply the ratio of the equilibrium fractionation factors for the hydrogen and oxygen isotopes (Marshall *et al.*, 2007). With reference to Figures 2.22 and 2.23, linear relationships were obtained from the mean $\delta^{18}O$ and δ^2H in rice as well as the mean predicted $\delta^{18}O$ and δ^2H values of precipitation at the rice cultivation regions of the respective countries during the rice growing months. Both the linear correlations were found to be different from the equation established in the meteoric water line. However, it should be noted that the relationship shown in Figure 2.22 is not very robust. If it were not for the Australian rice samples (the sample size of which is very small), there

would not be much of a correlation at all. Water is transferred from soil through the plant xylem to the leaf without significant fractionation (Marshall *et al.*, 2007). However, water is lost from the leaf through stomata during the process of evapo-transpiration prior to assimilation during photosynthesis. The extent of evapo-transpiration and subsequent fractionation of the isotopologues of water is dependent on the morphology of the plant and local climatic conditions, such as relative humidity, wind speed, altitude and direct sunlight. Ultimately there is significant enrichment in the water prior to fixation and there are subsequent further fractionations associated with plant metabolism. Consequently, a direct correlation with the meteoric water line is not expected. Furthermore, this deviation is possible if the precipitation occurs under relatively dry air and evaporates as it falls, therefore the kinetic fractionation affects the respective evaporation rates of DHO and $H_2^{18}O$ leading to a 'deuterium excess' and deviation from the MWL (Marshall *et al.*, 2007). Nevertheless, the data does show some correlation and the inclusion of the δ^2H values into the rice dataset for the CDA procedure did not contribute to any significant effect on the output results, showing that the hydrogen isotope information does not offer any additional discrimination to that obtained with oxygen isotope data, and was thus excluded from the geographical origin discriminant model for rice.
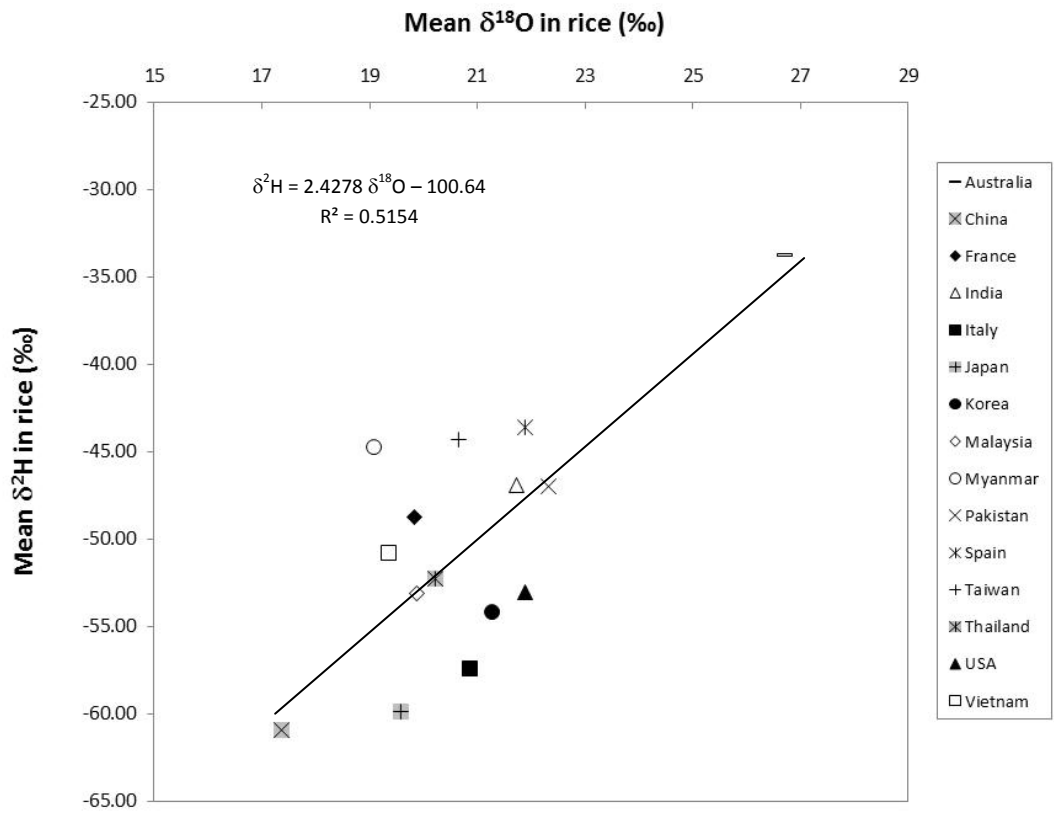
Figure 2.22 Mean values of $\delta^{18}O$ and δ^2H in rice by country of origin.
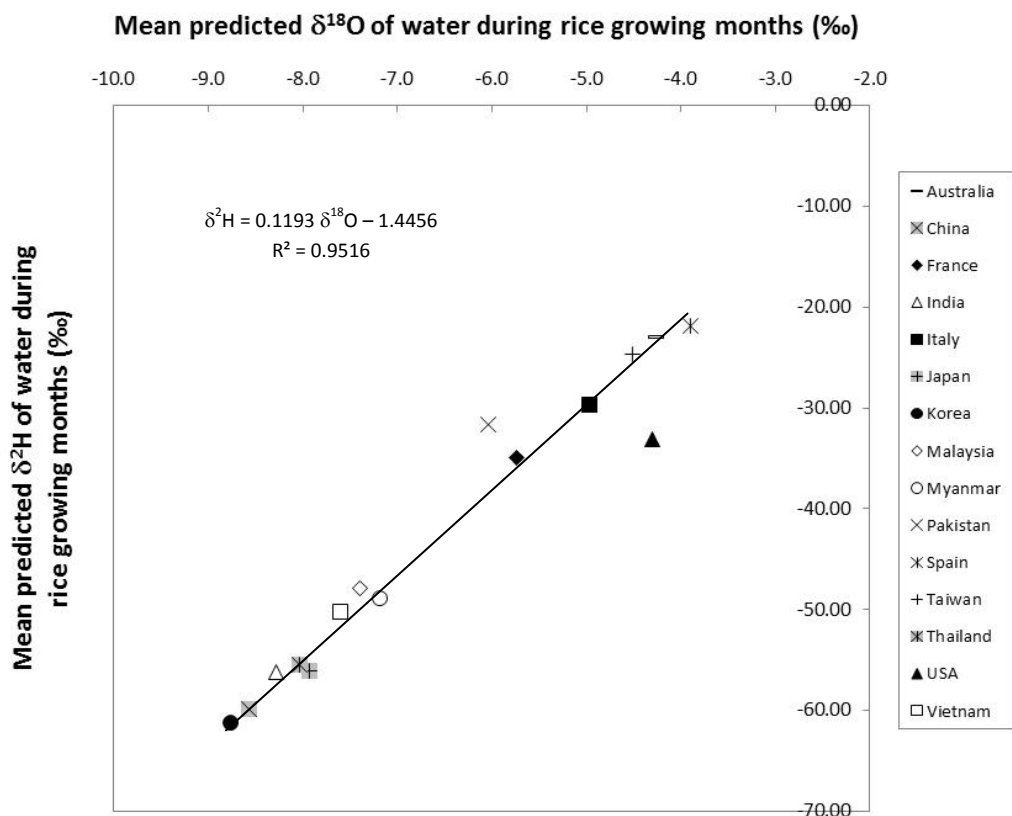
Figure 2.23 Mean predicted $\delta^{18}O$ and δ^2H values of precipitation at the rice cultivation regions of the respective countries of origin during the rice growing months.

2.5 Conclusions

The analysis of the stable isotope ratios in rice samples from a wide range of geographic regions has provided useful insights into the distribution of the isotopic ratios in rice samples from various countries. The results obtained in this study have demonstrated that the variations in carbon stable isotope ratio in rice may be attributed to the environmental conditions experienced by the rice plant. Changes in temperature, level of irradiance, and water vapour pressure have influences on the fractionation processes in plants which in turn can alter the carbon stable isotope ratio. As for the nitrogen stable isotope ratio in rice, agricultural practices such as the choice of fertilisers may affect the nitrogen isotopic ratio in the plant. Whereas for the oxygen and hydrogen

stable isotope ratios, the source water for plant growth and the precipitation levels at the rice growing regions during the rice growing months may contribute to the variations in the isotopic ratios for oxygen and hydrogen in the rice samples. In spite of the actual rice cultivation conditions and exact rice farm locations not being known, interesting relationships with the estimated climate data, geographical information and predicted isotopic ratios are indicated. Clearer relationships and greater data reliability could be achieved if the precise rice cultivation condition and location were available. Nonetheless, the stable isotope ratio analysis alone may not be sufficient for a comprehensive geographical origin distinction for rice as the individual stable isotope ratio studies for carbon, nitrogen, oxygen and hydrogen only suggested the differentiation of rice for very limited countries like Malaysia, Spain and Australia. Furthermore, these differentiations based on single stable isotope ratio analysis may not be conclusive. Other studies have demonstrated that the inclusion of elemental composition in rice grains is capable of providing good geographical discrimination of rice (Kelly *et al.*, 2002; Gonzálvez *et al.*, 2011).

Chapter 3 – Multi-element distribution of rice samples from ICP-MS analysis

3.1 Multi-element levels in rice

The rice samples (214) were subjected to microwave-assisted acid digestion prior to analysis by ICP-MS for simultaneous quantitative determination of 66 elements. About 25% of the rice samples were randomly selected for duplicate analysis as part of the quality control. 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 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).

3.1.1 Quality controls for multi-element analysis

The quality controls used in the trace element analysis consisted of three reference materials: NIST 1547 (peach leaves), NIST 1568a (rice flour) and ZC 73013 (spinach), and a blank spike for the 66 elements of interest. All the sample results were corrected for the blank spike recovery prior to comparison. The octopole reaction system (ORS) of the ICP-MS was operated in non-pressurised standard mode (Std) or was pressurised with either He or H_2 to reduce the effect of polyatomic interfering ions. The final limit of detection (LOD) used for each element was established by using the

median method LOD across all the analysis batches measured on seven different days. The method LOD for each element was calculated as three times the standard deviation of four replicate blank determinations divided by the weight of the sample used for the acid digestion in every analysis batch. A summary of the element analysis parameters, LOD and quality control results is provided in Appendix I.

3.1.2 Statistical analysis by canonical discriminant analysis (CDA)

The interrogation of the stable isotope ratios and multi-element data in rice by canonical discriminant analysis (CDA) was performed by a 'stepwise' analysis to select the most useful discriminating variables. As described by Klecka (1980), a forward stepwise procedure begins by selecting the individual variable which provides the largest univariate discrimination. The procedure then pairs this first variable with each of the remaining variables, one at a time, to find the combination which generates the greatest discrimination. The variable which contributed to the best pair is thus selected. The procedure is repeated to combine the first two variables with each of the remaining variables to form triplets. The best triplet determines the third variable to be selected. In summary, the procedure continues until all possible variables have been selected. The selected variables are then computed into different independent discriminant functions. The maximum number of functions that can be derived is equal to the number of groups minus one or the number of discriminating variables, whichever is smaller. Subsequently, the use of Wilks's lambda is employed as a measure of discrimination for the selection criterion by taking into consideration both the differences between groups and the cohesiveness or homogeneity within groups. Values of lambda which are close to 0 denote great discriminatory power but become progressively less discriminatory as

the lambda value increases to the maximum value of 1. No group differences are observed when lambda equals to 1.

Lastly, a 'leave-one-out' cross-validation discriminant analysis was performed to test the robustness of the model. In this approach, a known sample is removed from the dataset and classified using the model constructed using the remaining samples. This procedure was repeated for each sample in turn and evaluated for its success by comparison with the known origin (Heaton *et al.*, 2008).

3.1.3 Discussion on the multi-elemental composition in rice

The trace element composition in rice is primarily influenced by the water and soil composition in which it grows, as well as the composition of any fertiliser used. Thus, the element profile of the rice reflects the chemical composition of the soil which in turn is dependent on the underlying rock types from which the soil was formed. The innate differences in the rock types at the Earth's surface cause large variations in the trace element signatures of the soils. Despite the effects of erosion altering the relationship between the soil composition and the underlying lithology, unique elemental signatures are reflected in localised regions and this variation is translated into plant tissues and may be significant enough to result in a pronounced geographical distinction (Branch *et al.*, 2003).

In view of the large number of elements quantified for the rice samples in this study, the CDA procedure was applied to determine if a geographical differentiation could be achieved based solely on the elemental data. Two multivariate models were generated using a detailed classification by country and a broad classification by continent or group of countries. In the first model, the samples were grouped into their respective countries of origin (Figure 3.1). The results of the CDA are represented by

the group centroids of the respective countries of origin. A total of 65.0% of the original grouped cases correctly classified and 57.5% of the cross-validated grouped cases correctly classified (Figure 3.1). The 11 elements selected by the software for this multivariate discrimination were lithium (Li), sodium (Na), manganese (Mn), iron (Fe), cobalt (Co), copper (Cu), zinc (Zn), arsenic (As), molybdenum (Mo), cadmium (Cd) and bismuth (Bi). Function 1 (48.8% of variance) which provided the main separation between the sample groups was primarily associated with Fe, Cu, Zn, As and Mo while Function 2 (22.7% of variance) was mainly associated with Mn, Fe, Co, Cu and Zn.

Figure 3.1 A cross-plot showing the first two discriminant functions obtained from the stepwise canonical discriminant analysis for multi-element data using a detailed classification by country.

The discriminant analysis for the multi-element compositions revealed the rice samples from India, Pakistan and Thailand to be the most distinguishable from those of the other countries represented in the study (Figure 3.1). This suggests that the elemental variations in the rice samples of the 15 countries may not be significant enough to give good geographical distinction, and an increase in the sample size for some countries could possibly improve the discriminant study. A research into the local geologies of India, Pakistan and Thailand by Sillanpää (1982) showed that the nutrient status of the soils in these countries had relatively higher Cu and Zn levels and mid-range levels of Fe and Mn as compared to the other 27 countries (ranging from Europe, South America, Middle Eastern, African to Asian countries) in the study. The micronutrient levels in soil for India and Pakistan were also found to be fairly similar, possibly due to their closer geographical proximities. In addition, high Mo values were reported in the soils obtained at irrigated sites in Pakistan (Sillanpää, 1982) which coincided with the higher Mo levels found in the Pakistani rice samples.

The second model, where the rice samples were grouped into broad categories of Asia (China, Japan, Korea and Taiwan), Australia, Europe (France, Italy and Spain), India & Pakistan, North America (USA), and Southeast Asia (Malaysia, Myanmar, Thailand and Vietnam) was developed and the CDA plot generated (Figure 3.2).

Figure 3.2 A cross-plot showing the first two discriminant functions obtained from the stepwise canonical discriminant analysis with multi-element data using a detailed classification by continent or group of countries.

With reference to Figure 3.2, the results of the CDA are represented by the group centroids of the respective country groups with 84.1% of the original grouped cases correctly classified and 78.0% of the cross-validated grouped cases correctly classified. The 14 elements selected by the software for this multivariate discrimination were magnesium (Mg), aluminium (Al), potassium (K), manganese (Mn), iron (Fe), cobalt (Co), copper (Cu), zinc (Zn), gallium (Ga), arsenic (As), selenium (Se), molybdenum (Mo), cadmium (Cd) and bismuth (Bi). Function 1 (61.3% of variance) which provided the main separation between the sample groups was primarily associated with Mg, K, Zn, As and Mo, while Function 2 (24.3% of variance) was mainly associated with Mg, K, Mn, Co, Cu, Zn and As. Once again, these selected

elements would likely play a larger role in the discrimination of the rice growing countries based on the geological features of the soil.

A significant improvement to the geographical discrimination of the rice growing countries in the model is evident (Figure 3.2) where the rice samples from Southeast Asia, India & Pakistan and Europe are better discriminated from the rest. The higher classification percentages obtained indicated that the model has improved in its robustness and reliability. Another interesting observation is that the elements selected by the software are slightly different for the two models generated. Those elements which were identified in both models probably possessed higher discriminatory values for the rice samples examined in this study. It is also interesting to observe that the number of elements selected from the CDA procedure increased when the groups of interest changed from the individual countries to continents or groups of countries. A possible reason is that as more samples are classified into smaller groups of countries, greater variations in the elemental compositions are observed and since no single element was found to have uniquely significant discriminating ability, therefore the list of selected variables changes.

Based on the differences between the two models a study into some of the elements that were selected by the software for CDA classification may be able to provide an added understanding of the relationship between the compositions in the soil and the plant. Cadmium (Cd), a toxic trace element with no known metabolic function, occurs naturally at low levels in rock, soil and water (Herber, 1994). Cd is effectively absorbed by root and leaf systems and high levels of Cd can accumulate in soil organisms (Kabata-Pendias & Pendias, 2001; Morales-Rubio *et al*., 1993). In fact, almost 99% of Cd released into the atmosphere each year is a result of anthropogenic activities (Friberg *et al*., 1992), and Cd has been reported to be useful in investigations

of geographical origin (Lewis & Hird, 2007; Branch *et al*., 2003). A study on durum wheat grain (Norvell *et al*., 2000) showed that the accumulation of Cd in grain was strongly and positively associated with soil salinity. In addition, the concentration of Cd in plants was found to increase with increasing pollution in the plant cultivation area (Gonzálvez *et al*., 2011).

Selenium (Se) is an essential element in human health and is another useful marker in distinguishing different geographic origins of rice (Kelly *et al*., 2002) and in wheat (Branch *et al*., 2003). Cobalt (Co) is also an element which plays a significant role in the discriminant function. Studies have shown that Co enrichment in the soil results in elevated Co levels in plants (Gonzálvez *et al*., 2011; Kabata-Pendias & Pendias, 2001).

Likewise, Li *et al*. (2008) reported that the concentrations of copper (Cu) in rice grain and rice straw were significantly correlated with the Cu concentrations in the soil. The soil available Cu is in turn largely dependent on soil pH, where high soil pH tends to make Cu less available in the soil, while the soil adsorption capacity can affect the bioavailability of Cu in the soil. Moreover, the application of Cu-based fungicides has been reported to increase the level of Cu in agricultural soils (Komárek *et al*., 2009) which could lead to an enhanced Cu uptake by the plant. An example of such fungicide is the Bordeaux mixture ($CuSO_4$ + $Ca(OH)_2$) which has been found to be a good control for false smut in rice when applied at late booting to heading stage of the rice growth (IRRI, 2009).

Manganese (Mn) is required in plants as an enzyme cofactor and is involved in photosynthetic oxygen evolution. Mn deficiency is related to soil conditions, weather and crop species, and can be aggravated by low soil pH (< 6) and high organic content

(Hopkins & Hüner, 2004). This suggests that a higher utilisation of organic fertilisers may result in a reduced Mn content in the soil which can in turn lead to a lower Mn uptake by the plant.

Potassium (K) is another essential element for plant growth. It helps to strength plant cell walls and contributes to greater canopy photosynthesis and crop growth. K can also increase the number of spikelets per panicle and percentage of filled grain (IRRI, 2009). As the source of K for rice plants may come from the soil, crop residues, organic amendments, irrigation water and supplemental K from fertilisers, the K profile could be useful in providing insights into the geographical location of the rice growing region as well as the rice cultivation practices.

Rice is one of the highly sensitive crops to zinc (Zn) deficiency, especially that grown in lowland production regions, where the waterlogged soils are susceptible to Zn deficiency. It has been stated that after nitrogen, phosphorus and potassium, zinc and sulfur are the next most important mineral nutrients for rice (Alloway, 2008).

Interestingly, arsenic (As), being a toxic metalloid which can pollute water, land, crops and the environment (Zhao *et al*., 2010), was also identified as having some geographical discriminatory effect on the rice samples in this study. Garnier *et al*. (2010) reported As uptake by rice plants from the use of As-contaminated irrigation water. As a result, the trace As content found in the rice samples could be an indication of the degree of contamination in the rice cultivation region and a reflection of the extent of industrialisation around the region.

The essential plant macronutrient or micronutrients, magnesium (Mg), iron (Fe), sodium (Na) and molybdenum (Mo) (Hopkins & Hüner, 2004), are undoubtedly important elements to be absorbed from the soil for healthy plant growth. It is, therefore,

not unusual for these elements to be involved in the geographical discrimination of the rice samples. On the other hand, the effects of the remaining elements lithium (Li), aluminium (Al), gallium (Ga) and bismuth (Bi) on the geographical separation of rice samples cannot be easily interpreted, despite having a significant effect on the canonical discriminant functions that contributed to the geographical groupings in this particular study.

3.2 Conclusions

The discrimination of the geographical origins of the rice samples by CDA based only on the multi-element data proved to be useful in providing some distinction according to country of origin. An improvement in the differentiation was seen through the broader classification of origin into continents or groups of countries before applying the CDA procedure. It is possible that with the inclusion of the stable isotope ratio profile of these rice samples, an enhanced model could be constructed to identify the geographical origin of unknown rice samples across the different rice varieties.

Chapter 4 – Identification of the geographical origins of rice

4.1 Evaluation of the combination of stable isotope ratio and multi-element data

Similar to the treatment of the multi-element data for the rice samples, the combined isotope ratios (δ^{13}C, δ^{15}N and δ^{18}O) and multi-element dataset was interrogated by a 'stepwise' canonical discriminant analysis (CDA) in an attempt to construct an empirical model for rice samples from various countries of origin.

Two multivariate models were generated, the first using the detailed classification by country and the second using a broad classification by continent or group of countries. For the first model, the results of the CDA are represented by the group centroids of the respective countries of origin with 69.2% of the original grouped cases correctly classified and 60.7% of the cross-validated grouped cases correctly classified (Figure 4.1). The 10 variables selected by the software for this multivariate discrimination were δ^{13}C, δ^{15}N, δ^{18}O, lithium (Li), sodium (Na), copper (Cu), zinc (Zn), arsenic (As), molybdenum (Mo) and cadmium (Cd). Function 1 (43.7% of variance) which provided the main separation between the sample groups was primarily correlated with Cu, Zn, As and Mo while Function 2 (18.1% of variance) was mainly associated with δ^{13}C, Cu and Zn. Although the δ^{15}N and δ^{18}O results were excluded from the first two functions, it should be noted that the percentages of variance for the various functions were more spread out as compared to the other CDA outputs in this study. Hence the 10 variables that were identified would still likely take up a fairly important role in the discrimination study.
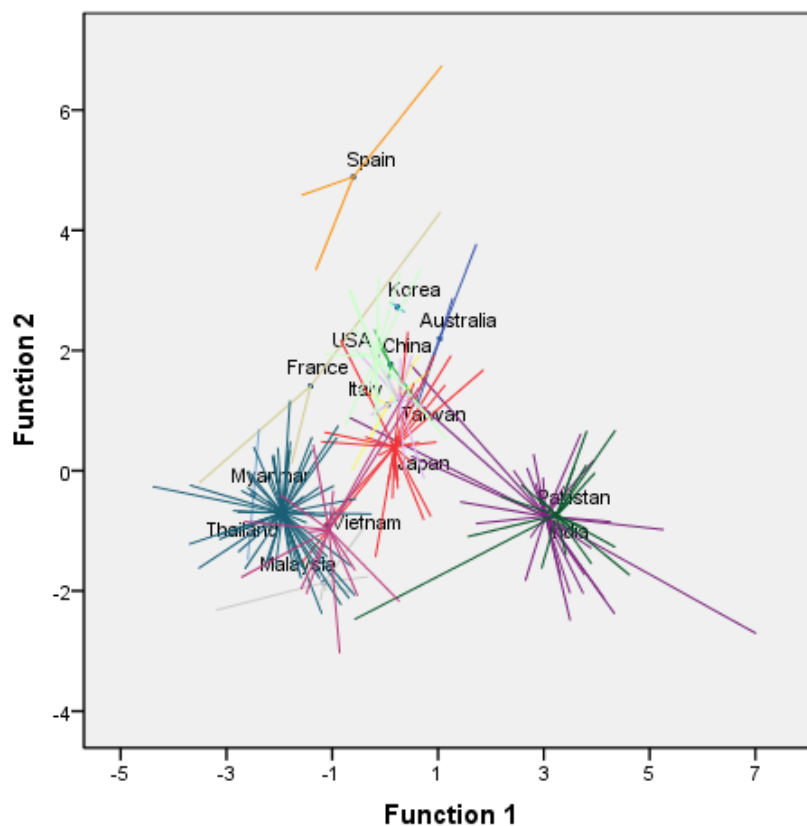
Figure 4.1 A cross-plot showing the first two discriminant functions obtained from the stepwise canonical discriminant analysis for stable isotope ratio and multi-element data using a detailed classification by country.

The combination of stable isotope ratios and multi-element concentrations resulted in samples from India, Pakistan and Spain being the only ones distinguishable from the samples from the other countries. This further demonstrates that the Indian and Pakistan rice samples possess characteristic signatures that differentiate them from the rest of the rice samples, regardless whether the distinction is based only on the multi-elemental profile or both the stable isotope ratio and multi-elemental data. Notably, this reflects function 1 being associated exclusively with inorganic elements. Although the rice samples from the remaining countries were not discriminated according to their

countries of origin, an improvement in the classification percentages was achieved by comparison with using only the multi-element data for discriminant analysis. It is possible that the variations in the rice samples may not be distinctive enough to give a good geographical differentiation, and an increase in the sample size for some countries could possibly improve the discriminant study. The second model where the rice samples were grouped into the broad categories of Asia (China, Japan, Korea and Taiwan), Australia, Europe (France, Italy and Spain), India & Pakistan, North America (USA), and Southeast Asia (Malaysia, Myanmar, Thailand and Vietnam) was generated (Figure 4.2).
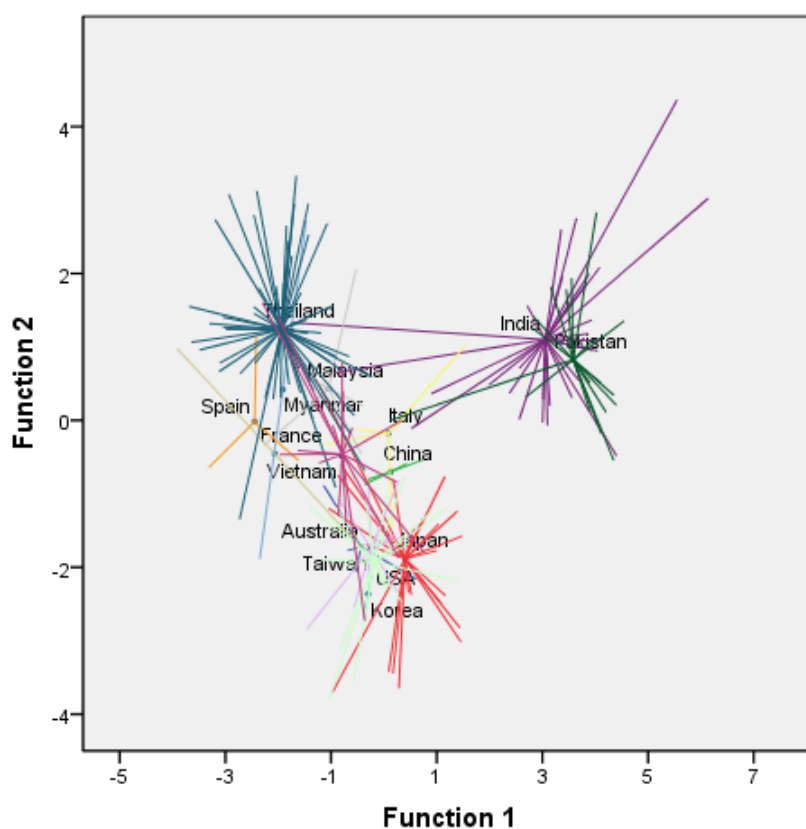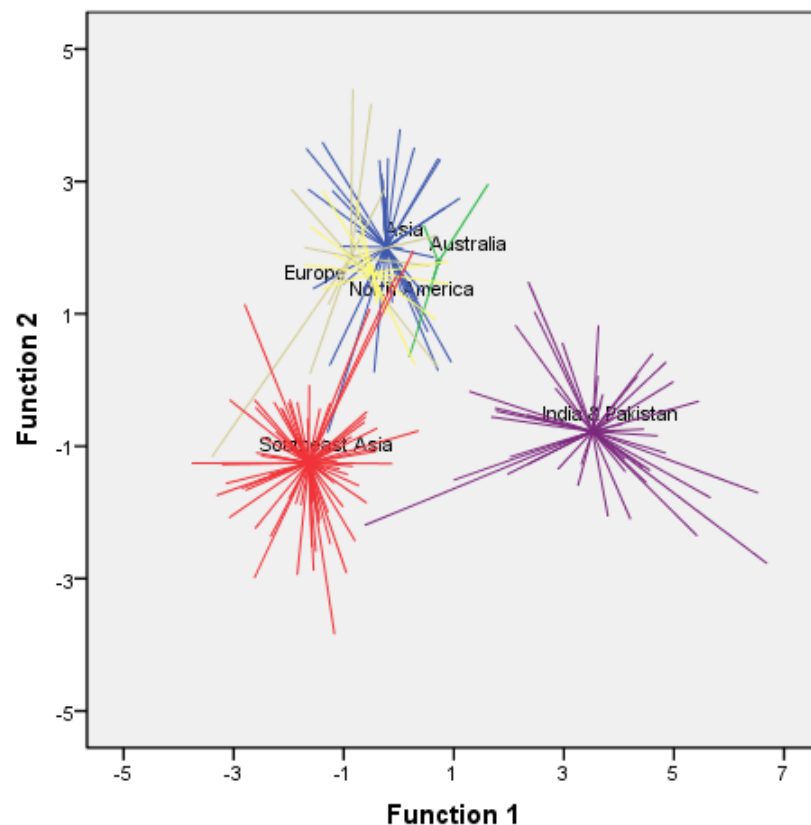

Figure 4.2 A cross-plot showing the first two discriminant functions obtained from the stepwise canonical discriminant analysis for stable isotope ratio and multi-element data using a detailed classification by continent or group of countries.

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 δ^{13}C, δ^{15}N, δ^{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 δ^{13}C, Mg, K, Cu and Zn (Figure 4.2).

As observed previously for the multi-element data analysis, a significant improvement in the model was obtained when the rice samples were grouped into broader categories. The rice samples from Southeast Asia and India & Pakistan were better discriminated from the rest. The classification percentages obtained for this model was the highest of all those obtained from CDA indicating that the model has the best robustness and reliability. Apart from the inclusion of the stable isotope ratios in the CDA procedure, the elements selected by the software to generate Figures 3.2 and 4.2 are very similar. The roles of these elements have been discussed in Chapter 3.

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.
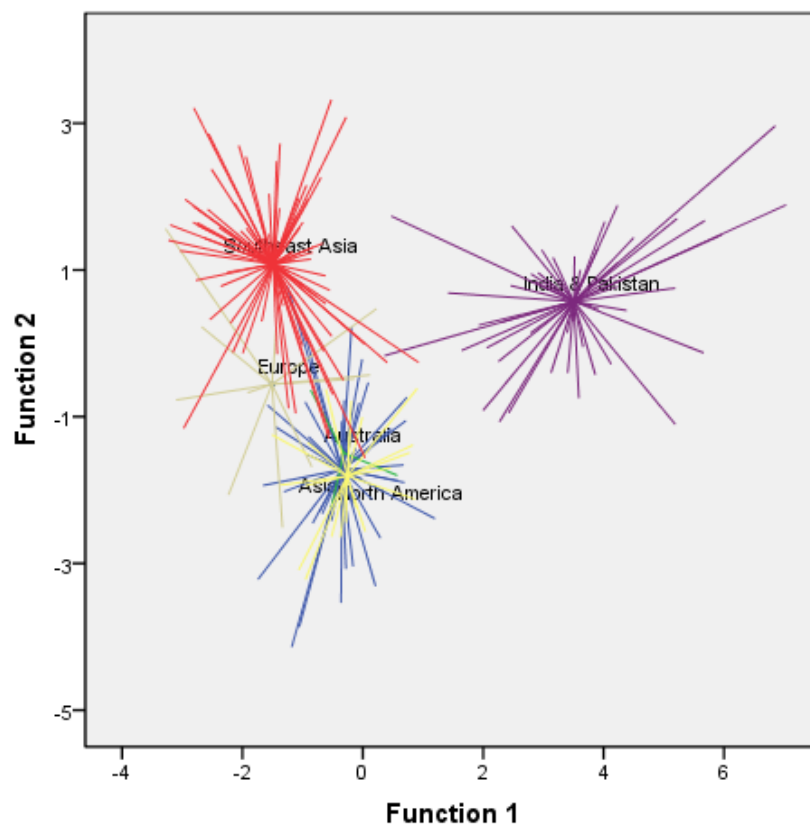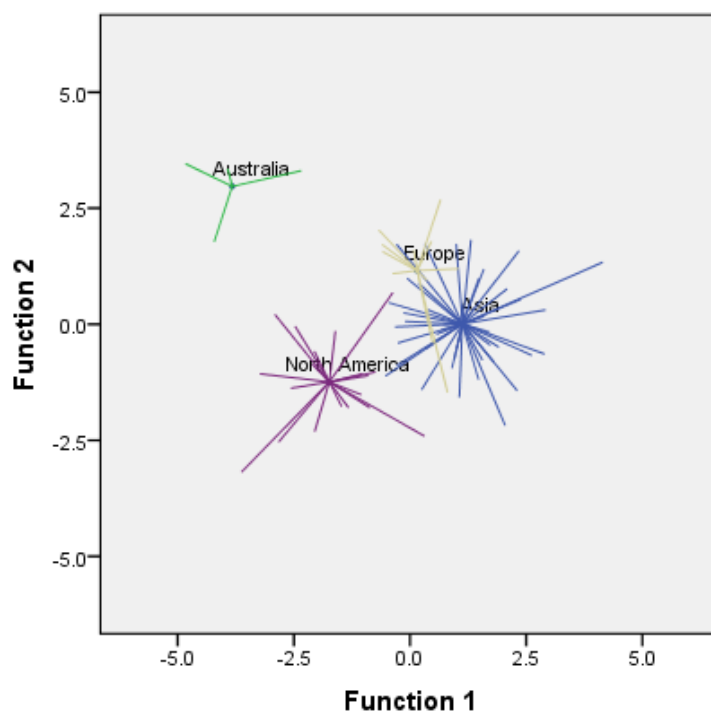
Figure 4.3 A cross-plot showing the first two discriminant functions obtained from the stepwise canonical discriminant analysis for stable isotope ratio and multi-element data for the Asian, Australian, European and North American rice samples.

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 4.3). The 6 elements selected by the software for this multivariate discrimination were δ^{15}N, δ^{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 δ^{15}N, δ^{18}O and Cd while Function 2 (29.7% of variance) was mainly associated with δ^{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 δ^{15}N and δ^{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 in the earlier chapter.

4.2 Identification of rice samples by rice types

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 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 4.4 shows the 'stepwise' CDA cross-plot for the Aromatic rice type.
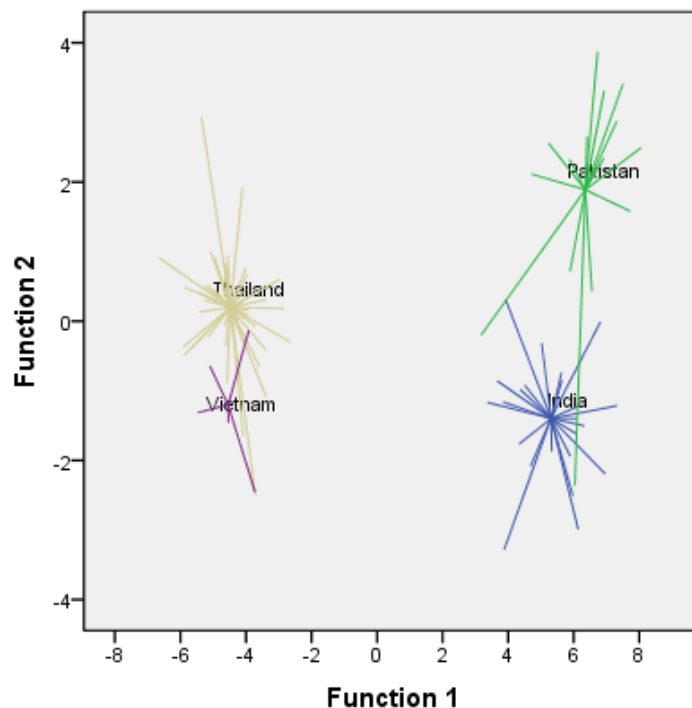
Figure 4.4 A cross-plot showing the first two discriminant functions obtained from the stepwise canonical discriminant analysis for stable isotope ratio and multi-element data for the Aromatic rice type.

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 4.5 shows the 'stepwise' CDA cross-plot for the Japonica rice type.
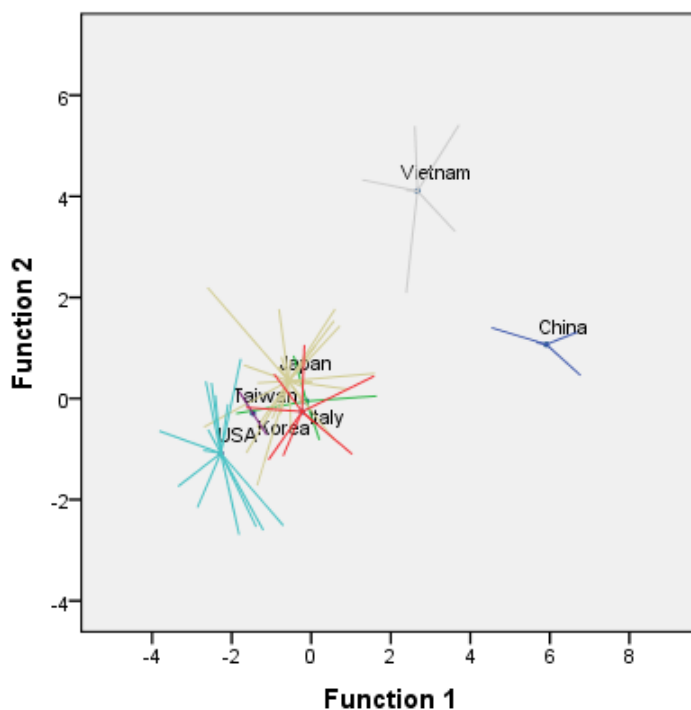
Figure 4.5 A cross-plot showing the first two discriminant functions obtained from the stepwise canonical discriminant analysis for stable isotope ratio and multi-element data for the Japonica rice type.

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 (Figure 4.5). The 10 elements selected by the software for this multivariate discrimination were δ^{15}N, δ^{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 δ^{15}N, Li, Ca, Zn, Cs and Os while Function 2 (36.5% of variance) was mainly associated with δ^{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.

4.3 Identification of rice samples with unknown countries of origins

The procurement of rice samples for this study revealed that some rice packages actually did not indicate the country of origin for the rice, despite them being sold in the retail market. A total of seven rice samples were found to have their countries of origin omitted from their packaging, and were therefore excluded from the dataset used in the previous discussions. These seven samples were analysed for their stable isotope ratio and multi-elemental compositions and their results were then applied into the model constructed from the CDA study of the other rice samples to predict their countries of origin.

Table 4.1 below displays the prediction results for the rice samples with unknown countries of origin. Although the actual country of origin for these samples

could not be verified to test the accuracy of the prediction, there are some indications that the prediction results could be accurate. For example, two samples of Koala brand Japonica rice purchased from different sellers gave the same predicted country of origin, China. Another example is of two Songhe brand unpolished rice which also gave the same predicted country of origin, Thailand. Notably, both samples contained unpolished red rice. Thus, the results suggest that it is possible to construct a model for the prediction of rice samples across different countries of origin and different rice types. It should also be noted that the method will have some limitations if the rice samples used in the database do not have a true traceability to its origin or if there have been notable changes in the rice agricultural practices by the different countries over time.

Table 4.1 Prediction results for the rice samples with unknown countries of origin.

Sample ID no.	Brand	Rice type	Predicted country of origin
34	Mamalov	Jasmine	Thailand
55	Koala	Japonica	China
93	Maxwill Gold	Jasmine	Thailand
110	Songhe	50% unpolished brown rice & 50% unpolished red rice	Thailand
111	Songhe	Unpolished red rice	Thailand
114	Koala	Japonica	China
211	Roshan	Idli	Pakistan

4.4 Conclusions

The inclusion of the stable isotope ratio results, together with the multi-elements data, in the geographical origin discrimination of the rice samples by CDA was found to be more powerful in providing distinction by country of origin. Comparing the cross-plots by countries with and without the stable isotope ratio data (Figures 4.1 and 3.1), a

slight improvement to the rice geographical differentiation, such as Spain, could be observed. This was supported statistically by the increase of the original grouped cases correctly classified from 65.0% to 69.2% and the cross-validated grouped cases correctly classified from 57.5% to 60.7%. The same applies to the comparison of the cross-plots by continents or group of countries with and without the stable isotope ratio data (Figures 4.2 and 3.2), a more significant improvement to the rice geographical differentiation, such as Europe, could be observed. This was also supported by the increase of the original grouped cases correctly classified from 84.1% to 90.7% and the cross-validated grouped cases correctly classified from 78.0% to 87.9%. It should be noted that the unavailability of published data on the percentages for the correct classification by CDA in rice makes it difficult for comparison with the results obtained in this study. A general rule is that the higher the percentage, the better is the model in the geographical classification.

The broader classification of the rice origin into continents or groups of countries before applying the CDA procedure was again shown to give an improved differentiation of geographical origin, similar to the observations made with the multi-element CDA study. A further investigation into the geographical origin discrimination of the rice samples by different rice types provided alternative options to construct the discriminant models. Furthermore, the prediction of rice samples with unknown countries of origin was possible with the discriminant models constructed in this study. However, it should be noted that the robustness of such models must be rigorously tested with rice samples with known countries of origin to support the accuracy of the prediction.

Chapter 5 – Overall conclusions and future work

The stable isotope ratio and multi-element compositions of rice from different geographical origins and including various rice types were determined by IRMS and ICP-MS, respectively. Rice samples from India & Pakistan and Southeast Asia were differentiated from the rice samples from Asia, Australia, Europe and North America by applying canonical discriminant analysis 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 2.3, 2.11, 2.15 and 2.21), 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 δ^2H 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 δ^2H 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 δ^2H 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 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 canonical discriminant analysis 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.

Future work could involve collaborations with international rice bodies or major rice producers to obtain authentic rice samples, soil and water sources during plant growth, monitor the specific rice growing conditions as well as gain access to the actual rice cultivation cycle and practices of individual rice farms. The majority of the published literature pertaining to the geographical origin of rice involved the analysis of the rice grain itself but it might be interesting to compare the differences in the stable

isotope ratio (if any) and multi-elemental compositions of the soil, plant leaves, rice grain husk and bran layer as well as the rice grain itself. Another interesting aspect of the future work could involve the analysis of processed rice products, like pre-cooked or microwavable rice, to assess its country of origin. However, it would be necessary to take into consideration that the water used in the rice cooking process could contribute to the stable isotope ratio and multi-elemental profile.

Chapter 6 – Experimental

6.1 Sources of rice samples

A total of 226 rice samples from fifteen different countries were used in this study (Appendix II). Of the rice samples obtained, 137 were long grain rice, 84 were short or medium grain rice while the remaining 5 were broken rice. The rice ranged from the regular long grain rice to specialty rice like Jasmine, Japonica and Basmati rice in both the polished and unpolished forms. The countries of origin for these rice samples were either provided directly by the rice importer or from the information available on the package label. These countries of origin included Australia, China, France, India, Italy, Japan, Korea, Malaysia, Myanmar, Pakistan, Spain, Taiwan, Thailand, USA and Vietnam. The package label information on the rice samples are generally assumed to be accurate for the purpose of this study, since most of the rice brands used have their company webpages available online to share about their rice product information. A detailed listing of these rice samples can be found in Appendix II.

6.1.1 Procurement of rice samples

The majority of the rice samples were purchased in Singapore from retailers including department stores, supermarkets and convenience shops. A total of 21 rice samples were purchased directly from a major rice importer, Chye Choon Foods Pte Ltd. Four of the Taiwanese rice samples and all of the Malaysian rice samples were purchased directly from retailers in Taiwan and Malaysia, respectively. Furthermore, fourteen rice samples were provided by the Crop and Food Security Department of the

Food and Environment Research Agency (FERA), UK, to add to the rice database in this study.

6.2 Preparation of rice samples

Approximately 30 g of each rice sample was packed into a glass container and sent by courier services to FERA, where the analyses by IRMS and ICP-MS were performed. A portion of each rice sample was milled to a fine powder for stable isotope ratio analysis while the remaining whole grains were acid digested for multi-elemental determination.

Figure 6.1 Containers used for rice sample storage.

6.2.1 Pulverisation of rice samples by ball milling

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. The ground rice powder was then placed into polypropylene tubs for storage prior to isotope ratio analysis.
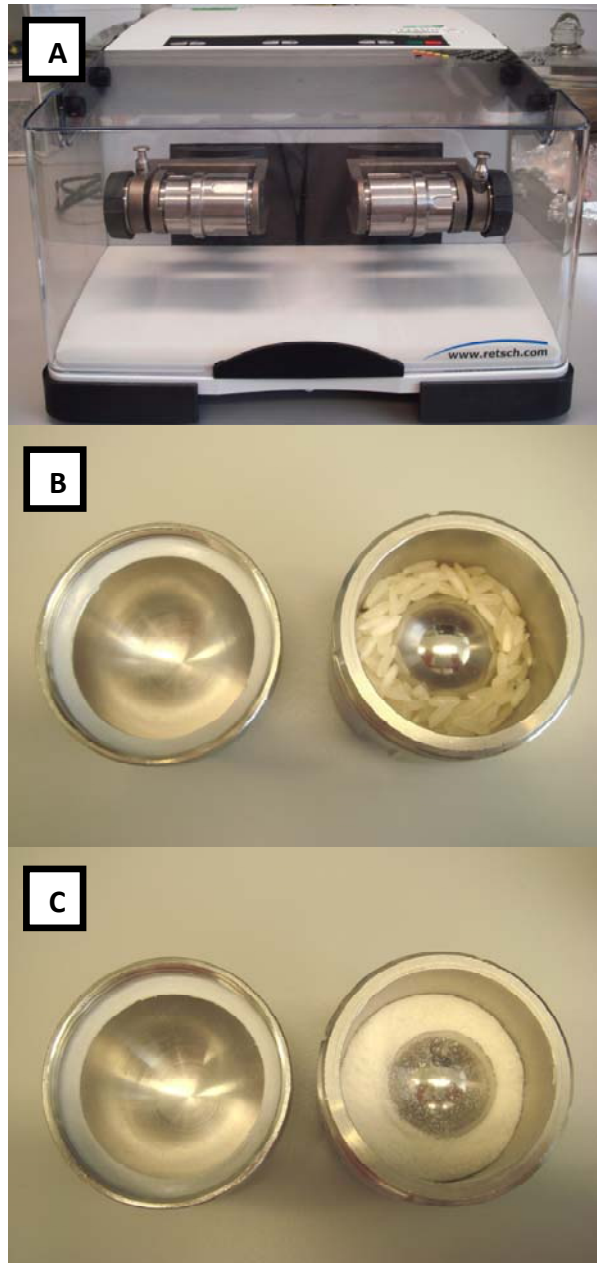
Figure 6.2 Rice pulverisation by ball milling. (A) Mixer Mill MM 400. (B) Rice sample before pulverisation. (C) Fine rice powder obtained after milling.

6.3 Isotope ratio analysis

Each of the stable isotope ratio analyses ($\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$ and δ^2H determinations) was carried out individually on the rice samples, recording the sample mass and analysis conditions used.

6.3.1 Preparation of rice samples for IRMS analysis

For carbon stable isotope ratio analysis, 1 mg of powdered rice sample was weighed into a tin capsule (4 x 6 mm, obtained from Elemental Microanalysis, Cambridge, UK). The capsule was folded and compressed into a spherical ball. Figure 6.3 illustrates the typical sample preparation procedure for IRMS analysis.
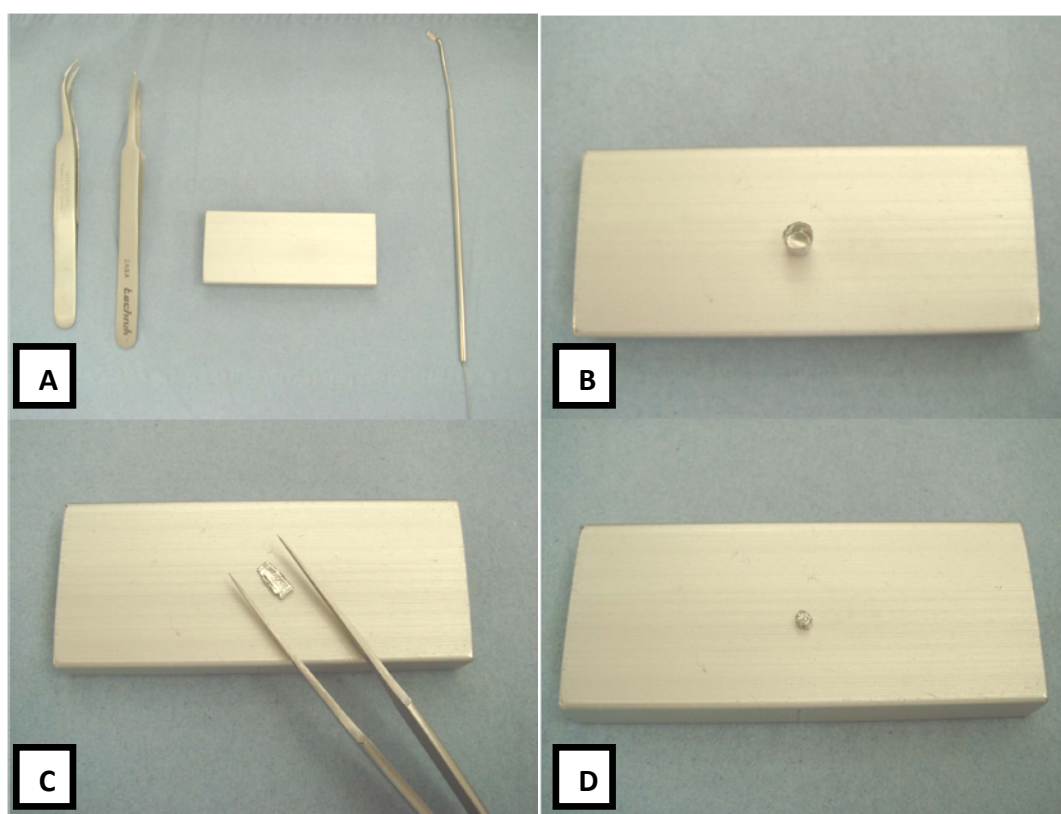
Figure 6.3 Typical sample preparation procedures for IRMS analysis. (A) Tools used for sample preparation. (B) Empty tin capsule (4x6 mm). (C) Folding of tin capsule with tweezers. (D) Tightly compressed spherical ball.

The above sample preparation process was repeated with 3 mg of powdered rice samples for nitrogen, and 2 mg of powdered rice samples for both the oxygen and hydrogen stable isotope ratio analysis.

6.3.2 Selection of stable isotope standards and reference materials

The stable isotope standards for calibration and the reference materials for quality control were selected based on their availability and suitability in matrix to the rice samples in the study.

6.3.2.1 Materials with known carbon isotopic compositions

A beet sugar laboratory reference material (JRC Code 99020037) with a known $\delta^{13}C$ ‰ value versus the Pee Dee Belemnite (PDB) scale was used to calibrate the $\delta^{13}C$ measurements of the rice samples by flash combustion EA-IRMS. This beet sugar reference material was previously used in the TRACE project and had an inter-laboratory collated $\delta^{13}C$ value of -26.8 ‰. The beet sugar was selected as the sugar beet plant is also a C_3 plant like rice. For quality control materials, the rice flour standard reference material NIST 1568 (Gaithersburg, Maryland, USA) and a fructose laboratory reference material (JRC Code 98050022) were included in each batch of analyses to assess the between batch precision of the $\delta^{13}C$ measurements. Although the NIST 1568 rice flour was meant to be an elemental reference material, its homogeneity and exact match of the rice sample matrix made it suitable for use in precision monitoring. The fructose laboratory reference material, like the beet sugar reference material, was also previously used in the TRACE project.

6.3.2.2 Materials with known nitrogen isotopic compositions

Two L-glutamic acid standard reference materials USGS40 and USGS41 (IAEA, Vienna, Austria) with known $\delta^{15}N$ values of -4.5 ‰ and 47.6 ‰ respectively versus air (N_2) scale, were used to calibrate the $\delta^{15}N$ measurements of the rice samples by flash combustion EA-IRMS. For the quality control materials, the NIST 1568 rice flour

sample and a wheat flour laboratory reference material (050818) were included in each batch of analyses to assess the between batch precision of the $\delta^{15}N$ measurements. The wheat flour reference material was previously used in the TRACE project and had an inter-laboratory collated $\delta^{15}N$ ‰ value.

6.3.2.3 Materials with known oxygen isotopic compositions

Two benzoic acid standard reference materials, IAEA-601 and IAEA-602 (IAEA, Vienna, Austria), with known $\delta^{18}O$ values of 23.3 ‰ and 71.4 ‰ respectively versus the Vienna Standard Mean Ocean Water (VSMOW) scale, were used to calibrate the $\delta^{18}O$ measurements of the rice samples by pyrolysis combustion EA-IRMS. For the quality control materials, the NIST 1568 rice flour sample and a sucrose reference material (IAEA-CH-6) were included in each batch of analyses to assess the between batch precision of the $\delta^{18}O$ measurements. As the sucrose reference material only has a certified $\delta^{13}C$ value, the mean $\delta^{18}O$ value adopted for this reference material was obtained from a study by Kornexl *et al*. (1999).

6.3.2.4 Materials with known hydrogen isotopic compositions

A wheat flour laboratory reference material (050818) with a known δ^2H value of -43.2 ‰ versus the Vienna Standard Mean Ocean Water (VSMOW) scale was used to calibrate the δ^2H measurements of the rice samples by pyrolysis combustion EA-IRMS. This wheat flour reference material was previously used in the TRACE project and had an inter-laboratory collated δ^2H ‰ value. For the quality control materials, the NIST 1568 rice flour sample and a rice reference material (FIT-PTS 2010 Round 3) were included in each batch of analyses to assess the between batch precision of the $\delta^{18}O$

measurements. The rice reference material from FIT-PTS (Food analysis using Isotopic Techniques – Proficiency Testing Scheme) has an inter-laboratory collated δ^2H ‰ value.

6.3.3 Measurement by IRMS

The isotope ratio measurements for $\delta^{13}C$ and $\delta^{15}N$ and those for δ^2H and $\delta^{18}O$ were carried out by the flash combustion EA-IRMS and high temperature pyrolysis EA-IRMS, respectively.

$\delta^{13}C$ AND $\delta^{15}N$ DETERMINATIONS

The capsules containing the rice samples were placed in the autosampler of the elemental analyser (Fisons, Ipswich, UK) and dropped into a vertical quartz tube maintained at a temperature of 1020 °C. As described by Rhodes *et al.* (2010), 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, Stockport, UK). The combustion gases were then passed over copper grains (Pelican Scientific, Stockport, UK) at a temperature of 650 °C, in a separate quartz tube, to remove residual oxygen and convert nitrous oxide 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 x 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 to account for the differences in C and N contents in rice. $\delta^{15}N$ results may experience greater variability among the replicates due to the low N content in rice. During each analysis, the reference gas was introduced before and after the sample peak. Rice generally contains about 10 times less N than C, thus the ionisation efficiency for N_2 may be compromised in the presence of CO_2. As a consequence, the $\delta^{13}C$ and $\delta^{15}N$ analysis were carried out in separate runs to avoid overloading the detectors with CO_2 and disturbing the baseline.

$\delta^{18}O$ DETERMINATION

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 product 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.

δ^2H DETERMINATION

The procedure for δ^2H determination is largely similar to the δ^{18}O determination apart from the elemental analyser and IRMS (Thermo Scientific, Massachusetts, 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 δ^2H determination.

6.3.4 Corrections applied to the instrumental data

Several mathematical corrections are typically applied to the acquired data obtained from the instrument software and data system. Electronic and chemical backgrounds are measured and subtracted in accordance with signal detection. In modern IRMS software systems, algorithms for the automatic correction for isobaric interferences have already been implemented (Gremaud & Hilkert, 2008).

6.3.4.1 Drift correction

The inclusion of reference materials with known isotopic values, at regular intervals within a sample batch, provides information on the instrumental drift. This drift correction can be done automatically by the software of the instrument or manually on an electronic spreadsheet. The corrections to the isotope ratio values of the samples were done according to the difference between the measured isotope ratio value of the reference material and its true value (as provided). The drift correction method was that described by Jamin *et al*. (2004). An example of the instrumental drift on a reference material is illustrated in Figure 6.4.
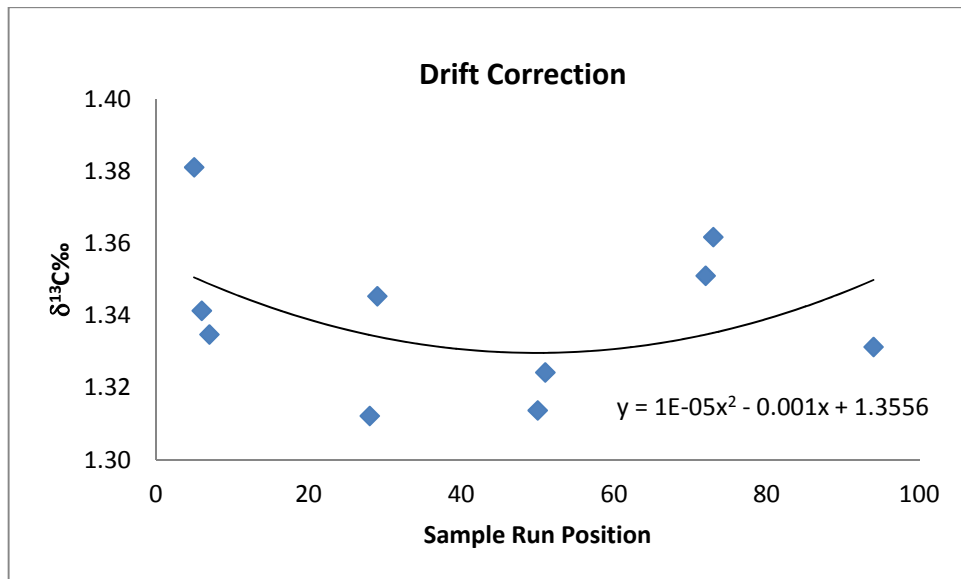
Figure 6.4 Example of drift correction from the $\delta^{13}C$ analysis run.

6.3.4.2 Delta linearity correction

The delta (δ) linearity correction is another type of corrections that can be applied to the data, apart from the usual drift correction. For example, in the $\delta^{15}N$ analysis, the sample results ranged from 0‰ to 12‰ while the available reference materials, USGS40 and USGS41, had known $\delta^{15}N$ values of -4.5‰ and 47.6‰, respectively. The delta linearity correction was carried out by plotting the measured $\delta^{15}N$ against the assigned $\delta^{15}N$ values for the USGS40 and USGS41. For some stable isotope ratio analysis, the linear correlation was applied to all the samples in the same batch run before carrying out the drift correction as discussed earlier.
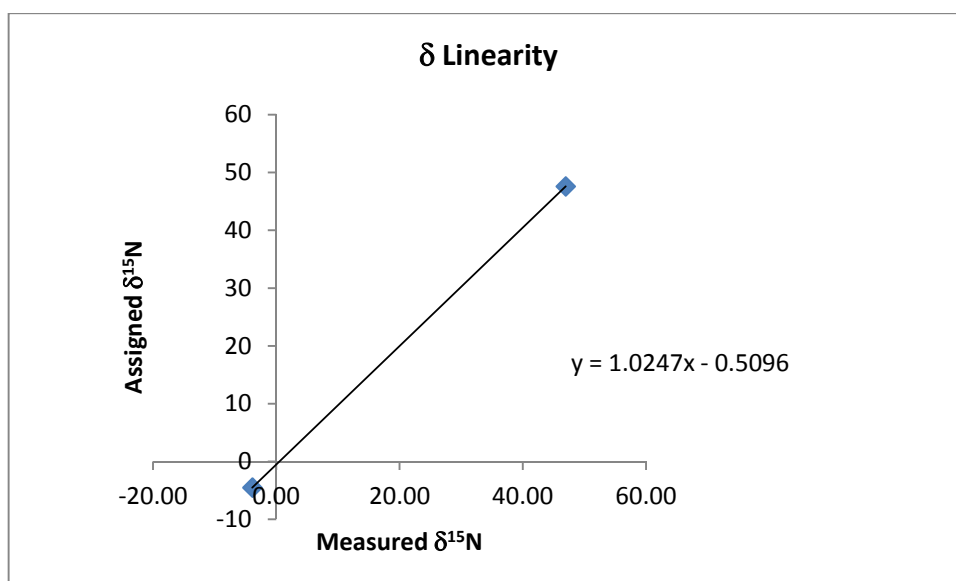
Figure 6.5 Example of delta linearity correction from the $\delta^{15}N$ analysis run.

6.3.4.3 H_3^+ correction

Protonation reactions taking place when H_2 is ionised in the ion source of the IRMS results in the unavoidable formation of H_3^+ ions. The presence of H_3^+ ions causes an isobaric interference to the hydrogen containing ^2H and ^1H. Source design can help to minimise the amount of H_3^+ formation but correction is often necessary to eliminate this interfering signal. The H_3^+ portion of the *m/z* 3 ion beam is determined as the H_3-Factor which is used to correct for the H_3^+ contribution. The determination of the H_3-Factor should be done prior to the analysis run. A low and stable H_3- Factor is required for a good δ^2H determination.

6.4 Multi-element analysis

A total of 66 elements were simultaneously quantified for the rice samples in the study by ICP-MS. The general procedure involved the microwave-assisted acid digestion of the rice grains followed by instrumental analysis using ICP-MS.

6.4.1 Preparation of rice samples for acid digestion

A 0.5 g of rice grain sample was digested in 4.75 mL concentrated nitric acid (BDH Laboratory Supplies, Poole, UK) and 0.25 mL concentrated hydrochloric acid (Fisher Scientific, Loughborough) using a microwave digestion system (Perkin Elmer, Massachusetts, USA) at a pressure of 75 bar. The microwave sample digestion programme used is shown in Table 6.1 below:

Table 6.1 Sample digestion programme for rice.

Step	Heating power (W)	Time (min)	Fan
1	0-500	2:00	1
2	500	5:00	1
3	500-1000	2:00	1
4	1000	20:00	1
4	0	15:00	3
Total		44:00	

The sample digest was first diluted to 10 mL with high purity deionised water (Millipore, Massachusetts, 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. The final solution was used for quantitative measurements by ICP-MS. For each digestion batch, four blank samples, three reference materials and one blank spike were included to ascertain detection limits, accuracy and recovery, respectively.

6.4.2 Selection of multi-element standards and reference materials

A combination of multi-element standards and single element standards were used for calibration in the ICP-MS analysis. Different calibration concentration ranges were prepared for the various elements in the analysis to cater to the huge variations

between the natural levels of certain elements in rice. The standards used are listed in Table 6.2 below.

Table 6.2 Multi-element and single element standards used in the study.

Calibration Standard	Supplier
Aluminium	VMR International, Leicestershire, UK
Antimony	BDH Laboratory Supplies, Poole, UK
Bismuth	BDH Laboratory Supplies, Poole, UK
Calcium	VMR International, Leicestershire, UK
Copper	VMR International, Leicestershire, UK
Indium	BDH Laboratory Supplies, Poole, UK
Iron	VMR International, Leicestershire, UK
Magnesium	VMR International, Leicestershire, UK
Manganese	VMR International, Leicestershire, UK
Mercury	VMR International, Leicestershire, UK
Osmium	Johnson Matthey, London, UK
Potassium	BDH Laboratory Supplies, Poole, UK
Scandium	BDH Laboratory Supplies, Poole, UK
Sodium	BDH Laboratory Supplies, Poole, UK
Tellurium	BDH Laboratory Supplies, Poole, UK
Tin	VMR International, Leicestershire, UK
Zinc	VMR International, Leicestershire, UK
Multi-element Solution 1 (Ce, Dy, Er, Eu, Gd, Ho, La, Lu, Nd, Pr, Sc, Sm, Tb, Th, Tm, Y, Yb)	*Glen Spectra Reference Materials*, Middlesex, UK
Multi-element Solution 2a (Ag, Al, As, Ba, Be, Ca, Cd, Co, Cr, Cs, Cu, Fe, Ga, K, Li, Mg, Mn, Na, Ni, Pb, Rb, Se, Sr, Tl, U, V, Zn)	*Glen Spectra Reference Materials*, Middlesex, UK
Multi-element Solution 3 (Au, Hf, Ir, Pd, Pt, Rh, Ru, Sb, Sn, Te)	*Glen Spectra Reference Materials*, Middlesex, UK
Multi-element Solution 4 (B, Ge, Mo, Nb, P, Re, S, Si, Ta, Ti, W, Zr)	*Glen Spectra Reference Materials*, Middlesex, UK

A rice flour standard reference material NIST 1568a (Gaithersburg, Maryland, USA), a peach leaves standard reference material NIST 1547 (Gaithersburg, Maryland, USA) and a spinach reference material ZC 73013 (China National Analysis Centre for Iron and Steel, Beijing, China) were used as the quality controls in the ICP-MS analysis to estimate the accuracy and precision of the analyses.

6.4.3 Measurement by ICP-MS

Sixty-six elements (Li, Be, B, Na, Mg, Al, K, Ca, Sc, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, Ge, As, Se, Rb, Sr, Y, Zr, Nb, Mo, Ru, Rh, Pd, Ag, Cd, Sn, Sb, Te, Cs, Ba, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, Hf, Ta, W, Re, Os, Ir, Pt, Au, Hg, Tl, Pb, Bi, Th, U) were analysed by ICP-MS (Agilent Technologies, Berkshire, UK). The ICP-MS was equipped with an octopole reaction system (ORS) to reduce the effects of polyatomic interfering ions on selected elements.

6.5 Data Analysis

Data analysis was carried out using canonical discriminant analysis (CDA) on the SPSS v17.0 (IBM, Middlesex, UK). CDA is the chosen multivariate method as it can be used to examine the differences between two or more groups with respect to 'predictor' variables (δ^{13}C, δ^{15}N, δ^2H, δ^{18}O and multi-elements), and in combination with the 'stepwise' approach, only a minimal number of selected variables would be sufficient to provide the maximum separation of the data into groups. The CDA approach makes use of "discriminant functions" which are able to combine the group characteristics in a way that will allow the identification of the group which a sample most closely resembles (Klecka, 1980).

Appendices

Appendix I Summary of the element analysis parameters, method LOD and quality control results for ICP-MS.

Element	Selected mass	Mode of ORS	LOD (ng/g)	NIST 1568a Rice flour Certified Value (ng/g)	Recovery (%) 17-Mar-11	13-Apr-11	14-Apr-11	05-May-11	13-May-11	18-May-11	19-May-11	NIST 1547 Peach leaves Certified Value (ng/g)	Recovery (%) 17-Mar-11	13-Apr-11	14-Apr-11	05-May-11	13-May-11	18-May-11	19-May-11
Li	7	Std	1.18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Be	9	Std	0.28	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B	11	Std	142	-	-	-	-	-	-	-	-	29000	120	141	77	85	99	85	88
Na	23	Std	279	6600	169	116	114	129	127	130	138	24000	177	121	134	130	134	127	125
Mg	24	Std	1215	560000	90	90	86	87	81	89	90	4320000	101	99	92	93	99	95	95
Al	27	Std	52.27	4400	87	81	83	85	78	86	85	249000	100	87	90	93	94	94	92
K	39	Std	7015	1280000	94	93	90	87	85	92	94	24300000	107	102	97	96	104	103	102
Ca	43	Std	1979	118000	69	100	88	88	84	85	86	15600000	66	102	97	98	104	99	99
Sc	45	He	3.98	-	-	-	-	-	-	-	-	40	75	96	142	79	92	80	94
Ti	47	He	39.37	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
V	51	He	17.27	7	20	160	129	35	6	44	-93	370	75	88	84	82	73	63	77
Cr	52	He	14.31	-	-	-	-	-	-	-	-	1000	77	51	101	82	63	70	78
Mn	55	Std	57.04	20000	94	91	89	89	84	90	92	98000	100	96	96	93	95	96	94
Fe	56	He	222	7400	91	76	70	96	90	85	98	218000	74	75	74	96	89	87	97
Co	59	Std	4.63	(18)	94	72	90	88	96	87	74	70	131	100	115	98	106	108	111
Ni	60	He	80.29	-	-	-	-	-	-	-	-	690	86	21	119	96	51	44	82
Cu	63	He	54.67	2400	91	87	87	85	88	86	103	3700	87	87	89	87	85	84	92
Zn	66	He	927	19400	97	97	100	98	91	95	112	17900	100	106	107	102	101	104	110
Ga	71	Std	2.14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ge	72	Std	16.38	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
As	75	He	1.75	290	117	91	98	99	86	92	104	60	610	770	879	765	683	684	729
Se	78	H₂	0.94	380	95	88	92	86	94	92	93	120	117	110	116	108	125	127	134
Rb	85	Std	19.24	6140	101	86	65	87	87	82	96	19700	108	86	69	92	102	88	103
Sr	88	Std	1.66	-	-	-	-	-	-	-	-	53000	108	101	106	101	108	105	108
Y	89	Std	0.06	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Zr	90	Std	1.09	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nb	93	Std	0.33	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mo	95	Std	14.50	1460	125	83	95	106	85	101	90	60	2043	475	712	437	213	1928	361
Ru	101	Std	0.57	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rh	103	Std	0.08	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pd	105	Std	0.20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ag	107	Std	0.67	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cd	111	Std	1.25	22	129	121	99	103	104	147	116	26	124	91	103	84	92	114	106

Note: (Number) refers to uncertified value provided by the supplier for information only.

| | | | | NIST 1568a Rice flour | | | | | | | | NIST 1547 Peach leaves | | | | | | | |
| | | | | Certified Value | Recovery (%) | | | | | | | Certified Value | Recovery (%) | | | | | | |
Element	Selected mass	Mode of ORS	LOD (ng/g)	(ng/g)	17-Mar-11	13-Apr-11	14-Apr-11	05-May-11	13-May-11	18-May-11	19-May-11	(ng/g)	17-Mar-11	13-Apr-11	14-Apr-11	05-May-11	13-May-11	18-May-11	19-May-11
Sn	118	Std	3.88	(4.7)	142	110	240	142	23	89	34	-	-	-	-	-	-	-	-
Sb	121	Std	1.28	(0.5)	283	-22	124	-32	-107	27	671	20	142	105	105	99	106	120	111
Te	125	Std	2.75	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cs	133	Std	0.17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ba	137	Std	3.13	-	-	-	-	-	-	-	-	124000	93	95	94	96	106	93	107
La	139	Std	0.10	-	-	-	-	-	-	-	-	9000	102	89	105	105	100	108	75
Ce	140	Std	0.13	-	-	-	-	-	-	-	-	10000	96	97	95	102	95	132	115
Pr	141	Std	0.04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nd	146	Std	0.22	-	-	-	-	-	-	-	-	7000	55	66	104	79	125	119	101
Sm	147	Std	0.22	-	-	-	-	-	-	-	-	1000	83	102	102	125	129	93	86
Eu	153	Std	0.05	-	-	-	-	-	-	-	-	170	104	162	120	98	116	114	98
Gd	157	Std	0.17	-	-	-	-	-	-	-	-	1000	175	99	110	83	116	164	107
Tb	159	Std	0.02	-	-	-	-	-	-	-	-	100	94	107	111	101	120	120	104
Dy	163	Std	0.15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ho	165	Std	0.03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Er	166	Std	0.08	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tm	169	Std	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Yb	172	Std	0.08	-	-	-	-	-	-	-	-	200	83	68	60	82	46	82	59
Lu	175	Std	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hf	178	Std	0.12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ta	181	Std	2.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
W	182	Std	0.95	(1.2)	914	22	-33	463	707	33	91	-	-	-	-	-	-	-	-
Re	185	Std	0.06	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Os	189	Std	0.32	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ir	193	Std	0.06	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pt	195	Std	0.28	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Au	197	Std	0.08	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hg	202	Std	0.45	5.8	94	87	98	102	110	96	99	31	98	97	97	103	101	99	104
Tl	205	Std	0.13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pb	208	Std	1.96	-	-	-	-	-	-	-	-	870	73	76	77	80	82	76	81
Bi	209	Std	0.13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Th	232	Std	0.05	-	-	-	-	-	-	-	-	50	80	80	99	78	86	91	87
U	238	Std	0.08	(0.3)	93	21	44	76	89	75	59	15	69	62	65	66	66	69	71

Note: (Number) refers to uncertified value provided by the supplier for information only.

Element	Selected mass	Mode of ORS	LOD (ng/g)	ZC 73013 Spinach								Blank Spike							
				Assigned Value (ng/g)	Recovery (%)							Spike Amount (ng)	Recovery (%)						
					17-Mar-11	13-Apr-11	14-Apr-11	05-May-11	13-May-11	18-May-11	19-May-11		17-Mar-11	13-Apr-11	14-Apr-11	05-May-11	13-May-11	18-May-11	19-May-11
Li	7	Std	1.18	-	-	-	-	-	-	-	-	50	96	100	125	102	105	109	108
Be	9	Std	0.28	-	-	-	-	-	-	-	-	50	88	99	114	101	101	105	106
B	11	Std	142	25000	137	160	77	96	99	97	94	50	82	68	540	109	96	101	104
Na	23	Std	279	15000000	120	90	93	90	93	94	96	1000	80	109	121	102	97	97	97
Mg	24	Std	1215	5520000	98	100	88	91	90	93	93	100000	99	98	116	102	102	103	102
Al	27	Std	52.27	610000	68	63	60	66	63	65	63	1000	91	100	109	93	96	93	95
K	39	Std	7015	24900000	108	109	100	99	106	103	107	100000	97	95	109	103	97	98	100
Ca	43	Std	1979	6600000	69	108	99	100	102	96	94	10000	219	92	107	99	98	101	100
Sc	45	He	3.98	(93)	120	72	134	97	120	81	107	50	92	130	83	97	100	137	93
Ti	47	He	39.37	(28000)	114	26	42	120	52	137	57	50	49	143	130	31	98	35	89
V	51	He	17.27	870	73	81	70	81	69	74	76	50	113	105	103	95	117	116	102
Cr	52	He	14.31	1400	78	51	109	80	58	78	86	50	105	147	77	98	124	112	94
Mn	55	Std	57.04	41000	101	99	97	93	95	95	97	10000	100	99	103	102	99	101	103
Fe	56	He	222	540000	74	75	68	93	83	90	97	1000	104	98	127	96	98	103	91
Co	59	Std	4.63	220	104	88	112	89	91	90	93	50	92	105	96	100	99	99	98
Ni	60	He	80.29	920	83	24	347	89	50	51	92	50	103	287	56	92	144	170	82
Cu	63	He	54.67	8900	87	88	96	80	85	93	102	1000	104	103	95	103	96	98	88
Zn	66	He	927	35300	103	106	106	102	104	111	116	10000	101	95	91	97	86	91	87
Ga	71	Std	2.14	-	-	-	-	-	-	-	-	50	101	101	105	104	95	93	101
Ge	72	Std	16.38	(20)	440	255	180	249	184	268	266	50	101	92	93	91	99	98	94
As	75	He	1.75	230	124	110	113	115	105	111	131	50	89	108	95	97	96	96	85
Se	78	H$_2$	0.94	92	91	82	103	82	97	84	115	50	87	95	92	94	84	91	93
Rb	85	Std	19.24	30000	104	87	69	92	102	84	105	50	90	106	138	102	94	111	92
Sr	88	Std	1.66	87000	96	91	95	90	95	92	100	50	96	100	98	99	94	98	95
Y	89	Std	0.06	200	72	63	70	61	83	73	70	1	92	102	96	105	84	81	95
Zr	90	Std	1.09	-	-	-	-	-	-	-	-	50	96	96	92	96	95	95	95
Nb	93	Std	0.33	(60)	99	60	68	68	73	65	68	50	75	93	95	78	92	92	96
Mo	95	Std	14.50	470	223	292	254	129	213	140	250	50	74	107	90	82	96	85	95
Ru	101	Std	0.57	-	-	-	-	-	-	-	-	1	122	137	38	103	75	79	50
Rh	103	Std	0.08	-	-	-	-	-	-	-	-	1	68	91	77	90	94	93	116
Pd	105	Std	0.20	-	-	-	-	-	-	-	-	1	124	77	75	92	176	145	148
Ag	107	Std	0.67	-	-	-	-	-	-	-	-	50	97	102	97	98	97	103	96
Cd	111	Std	1.25	150	127	133	132	129	124	139	138	50	105	95	91	97	93	88	89

Note: (Number) refers to uncertified value provided by the supplier for information only.

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Element	Selected mass	Mode of ORS	LOD (ng/g)	ZC 73013 Spinach Assigned Value (ng/g)	Recovery (%) 17-Mar-11	13-Apr-11	14-Apr-11	05-May-11	13-May-11	18-May-11	19-May-11	Blank Spike Spike Amount (ng)	Recovery (%) 17-Mar-11	13-Apr-11	14-Apr-11	05-May-11	13-May-11	18-May-11	19-May-11
Sn	118	Std	3.88	-	-	-	-	-	-	-	-	50	97	110	109	98	100	97	98
Sb	121	Std	1.28	43	81	82	73	69	79	78	72	50	94	100	94	95	98	95	98
Te	125	Std	2.75	-	-	-	-	-	-	-	-	50	97	92	108	85	98	85	95
Cs	133	Std	0.17	130	93	88	91	89	96	89	94	50	96	99	96	99	93	98	96
Ba	137	Std	3.13	9000	82	85	81	79	95	81	91	50	101	100	103	101	91	97	89
La	139	Std	0.10	350	77	68	75	78	82	76	55	1	94	106	95	95	99	79	134
Ce	140	Std	0.13	660	76	77	71	79	81	100	91	1	98	107	100	108	113	71	89
Pr	141	Std	0.04	75	80	83	103	69	92	93	113	1	107	106	76	119	102	84	74
Nd	146	Std	0.22	280	51	62	88	70	123	108	91	1	175	139	90	116	83	81	85
Sm	147	Std	0.22	56	70	81	76	95	97	69	66	1	107	94	98	90	71	125	138
Eu	153	Std	0.05	11.1	94	135	85	79	80	97	74	1	98	72	95	106	102	96	117
Gd	157	Std	0.17	54	145	82	87	68	96	130	85	1	58	103	100	114	87	60	104
Tb	159	Std	0.02	7.2	72	74	75	69	92	84	72	1	114	104	98	103	96	91	113
Dy	163	Std	0.15	41	59	83	90	67	53	54	102	1	112	90	75	97	137	135	76
Ho	165	Std	0.03	8.1	79	66	68	72	71	74	70	1	89	106	93	95	107	98	94
Er	166	Std	0.08	17	82	74	99	83	89	82	112	1	93	123	93	113	112	119	78
Tm	169	Std	0.02	3.1	53	74	71	62	63	62	68	1	97	95	88	111	102	99	96
Yb	172	Std	0.08	19	94	70	63	83	49	98	64	1	69	88	115	74	139	76	110
Lu	175	Std	0.02	3	64	58	51	62	56	61	58	1	89	118	97	87	94	105	92
Hf	178	Std	0.12	(40)	14	17	23	16	16	16	25	1	116	102	86	94	105	116	74
Ta	181	Std	2.00	-	-	-	-	-	-	-	-	50	16	27	26	22	32	31	32
W	182	Std	0.95	-	-	-	-	-	-	-	-	50	14	93	94	30	106	102	91
Re	185	Std	0.06	-	-	-	-	-	-	-	-	50	96	99	100	101	106	100	95
Os	189	Std	0.32	-	-	-	-	-	-	-	-	50	38	15	34	63	86	26	27
Ir	193	Std	0.06	-	-	-	-	-	-	-	-	1	81	95	108	125	104	78	105
Pt	195	Std	0.28	-	-	-	-	-	-	-	-	1	84	25	128	132	45	104	122
Au	197	Std	0.08	-	-	-	-	-	-	-	-	1	26	36	29	24	10	36	45
Hg	202	Std	0.45	20	119	118	111	120	115	121	123	50	93	99	96	94	99	94	90
Tl	205	Std	0.13	(49)	77	80	74	83	74	80	78	50	98	100	100	96	106	98	95
Pb	208	Std	1.96	11100	82	92	86	88	86	92	94	50	104	107	104	99	101	99	97
Bi	209	Std	0.13	13.5	101	81	79	82	78	82	89	50	96	106	99	102	105	97	94
Th	232	Std	0.05	114	72	71	76	66	76	82	69	1	105	115	84	114	107	96	99
U	238	Std	0.08	89	81	80	75	83	78	83	84	50	97	103	100	99	105	99	93

Note: (Number) refers to uncertified value provided by the supplier for information only.

Note: The method LOD was obtained from the median LOD from all the analysis batches. The LOD for each element from each analysis batch was calculated from the multiplication of the standard deviation of all the blanks for each element by 3 and divided by the sample weight used.

Appendix II Detailed listing of the rice samples used in the study.

Sample ID no.	Brand	Rice type	Country of origin (from package)	Country of purchase	Specific place of purchase
1	Golden Phoenix	Thai Hom Mali Rice	Thailand	Singapore	Chye Choon Foods
2	Golden Twin Lion	Mixed Fragrant Rice (Jasmine rice min 80% & white rice max 20%)	Thailand	Singapore	Chye Choon Foods
3	Golden Royal Palace	Thai Hom Mali Rice	Vietnam	Singapore	Chye Choon Foods
4	Golden Lion King	Premium Thai Hom Mali Fragrant Rice	Thailand	Singapore	Chye Choon Foods
5	Golden Blossom	Thai Hom Mali Fragrant Rice	Thailand	Singapore	Chye Choon Foods
6	Kai	Koshihikari Short Grain Japonica Rice	China	Singapore	Chye Choon Foods
7	Sun West	Calrose Rice	Vietnam	Singapore	Chye Choon Foods
8	Peacock	Jasmine Rice	Vietnam	Singapore	Chye Choon Foods
9	Blue Anchor	Thai Fragrant White Rice	Thailand	Singapore	Chye Choon Foods
10	Golden Flying Horse	Fragrant White Rice	Vietnam	Singapore	Chye Choon Foods
11	Golden Prosperity	Thai Hom Mali Fragrant Rice	Thailand	Singapore	Chye Choon Foods
12	Anchor	India Ponni Rice	India	Singapore	Chye Choon Foods
13	Anchor	Parboiled Rice	Thailand	Singapore	Chye Choon Foods
14	Vita	Hananomai Short Grain Japonica Rice	Vietnam	Singapore	Chye Choon Foods
15	Vita	Premium Brown Rice	Vietnam	Singapore	Chye Choon Foods
16	Peacock	Fragrant Basmati Rice	Pakistan	Singapore	Chye Choon Foods
17	Golden Leaf	Thai Supreme Hom Mali Fragrant Rice	Thailand	Singapore	Chye Choon Foods
18	Green Double Lion	Broken White Rice (Old Crop)	Thailand	Singapore	Chye Choon Foods
19	Anchor	Broken White Rice	Vietnam	Singapore	Chye Choon Foods
20	IRRI-6	Broken Rice	Pakistan	Singapore	Chye Choon Foods
21	Zeera (BBB)	Broken Rice	Myanmar	Singapore	Chye Choon Foods
22	Golden Pineapple	Basmati Rice	Pakistan	Singapore	Cold Storage
23	Songhe	Thai Hom Mali Rice (New Crop)	Thailand	Singapore	Cold Storage
24 *	三好米	特級精米	Taiwan	Taiwan	Wellcome
25 *	御膳米	粳白米	Taiwan	Taiwan	7 Eleven

* English translation was unavailable on the rice packaging

Sample ID no.	Brand	Rice type	Country of origin (from package)	Country of purchase	Specific place of purchase
26 *	中华米	顶级越光米 (Koshihikari Rice)	Taiwan	Taiwan	Wellcome
27 *	御膳米	粳白米	Taiwan	Taiwan	7 Eleven
28 *	Cap Dua Udang	Beras Super Special (Tempatan)	Malaysia	Malaysia	-
29 *	Jasmine	Beras Super Special Tempatan	Malaysia	Malaysia	-
30 *	Cap Utara	Super Special Tempatan	Malaysia	Malaysia	-
31 *	Jati	Beras Super Special	Malaysia	Malaysia	-
32 *	Xuite	蓬莱米	Taiwan	Singapore	First Emporium
33 *	钟爱一生	飞燕米	Taiwan	Singapore	First Emporium
34	Mamalov	Fragrant Rice	Unknown	Singapore	First Emporium
35	Paddy Land	Crystalline Fragrant Rice	Thailand	Singapore	First Emporium
36	Golden Wheel	Mixed Fragrant Rice (min 80% Thai Hom Mali Rice & max 20% white rice)	Thailand	Singapore	First Emporium
37	Akita Komachi	Japonica Rice	Vietnam	Singapore	Yue Hwa
38 *	中兴米	花东台粳九号	Taiwan	Singapore	First Emporium
39 *	一桶金	Superior Fragrant Rice	Thailand	Singapore	First Emporium
40 *	天禾	东北大米	China	Singapore	Yue Hwa
41 *	新屋	芋香米	Taiwan	Singapore	First Emporium
42 *	米屋	台粳九号	Taiwan	Singapore	First Emporium
43 *	松花江	珍珠米	China	Singapore	First Emporium
44	Double FP 双虹	Thai Hom Mali Rice	Thailand	Singapore	NTUC
45	Songhe	Thai Hom Mali Rice	Thailand	Singapore	NTUC
46	Royal Umbrella	Thai Hom Mali Rice	Thailand	Singapore	NTUC
47	Double FP 双飞	Thai Hom Mali Rice	Thailand	Singapore	Carrefour
48	Red Ruby	Short Grain Rice	Myanmar	Singapore	Peninsular Plaza
49	Super Q	Fragrant White Rice	Thailand	Singapore	Carrefour
50	Agir Carrefour Bio Riz Thai	Thai Rice Long Superior (Organic)	France	Singapore	Carrefour

* English translation was unavailable on the rice packaging

Sample ID no.	Brand	Rice type	Country of origin (from package)	Country of purchase	Specific place of purchase
51	New Moon	Thai Hom Mali Rice (New Crop)	Thailand	Singapore	Carrefour
52	Golden Phoenix	Thai Hom Mali Rice (New Crop)	Thailand	Singapore	Carrefour
53	Heavenly	Grade A Thai Hom Mali Rice (New Crop)	Thailand	Singapore	Carrefour
54	Good Rice	Gold Rice	Korea	Singapore	Carrefour
55	Koala	Super Premium Japonica Rice	Unknown	Singapore	Carrefour
56	Gallo	Italian Long Grain Rice	Italy	Singapore	Carrefour
57	Diamond G	Calrose Rice (New Crop)	USA	Singapore	Carrefour
58	Shokaku	Premium Rice	USA	Singapore	Carrefour
59	ecoBrown's	Unpolished Brown Rice	Malaysia	Singapore	Carrefour
60	New Moon	Thai Hom Mali Rice (Organic)	Thailand	Singapore	Carrefour
61	Diamond G	California Koshihikari Super Premium Rice	USA	Singapore	Carrefour
62	Kangaroo Brand	Premium Calrose Rice	Australia	Singapore	Carrefour
63	Paddy King	Premium Thai Hom Mali Fragrant Rice	Thailand	Singapore	Carrefour
64	Super Q	Premium Thai Hom Mali Jasmine Rice	Thailand	Singapore	Carrefour
65	Paddy King	Premium Calrose Rice	USA	Singapore	Carrefour
66	Paddy King	Premium Sushi Rice (Koshihikari variety of Japan)	USA	Singapore	Carrefour
67	Heavenly	Thai Hom Mali Standard Rice	Thailand	Singapore	Carrefour
68	New Moon	Mixed Thai Hom Mali Rice (80% Thai Hom Mali Rice & 20% Thai White Rice)	Thailand	Singapore	Carrefour
69	Carrefour	Premium Thai Hom Mali Fragrant Rice	Thailand	Singapore	Carrefour
70	Carrefour	Thai White Rice	Thailand	Singapore	Carrefour
71	Heavenly	Premium Thai Hom Mali Fragrant Rice	Thailand	Singapore	Carrefour
72	Harmuni	Thai Hom Mali Rice	Thailand	Singapore	Carrefour
73	Jin Huang	Thai Hom Mali Rice	Thailand	Singapore	Carrefour
74	Golden Rooster	Thai Hom Mali Rice	Thailand	Singapore	Carrefour
75	Harmuni	Premium Thai Rice	Thailand	Singapore	Carrefour

Sample ID no.	Brand	Rice type	Country of origin (from package)	Country of purchase	Specific place of purchase
76	Golden Peony	Thai Hom Mali Rice	Thailand	Singapore	Carrefour
77	New Moon	Thai Hom Mali Rice (Brown Rice)	Thailand	Singapore	Carrefour
78 *	纯情米	Short Grain Rice	Japan	Singapore	Carrefour
79 *	岩手	江利金札米	Japan	Singapore	Carrefour
80	Carrefour Discount	Riz Long Grain	France	Singapore	Carrefour
81	Golden Peony	Thai Hom Mali Rice (Brown Rice)	Thailand	Singapore	Carrefour
82	New Moon	Premium Red Cargo Rice	Thailand	Singapore	Carrefour
83	Diamond G	Brown Rice	USA	Singapore	Carrefour
84	Earth Komachi	Japanese Short Grain Rice	Japan	Singapore	Carrefour
85 *	国宝	Kokuho Rice	USA	Singapore	Carrefour
86	Golden Pineapple	Thai Hom Mali Rice	Thailand	Singapore	Carrefour
87	AAA	Thai Fragrant White Rice	Thailand	Singapore	Carrefour
88	Golden Eagle	Thai Hom Mali Rice	Thailand	Singapore	Carrefour
89	Golden Eagle	Thai Fragrant White Rice (New Crop)	Thailand	Singapore	Carrefour
90	Paddy King	Premium Thai Hom Mali Fragrant Rice	Thailand	Singapore	Carrefour
91	AAA	Thai White Rice	Thailand	Singapore	Carrefour
92	Koshihikari	Koshihikari Super Premium Rice	Vietnam	Singapore	Carrefour
93	Maxwill Gold	Jasmine Fragrant Rice	Unknown	Singapore	Carrefour
94	Fairprice	Thai Fragrant White Rice	Thailand	Singapore	NTUC
95	Fairprice	Thai White Rice	Thailand	Singapore	NTUC
96	Fairprice Gold	Thai Hom Mali Superior Fragrant Rice	Thailand	Singapore	NTUC
97	Fairprice	Jasmine Fragrant Rice	Vietnam	Singapore	NTUC
98	Fairprice	Japonica Rice	Vietnam	Singapore	NTUC
99	Fairprice	Basmati Rice	Pakistan	Singapore	NTUC
100	Fairprice	Brown Unpolished Rice	Thailand	Singapore	NTUC

* English translation was unavailable on the rice packaging

Sample ID no.	Brand	Rice type	Country of origin (from package)	Country of purchase	Specific place of purchase
101	Fairprice	Red Unpolished Rice	Thailand	Singapore	NTUC
102	Fairprice	Healthy Rice (80% fragrant rice, 10% red & 10% brown unpolished rice)	Thailand	Singapore	NTUC
103	Temasek Gold	Basmati Rice	Pakistan	Singapore	NTUC
104	Fairprice	Calrose Rice	Australia	Singapore	NTUC
105	Golden Royal Dragon	Mixed Thai Hom Mali Rice (80% Thai Hom Mali Rice & 20% Thai White Rice)	Thailand	Singapore	NTUC
106	Fairprice	Ponni Rice Parboiled	India	Singapore	NTUC
107	Naturel	Hom Mali Brown Rice (Organic)	Thailand	Singapore	NTUC
108	Budget	Long Grain White Rice AAA	Vietnam	Singapore	NTUC
110	Songhe	Healthy Rice (50% brown unpolished rice & 50% red unpolished rice)	Unknown	Singapore	Cold Storage
111	Songhe	Red Unpolished Rice (Healthy Rice)	Unknown	Singapore	Cold Storage
112	御品皇 (Yu Pin King)	Thai Hom Mali Rice	Thailand	Singapore	Cold Storage
113	No Frills	Thai Fragrant Rice	Thailand	Singapore	Cold Storage
114	Koala	Super Premium Japonica Rice	Unknown	Singapore	Cold Storage
115	Fairprice	Glutinous Rice	Thailand	Singapore	NTUC
116	Uncle Ben's	Whole Grain Parboiled Brown Rice	USA	Singapore	Market Place
117	Fantastic	Basmati Rice	India	Singapore	Market Place
118	Guard	Basmati Rice	Pakistan	Singapore	Cold Storage
119	Tsuru	Calrose Rice	USA	Singapore	Market Place
120	Akita-San Shimai Akitamachi	Japanese Rice	Japan	Singapore	Market Place
121	First Choice	Thai Hom Mali Rice	Thailand	Singapore	Market Place
122	Koshihikari USA	Imperial Quality Short Grain Rice	USA	Singapore	Market Place
123	Nigatasan Shinmai Koshihikari	Japanese Rice	Japan	Singapore	Market Place
124	Ja-Rice	Hinohikari Japanese Rice	Japan	Singapore	Meidi-Ya
125	Ja-Rice	Akitakomachi Japanese Rice	Japan	Singapore	Meidi-Ya
126	California Akita Komachi Genmai	Superior Organic BrownRice	USA	Singapore	Meidi-Ya

Sample ID no.	Brand	Rice type	Country of origin (from package)	Country of purchase	Specific place of purchase
127	Koshihikari	Premium Grade Koshihikari Rice	Vietnam	Singapore	Meidi-Ya
128	Bamboo Hill	Thai Hom Mali Rice	Thailand	Singapore	Meidi-Ya
129	Meidi-Ya	Koshihikari Rice (Japonica Rice)	Vietnam	Singapore	Meidi-Ya
130	Fancl	Germinated Brown Rice	Japan	Singapore	Meidi-Ya
131	Niigata Oeaachan No Osusume Koshihikari	Japanese Short Grain Rice	Japan	Singapore	Meidi-Ya
132	Koshihikari	Koshihikari Rice	USA	Singapore	Meidi-Ya
133	Kamada Niigata Wataboshi	Japanese Sweet Rice	Japan	Singapore	Meidi-Ya
134	Ja-Rice	Tokushima-Koshihikari Rice	Japan	Singapore	Meidi-Ya
135	Iwate Hitomebore	Japonica Rice	Japan	Singapore	Meidi-Ya
136	Ja-Rice	Aomori-Tsugaru Roman Rice	Japan	Singapore	Meidi-Ya
137	Kitoku Shinryo	Japanese Rice	Japan	Singapore	Meidi-Ya
138	Seimind	Mitsuhikari (Japonica White Rice)	Japan	Singapore	Meidi-Ya
139	Ja-Rice	Nanatsuboshi Japanese Rice	Japan	Singapore	Meidi-Ya
140	Ja-Rice	Niigata Koshihikari Japanese Rice	Japan	Singapore	Meidi-Ya
141	Ja-Rice	Akita-Hitomebore Rice	Japan	Singapore	Meidi-Ya
142	Seimind	Kinuhikari (Unpolished Japonica Brown Rice)	Japan	Singapore	Meidi-Ya
143	Lavorazione Artigianale (Rustichella d'abruzzo)	Riso Superfino - Arborio	Italy	Singapore	Meidi-Ya
144	Lavorazione Artigianale (Rustichella d'abruzzo)	Riso Superfino - Carnaroli	Italy	Singapore	Meidi-Ya
145	Lavorazione Artigianale (Rustichella d'abruzzo)	Riso Superfino - Vialone Nano	Italy	Singapore	Meidi-Ya
146	Just	Organic Brown Rice	Australia	Singapore	Meidi-Ya
147	Niigata Tochio Koshi Hikari	Japanese Short Grain Rice	Japan	Singapore	Meidi-Ya
148	Kameda Niigata Kkoshiibuki	Niigata Japanese Short Grain Rice	Japan	Singapore	Meidi-Ya
149	Tamaki Classic	California Premium Short Grain	USA	Singapore	Meidi-Ya
150	Tamaki Gold	California Koshihikari Short Grain	USA	Singapore	Meidi-Ya
151	Akita Komachi	Akita Komachi Rice	USA	Singapore	Meidi-Ya

Sample ID no.	Brand	Rice type	Country of origin (from package)	Country of purchase	Specific place of purchase
152	Seimind	Koshihikari (Unpolished Japonica Brown Rice)	Japan	Singapore	Meidi-Ya
153	Kameda Seika Uonuma Koshiibuki	Japanese Rice	Japan	Singapore	Meidi-Ya
154	Niigata Koshihikari Musenmai	Japanese Rice	Japan	Singapore	Meidi-Ya
155	Bamboo Hill	Organic Thai Hom Mali Rice	Thailand	Singapore	Meidi-Ya
156	Bamboo Hill	Organic Thai Hom Mali Brown Rice	Thailand	Singapore	Meidi-Ya
157	Kitoku Shinryo	Japanese Rice	Japan	Singapore	Meidi-Ya
158	Tamanishiki	Super Premium Short Grain Rice	USA	Singapore	Meidi-Ya
159	Seimind	Kinumusume (Unpolished Japonica Brown Rice)	Japan	Singapore	Meidi-Ya
160	Lundberg	Organic Long Grain Brown Rice	USA	Singapore	Meidi-Ya
162	Divella	Riso Superfino - Arborio	Italy	Singapore	Meidi-Ya
163	Gibbs	Wild Rice	USA	Singapore	Meidi-Ya
164	Maharani	Diabetics Basmati Rice (Processed Parboiled Rice)	India	Singapore	Mustafa
165	Charminar	Basmati Rice	India	Singapore	Mustafa
166	Kohinoor	Extra Long Parboiled Basmati Rice	India	Singapore	Mustafa
167	National	Basmati Rice	Pakistan	Singapore	Mustafa
168	Tilda	Pure Basmati Rice	India	Singapore	Mustafa
169	Golden Chhap	Aged Basmati Rice	India	Singapore	Mustafa
170	Chanda	Basmati Rice	Pakistan	Singapore	Mustafa
171	Red Sun	Thai Hom Mali Rice (Thai Jasmine Rice)	Thailand	Singapore	Mustafa
172	Qilla	Premium Basmati Rice	India	Singapore	Mustafa
173	Lehar	Indian Basmati Rice	India	Singapore	Mustafa
174	Aeroplane La-Taste	Indian Basmati Rice	India	Singapore	Mustafa
175	Daawat	Basmati Rice	India	Singapore	Mustafa
176	Guard	Basmati Rice	Pakistan	Singapore	Mustafa
177	Okome	Shortgrain Rice	USA	Singapore	Mustafa

Sample ID no.	Brand	Rice type	Country of origin (from package)	Country of purchase	Specific place of purchase
178	Trophy Royale	Basmati Rice	India	Singapore	Mustafa
179	Kohinoor	Basmati Rice	India	Singapore	Mustafa
180	Uncle Cook's	Parboiled Ponni Rice	India	Singapore	Mustafa
181	Pattu	Broken Rice	Malaysia	Singapore	Mustafa
182	Maharani	Parboiled Rice for Diabetics	India	Singapore	Mustafa
183	Trophy Gold	Basmati Rice	India	Singapore	Mustafa
184	Laziza International	Super Kernel Basmati Rice	Pakistan	Singapore	Mustafa
185	India Gate	Brown Basmati Rice	India	Singapore	Mustafa
186	India Gate	Classic Basmati Rice	India	Singapore	Mustafa
187	Dragonfly	Organic Black Rice	Thailand	Singapore	Mustafa
188	Chanda	Premium Basmati Rice	Pakistan	Singapore	Mustafa
189	Kohinoor	Extra Long Basmati Rice	India	Singapore	Mustafa
190	Sun Rice	Doongara Clever Rice (For Diabetics)	Australia	Singapore	Mustafa
191	India Gate	Premium Basmati Rice	India	Singapore	Mustafa
192	Gold Seal Indus Valley	Indian Basmati Rice	India	Singapore	Mustafa
193	Maharani	Traditional Premium Basmati Rice	India	Singapore	Mustafa
194	Lal Qilla	Traditional Royal Basmati Rice	India	Singapore	Mustafa
195	Anarkali	Basmati Rice	Pakistan	Singapore	Mustafa
196	Nishiki	Musenmai Rice (New Crop)	USA	Singapore	Yamakawa
197	Two Coconut Trees	Rice	Pakistan	Singapore	Sheng Siong
198	India Gate	Basmati Rice Super (1 Year Old Aged Rice)	India	Singapore	Roshan
199	Daawat	Basmati Rice	India	Singapore	Roshan
200	Chang	Thai Organic Hom Mali Brown Rice	Thailand	Singapore	Sheng Siong
201	Big Bridge Rice	Sushi Rice (台粳9号）	Taiwan	Singapore	Sheng Siong
202	Roshan	Basmati Rice	India	Singapore	Roshan

Sample ID no.	Brand	Rice type	Country of origin (from package)	Country of purchase	Specific place of purchase
203	Roshan	Raw Rice	India	Singapore	Roshan
204	Malabar	Basmati Rice	Pakistan	Singapore	Sheng Siong
205	Nur Jahan	Basmati Rice	India	Singapore	Roshan
206	Raj	White Parboiled Ponni Rice	India	Singapore	Roshan
207	Flying Man	Crystalline Fragrant Rice	Thailand	Singapore	Tan Minimart
208	Happy Family	Whole Grain Rice	Thailand	Singapore	Sheng Siong
209	Nang Rum Thai	Thai Hom Mali Rice	Thailand	Singapore	Sheng Siong
210	No. 717	Pure Kernel Basmati Rice	Pakistan	Singapore	Sheng Siong
211	Roshan	Idli Rice	Unknown	Singapore	Roshan
212	Golden Knife	White Rice	Vietnam	Singapore	Roshan
213	Golden Knife	Fragrant Rice	Vietnam	Singapore	Roshan
214	Good Rice	Gold Rice	Korea	Singapore	Seoul Mart
215	Barkat Premium Basmati Rice	Basmati Rice	Pakistan	Provided by FERA	
216	Shhaha Mahifiz Basmati Rice No.2	Basmati Rice	Pakistan	Provided by FERA	
217	Shhaha Mahifiz Basmati Rice No.3	Basmati Rice	Pakistan	Provided by FERA	
218	Tazaki 'Akitokomachi'	Short Grain Rice	Spain	Provided by FERA	
219	Itochu 'Kirara'	Short Grain Rice	China	Provided by FERA	
220	Veetee 'Lido' (gunstones Standard)	Short Grain Rice	France	Provided by FERA	
221	Okura 'Akitomachi'	Short Grain Rice	Spain	Provided by FERA	
222	Nichimen 'Zen'	Short Grain Rice	Japan	Provided by FERA	
223	Suntra 'Guadiamar'	Brown Milled Rice	Spain	Provided by FERA	

List of abbreviations and units

‰	Per mil
3-PGA	3-phosphoglycerate
ATP	Adenosine triphosphate
BSIA	Bulk stable isotope analysis
ca.	Circa
CDA	Canonical discriminant analysis
CDT	Cañon Diabolo Troilite
CE	Capillary electrophoresis
CRDS	Cavity ring down spectroscopy
DNA	Deoxyribonucleic acid
DPW	Deepwater ecology
EA	Elemental analyser
EC	European Council
EU	European Union
FAO	Food and Agriculture Organisation
FERA	Food and Environment Research Agency

FIT-PTS   Food analysis using Isotopic Techniques Proficiency Testing Scheme

FSA   Food Standards Agency

GC   Gas chromatography

GI   Glycaemic index

HPLC   High performance liquid chromatography

Hz   Hertz

i.d.   Internal diameter

IAEA   International Atomic Energy Agency

ICP-HRMS   Inductively coupled plasma high resolution mass spectrometry

ICP-MS   Inductively coupled plasma mass spectrometry

ICP-OES   Inductively coupled plasma optical emission spectrometry

IIASA   International Institute for Applied Systems Analysis

IR   Infrared

IRMS   Isotope ratio mass spectrometry

IRR   Irrigated ecology

IRRI   International Rice Research Institute

LC   Liquid chromatography

LOD   Limit of detection

m/z	Mass-to-charge ratio
WL	Meteoric water line
N.A.	Not applicable
NADPH	Nicotinamide adenine dinucleotide phosphate
NBS	National Bureau of Standards
NIST	National Institute for Standards and Technology
NMR	Nuclear magnetic resonance
ORS	Octopole reaction system
PCA	Principal component analysis
PDB	Pee Dee Belemnite
PDO	Protected designation of origin
PGI	Protected geographical indication
PTR-MS	Proton transfer reaction mass spectrometry
rf	Radio frequency
RFL	Rainfed lowland ecology
RPP	Reductive pentose phosphate
SMOW	Standard Mean Ocean Water
TDL	Tidal wetland ecology

TSG	Traditional specialty guaranteed
UK	United Kingdom
UPL	Upland ecology
USA	United States of America
VCDT	Vienna Cañon Diabolo Troilite
VPDB	Vienna Pee Dee Belemnite
VSMOW	Vienna Standard Mean Ocean Water

List of References

Abu-Samra A., Morris J. S. & Koirtyohann S. R. (1975). Wet ashing of some biological samples in a microwave oven. *Analytical Chemistry*, 47, 1475-1477.

Alloway B. J. (2008). Zinc in soils and crop nutrition. International Zinc Association of Brussels and International Fertiliser Industry Association of Paris. Available at http://www.fertilizer.org/ifa/HomePage/LIBRARY/Our-selection2/Fertilizer-use.html. Accessed on 17 August 2011.

Anderson K. A. & Smith B. W. (2002). Chemical profiling to differentiate geographic growing origins of coffee. *Journal of Agricultural and Food Chemistry*, 50, 2068-2075.

Angerosa F., Breas O., Contento S., Guillou C., Reniero F. & Sada E. J. (1999). Application of stable isotope ratio analysis to the characterisation of the geographical origin of olive oils. *Journal of Agricultural and Food Chemistry*, 47, 1013-1017.

Aprea E., Carlin S., Versini G., Märk T. D. & Gasperi F. (2007). Assessing truffle origin by PTR-MS fingerprinting and comparison with GC-MS for the headspace analysis. 3rd International Conference on Proton Transfer Reaction Mass Spectrometry and its Application. Available at http://www.ptrms.com/downloads/PTRMS_Conference2007_Web.pdf.!Accessed on 26 March 2011.

Arana I., Jaren C. & Arazuri S. (2005). Maturity, variety and origin determination in white grapes (*Vitis Vinifera* L.) using near infrared reflectance technology. *Journal of Near Infrared Spectroscopy*, 13, 349-357.

Aras N. K. & Ataman O. Y. (2006). *Trace Element Analysis of Food and Diet*. Cambridge: The Royal Society of Chemistry, Chapter 7, 136-163.

Ariyama K., Nishida T., Noda T., Kadokura M. & Yasui A. (2006). Effects of fertilisation, crop year, variety, and provenance factors on mineral concentrations in onions. *Journal of Agricultural and Food Chemistry*, 54, 3341-3350.

Ariyama K., Aoyama Y., Mochizuki A., Homura Y., Kadokura M. & Yasui A. (2007). Determination of the geographic origin of onions between three main production areas in Japan and other countries by mineral composition. *Journal of Agricultural and Food Chemistry*, 55, 347-354.

Asher C. J. (1978). Natural and synthetic culture media for spermatophytes. In M. J. Rechigl (Ed.), *CRC Handbook Series in Nutrition and Food*, vol 3, (pp. 575-609). USA: CRC Press Inc.

Australian Government Bureau of Meteorology. (2011). Glossary. Available at http://www.bom.gov.au/lam/glossary/wpagegl.shtml. Accessed on 26 July 2011.

Bahar B., Schmidt O., Moloney A. P., Scrimgeour C. M., Begley I. S. & Monahan F. J. (2008). Seasonal variation in the C, N and S stable isotope composition of retail organic and conventional Irish beef. *Food Chemistry*, 106, 1299-1305.

Barber S. A. (1995). *Soil Nutrient Bioavailability. A Mechanistic Approach*. (2nd ed.). New York: John Wiley & Sons, Inc., Chapter 4, 85-109.

Bateman A. S. & Kelly S. D. (2007). Fertiliser nitrogen isotope signatures. *Isotopes in Environmental and Health Studies*, 43, 237-247.

Bellí N., Marín S., Duaigües A., Ramos A. J. & Sanchis V. (2002). Ochratoxin A in wines, musts and grape juices from Spain. *Journal of the Science of Food and Agriculture*, 84, 591-594.

Bender M. M. & Berge A. J. (1979). Influence of N and K fertilisation and growth temperature on $^{13}C/^{12}C$ ratios of Timothy (*Phleum pratense* L.). *Oecologia*, 44, 117-118.

Bonizzi I., Feligini M., Aleandri R. & Enne G. (2006). Genetic traceability of the geographical origin of typical Italian water buffalo Mozzarella cheese: A preliminary approach. *Journal of Applied Microbiology*, 102, 667-673.

Bowen G. (2011). OIPC for Google Earth. Available at http://wateriso.eas.purdue.edu/waterisotopes/pages/data_access/da_main.html. Accessed on 30 July 2011.

Branch S., Burke S., Evans P., Fairman B. & Wolff Briche C. S. J. (2003). A preliminary study in determining the geographical origin of wheat using isotope ratio inductively coupled plasma mass spectrometry with ^{13}C, ^{15}N mass spectrometry. *Journal of Analytical Atomic Spectrometry*, 18, 17-22.

Brand W. A. (2007). Mass spectrometer hardware for analysing stable isotope ratios. In P. A. De Groot (Ed.), *Handbook of Stable Isotope Analytical Techniques* (pp. 835-856). Amsterdam: Elsevier Inc.

Brand W. A., Huang L., Mukai H., Chivulescu A., Richter J. M. & Rothe M. (2009). How well do we knoe VPDB? Variability of $\delta^{13}C$ and $\delta^{18}O$ in CO_2 generated from NBS19-calcite. *Rapid Communications in Mass Spectrometry*, 23, 915-926.

Breas O., Guillou C., Reniero F., Sada E. & Angerosa F. (1998). Oxygen-18 measurement by continuous flow pyrolysis/isotope ratio mass spectrometry of vegetable oils. *Rapid Communications in Mass Spectrometry*, 12, 188-192.

Brescia M. A., Košir I. J., Caldarola V., Kidrič J. & Sacco A. (2003). Chemometric classification of Apulian and Slovenian wines using ^1H NMR and ICP-OES together with HPICE data. *Journal of Agricultural and Food Chemistry*, 51, 21-26.

Brescia M. A., Monfreda M., Buccolieri A. & Carrino C. (2005). Characterisation of the geographical origin of buffalo milk and mozzarella cheese by means of analytical and spectroscopic determinations. *Food Chemistry*, 89, 139-147.

Brooks J. R., Buchmann N., Phillips S., Ehleringer B., Evans R. D., Lott M. Martinelli L. A., Pockman W. T., Sandquist D., Sparks J. P., Sperry L., Williams D. & Ehleringer J. R. (2002). Heavy and light beer: a carbon isotope approach to detect C_4 carbon in beers of different origins, styles, and prices. *Journal of Agricultural and Food Chemistry*, 50, 6413-6418.

Butt M. S., Anjum F. M., Salim-ur-Rehman, Tahir-Nadeem M., Sharif M. K. & Anwer M. (2008). Selected quality attributes of fine basmati rice: effect of storage history and varieties. *International Journal of Food Properties*, 11, 698-711.

Buttery R. G., Ling L. C., Juliano B. O. & Turnbaugh J. G. (1983). Cooked rice aroma and 1-acetyl-1-pyrroline. *Journal of Agricultural and Food Chemistry*, 31, 823-826.

Calderone G. & Guillou C. (2008). Analysis of isotopic ratios for the detection of illegal watering of beverages. *Food Chemistry*, 106, 1399-1405.

California Rice Commission. (2010). California rice: master preparation through world flavors. Available at http://www.calrice.org/pdf/ACF-Presentation.pdf. Accessed on 8 February 2011.

Chesson L. A., Valenzuela L. O., O'Grady S. P., Cerling T. E. & Ehleringer J. R. (2010). Hydrogen and oxygen stable isotope ratios of milk in the United States. *Journal of Agricultural and Food Chemistry*, 58, 2358-2363.

Codex Standard 198-1995. Codex standard for rice. Available at www.codexalimentarius.net/download/standards/61/CXS_198e.pdf. *Accessed on 11 February 2011.*

Coetzee P. P., Steffens F. E., Eiselen R. J., Augustyn O. P., Balcaen L. & Vanhaecke F. (2005). Multi-element analysis of South African wines by ICP-MS and their classification according to geographical origin. Journal of Agricultural and Food Chemistry, 53, 5060-5066.

Collomb M., Bütikofer U., Sieber R., Jeangros B. & Bosset J.-O. (2002). Composition of fatty acids in cow's milk fat produced in the lowlands, mountains and highlands of Switzerland using high-resolution gas chromatography. *International Dairy Journal*, 12, 649-659.

Cornell S., Rendell A. & Jickells T. (1995). Atmospheric inputs of dissolved organic nitrogen to the oceans. *Nature,* 376, 243-246.

Council of Agriculture Executive Yuan R.O.C. (Taiwan). AG. Statistics Yearbook. Available at http://eng.coa.gov.tw/htmlarea_file/web_articles/coa/12268/024.pdf. Accessed on 25 July 2011.

Craig H. (1961a). Standards for reporting concentrations of deuterium and oxygen-18 in natural waters. *Science,* 133, 1833-1834.

Craig H. (1961b). Isotopic variations in meteoric waters. *Science*, 133, 1702-1703.

Crittenden R. G., Andrew A. S., LeFournour M., Young M. D., Middleton H. & Stockmann R. (2007). Determining the geographic origin of milk in Australasia using multi-element stable isotope ratio analysis. *International Dairy Journal*, 17, 421-428.

D'Ilio S., Alessandrelli M., Cresti R., Forte G. & Caroli S. (2002). Arsenic content of various types of rice as determined by plasma-based techniques. *Microchemical Journal*, 73, 195-201.

Dass C. (2007). *Fundamentals of Contemporary Mass Spectrometry*. New Jersey: John Wiley & Sons, Inc., Chapter 7, 263-285.

Dawson T. E. & Ehleringer J. R. (1993). Isotopic enrichment of water in the "woody" tissues of plants: implications for plant water source, water uptake and other studies which use the stable isotopic composition of cellulose. *Geochimica et Cosmochimica Acta*, 57, 3487-3492.

Di Cagno R., Banks J., Sheehan L., Fox P. F., Brechany E. Y., Corsetti A. & Gobbetti M. (2003). Comparison of the microbiological, compositional, biochemical, volatile profile and sensory characteristics of three Italian PDO ewes' milk cheeses. *International Dairy Journal*, 13, 961-972.

Directorate General for Agriculture and Rural Development of the European Commission. What is organic farming? Available at http://ec.europa.eu/agriculture/organic/organic-farming/what-organic_en. Accessed on 26 July 2011.

Downey G., Mclntyre P. & Davies A. N. (2003). Geographic classification of extra virgin olive oils from the eastern Mediterranean by chemometric analysis of visible and near-infrared spectroscopic data. *Applied Spectroscopy*, 57, 158-163.

Dupuy N., Le Dréau Y., Ollivier D., Artaud J., Pinatel C. & Kister J. (2005). Origin of French virgin olive oil registered designation of origins predicted by chemometric analysis of synchronous excitation-emission fluorescence spectra. *Journal of Agricultural and Food Chemistry*, 53, 9361-9368.

Elflein L. & Raezke K.-P. (2008). Improved detection of honey adulteration by measuring differences between $^{13}C/^{12}C$ stable carbon isotope ratios of protein and sugar compounds with a combination of elemental analyser – isotope ratio mass spectrometry and liquid chromatography – isotope ratio mass spectrometry (δ^{13}C-EA/LC-IRMS). *Apidologie*, 39, 574-587.

Etièvant P., Schlich P., Cantagrel R., Bertrand M. & Bouvier J.-C. (1989). Varietal and geographic classification of French red wines in terms of major acids. *Journal of the Science of Food and Agriculture*, 46, 421-438.

FAO (Food and Agriculture Organisation of the United Nations). (2000). FAO rice information, volume 2. Available at http://www.fao.org/ag/AGP/agpc/doc/field/commrice/ricepdf/faorice.pdf. Accessed on 30 January 2011.

FAO (Food and Agriculture Organisation of the United Nations). (2002). Web LocClim, Local Monthly Climate Estimator. Available at http://www.fao.org/sd/locclim/srv/locclim.home. Accessed on 25 July 2011.

FAO (Food and Agriculture Organisation of the United Nations). (2004). International Year of Rice 2004. Available at http://www.fao.org/rice2004/en/world.htm. Accessed on 26 July 2011.

FAO (Food and Agriculture Organisation of the United Nations). (2007). SDdimensions. Available at http://www.fao.org/WAICENT/FAOINFO/SUSTDEV/EIdirect/CLIMATE/EIsp0002.htm. Accessed on 23 June 2011.

FAO (Food and Agriculture Organisation of the United Nations). (2008). FAO expects rice production to rise by 1.8 percent in 2008. FAO Newsroom. Available at http://www.fao.org/newsroom/en/news/2008/1000820/index.html. Accessed on 13 February 2011.

FAO (Food and Agriculture Organisation of the United Nations). (2011). Rice Market Monitor (RMM). Available at http://www.fao.org/economic/est/publications/rice-publications/rice-market-monitor-rmm/en/. Accessed on 13 February 2011.

Farquhar G. D. & Sharkey T. D. (1982). Stomatal conductance and photosynthesis. *Annual Review of Plant Physiology*, 33, 317-345.

Fay L. B. & Kussmann M. (2010). Mass spectrometry technologies. In L. B. Fay & M. Kussmann (Eds.) *Mass Spectrometry and Nutrition Research* (pp. 3-47) Cambridge: The Royal Society of Chemistry.

Francey R.J. & Farquhar G.D. (1982). An explanation of ^{13}C/^{12}C variations in tree rings. *Nature*, 297, 28-31.

Frías S., Trujillo J. P. P., Peña E. M. & Conde J. E. (2001). Classification and differentiation of bottled sweet wines of Canary Islands (Spain) by their metallic content. *European Food Research and Technology*, 213, 145-149.

Friberg L., Elinder C. & Kjellstrom T. (1992). Environmental Health Criteria 134, Cadmium. World Health Organisation, Geneva. Available at http://www.inchem.org/documents/ehc/ehc/ehc134.htm. Accessed on 16 August 2011.

FSA (Food Standards Agency). (2004). Survey on Basmati rice. Available at http://www.food.gov.uk/multimedia/pdfs/fsis4704basmati.pdf. Accessed on 14 February 2011.

Galle S. A., Koot A., Soukoulis C., Cappellin L., Biasioli F., Alewijn M. & van Ruth S. M. (2011). Typicality and geographical origin markers of protected origin cheese from The Netherlands revealed by PTR-MS. *Journal of Agricultural and Food Chemistry*, 59, 2554-2563.

Garnier J.-M., Travassaz F., Lenoble V., Rose J., Zheng Y., Hossain M. S., Chowdhury S. H., Biswas A. K., Ahmed K. M., Cheng Z. & van Geen A. (2010). Temporal variations in arsenic uptake by rice plants in Bangladesh: The role of iron plaque in paddy fields irrigated with groundwater. *Science of the Total Environment*, 408, 4185–4193.

Georgi M., Voerkelius S., Rossmann A., Graßmann J. & Schnitzler W. H. (2005). Multielement isotope ratios of vegetables from integrated and organic production. *Plant and Soil*, 275, 93-100.

Gilg A. & Battershill M. (1998). Quality farm food in Europe: a possible alternative to the industrialised food market and to current agrienvironmental policies: lessons from France. *Food Policy*, 23, 25-40.

Global Information and Early Warning System (GIEWS) on food and agriculture. Available at http://www.fao.org/giews/countrybrief/index.jsp. Accessed on 26 July 2011.

Global Times. (2010). Counterfeit Thai rice floods country. Available at http://business.globaltimes.cn/industries/2010-07/553062.html. Accessed on 14 February 2011.

Gohil H. L., Correll M. J. & Sinclair T. (2011). Predicting the effects of gas diffusivity on photosynthesis and transpiration of plants grown under hypobaria. *Advances in Space Research*, 47, 49-54.

Gómez-Ariza J. L., Arias-Borrego A. & García-Barrera T. (2006). Multielemental fractionation in pine nuts (*Pinus pinea*) from different geographic origins by size-exclusion chromatography with UV and inductively coupled mass spectrometry detection. *Journal of Chromatography A*, 1121, 191-199.

Gonfiantini R., Stichler W. & Rozanski K. (1993). Standards and intercomparison materials distributed by the International Atomic Energy Agency for stable isotope measurements. In IAEA-TECDOC-825 (pp 13-29). Available at http://www-naweb.iaea.org/napc/ih/documents/TECDOCS/TECDOC%200825%20Reference%20materials%20Intercomparison%201995.PDF. Accessed on 24 February 2011.

Gonzálvez A., Armenta S. & de la Guardia M. (2011). Geographical traceability of "Arròs de Valencia" rice grain based on mineral element composition. *Food Chemistry*, 126, 1254-1260.

Gremaud G. & Hilkert A. (2008). Isotopic-spectroscopic technique: stable isotope ratio mass spectrometry (IRMS). In D-W. Sun (Ed.), *Modern Techniques for Food Authentication* (pp. 269-320). Amsterdam: Elsevier Inc.

Grist D. H. (1975). *Rice*. (5th ed.). London: Longman Group Ltd, (Chapter 5).

Guadarrama A., Rodríguez-Méndez M. L., Sanz C., Ríos J. L. & de Saja J. A. (2001). Electronic nose based on conducting polymers for the quality control of the olive oil

aroma - Discrimination of quality, variety of olive and geographic origin. *Analytica Chimica Acta*, 432, 283-292.

Guo B. L., Wei Y. M., Pan J. R. & Li Y. (2010). Stable C and N isotope ratio analysis for regional geographical traceability of cattle in China. *Food Chemistry*, 118, 915-920.

Hattori R., Yamada K., Hasegawa K., Ishikawa Y., Ito Y., Sakamoto Y. & Yoshida N. (2008). An improved method for the measurement of the isotope ratio of ethanol in various samples, including alcoholic and non-alcoholic beverages. *Rapid Communications in Mass Spectrometry*, 22, 3410-3414.

Heaton K., Kelly S. D., Hoogewerff J. & Woolfe M. (2008). Verifying the geographical origin of beef: the application of multi-element isotope and trace element analysis. *Food Chemistry*, 107, 506-515.

Héberger K., Csomós E. & Simon-Sarkadi L. (2003). Principal component and linear discriminant analyses of free amino acids and biogenic amines in Hungarian wines. *Journal of Agricultural and Food Chemistry*, 51, 8055-8060.

Herber R. F. M. (1994). Cadmium. In H. G. Seiler, A. Sigel, & H. Sigel (Eds.), *Handbook on Metals in Clinical and Analytical Chemistry*, (Chapter 19). New York: Marcel Dekker, Inc.

Hernández C. V. & Rutledge D. N. (1994). Multivariate statistical analysis of gas chromatograms to differentiate cocoa masses by geographical origin and roasting conditions. *Analyst*, 119, 1171-1176.

Herruzo A.C. & Morote F. Regional advantages and economies of size in Spanish rice farming. Available at http://ressources.ciheam.org/om/pdf/c15-2/CI011052.pdf. Accessed on 25 July 2011.

Hintze K. J., Lardy G. P., Marchello M. J. & Finley J. W. (2001). Areas with high concentrations of selenium in the soil and forage produced beef with enhanced concentrations of selenium. *Journal of Agricultural and Food Chemistry*, 49, 1062-1067.

Hoefs J. (2009). *Stable Isotope Geochemistry*. (6th ed.). Berlin: Springer, Chapter 1, 1-34.

Hofstetter T. B. & Berg M. (2011). Assessing transformation processes of organic contaminants by compound-specific stable isotope analysis. *Trends in Analytical Chemistry*, 30, 618-627.

Hopkins W. G. & Hüner N. P. A. (2004). *Introduction to Plant Physiology*. (3rd ed.). USA: John Wiley & Sons, Inc., (Chapter 12).

Horacek M., Eisinger E. & Papesch W. (2010). Reliability of stable isotope values from meat juice for the determination of the meat origin. *Food Chemistry*, 118, 910-914.

Horwitz W. (1982). Evaluation of analytical methods used for regulation of foods and drugs. *Analytical Chemistry*, 54, 67-76.

Husted S., Mikkelsen B. F., Jensen J. & Nielsen N. E. (2004). Elemental fingerprint analysis of barley (*Hordeum vulgare*) using inductively coupled plasma mass spectrometry, isotope-ratio mass spectrometry, and multivariate statistics. *Analytical and Bioanalytical Chemistry*, 378, 171-182.

Iizuka K. & Aishima T. (1999). Differentiation of soy sauce by pattern recognition analysis of mid- and near-IR spectra. *Journal of Food Composition and Analysis*, 12, 197-209.

Ilbery B. & Kneafsey M. (1998). Product and place: promoting quality products and services in the lagging rural regions of the European Union. *European Urban and Regional Studies*, 5, 329-341.

International Business Times. (2011). China makes fake rice from plastic: report. Available at http://uk.ibtimes.com/articles/108582/20110203/china-makes-fake-rice-from-plastic-report.htm. Accessed on 14 February 2011.

IRRI (International Rice Research Institute). (1987). World Rice Statistics. Available at http://dspace.irri.org:8080/dspace/bitstream/10269/262/2/9711042045_content.pdf. Accessed on 26 July 2011.

IRRI (International Rice Research Institute). (2004). Rice fact sheets. Organic rice. Available at http://www.knowledgebank.irri.org/factsheetsPDFs/fs_organicRice.pdf. Accessed on 9 February 2011.

IRRI (International Rice Research Institute). (2006). Rice breeding course. Breeding program management. Available at http://www.knowledgebank.irri.org/ricebreedingcourse/Grain_quality.htm. Accessed on 6 February 2011.

IRRI (International Rice Research Institute). (2009). Rice knowledge bank. Available at http://irri.org/knowledge/training/knowledge-bank. Accessed on 4 February 2011.

IRRI (International Rice Research Institute). Available at http://irri.org/. Accessed on 30 January 2011.

Itoh M. (2010). Not all white rice tastes the same. *The Japan Times*. Available at http://search.japantimes.co.jp/cgi-bin/fg20101029f1.html. Accessed on 10 February 2011.

IWRA (International Wild Rice Association). Wild rice history. Available at http://www.wildrice.org/iwrawebsite/html/wildricehistory.html. Accessed on 8 February 2011.

Jamin E., Gonzalez J., Remaud G., Naulet N., Martin G. G., Weber D., Rossmann A. & Schmidt H.-L. (1997). Improved detection of sugar addition to apple juices and concentrates using internal standard ^{13}C IRMS. *Analytica Chimica Acta*, 347, 359-368.

Jamin E., Martin F. & Martin G. G. (2004). Determination of the $^{13}C/^{12}C$ ratio of ethanol derived from fruit juices and maple syrup by isotope ratio mass spectrometry: collaborative study. *Journal of AOAC International*, 87, 621-631.

Juliano B. O. (1993) Rice in human nutrition. Available at http://www.fao.org/docrep/t0567e/T0567E00.htm#Contents*. Accessed on 11 February 2011.*

Kabata-Pendias, A. & Pendias, H. (2001). *Trace Elements in Soils and Plants*. Boca Raton: CRC Press LLC.

Karoui R., Dufour E., Pillonel L., Picque D., Cattenoz T. & Bosset J.-O. (2004). Determining the geographic origin of Emmental cheeses produced during winter and summer using a technique based on the concentration of MIR and fluorescence spectroscopic data. *European Food Research and Technology*, 219, 184-189.

Karoui R., Bosset J.-O., Mazerolles G., Kulmyrzaev A. & Dufour E. (2005). Monitoring the geographic origin of both experimental French Jura hard cheeses and Swiss Gruyère and L'Etivaz PDO cheeses using mid-infrared and fluorescence spectroscopies: a preliminary investigation. *International Dairy Journal*, 15, 275-286.

Karoui R., Martin B. & Dufour E. (2005). Potentiality of front-face fluorescence spectroscopy to determine the geographic origin of milks from the Haute-Loire department (France). *Lait*, 85, 223-236.

Kawasaki A., Oda H. & Hirata T. (2002). Determination of strontium isotope ratio of brown rice for estimating its provenance. Soil Science and Plant Nutrition, 48, 635-640.

Kelly S., Baxter M., Chapman S., Rhodes C., Dennis J. & Brereton, P. (2002). The application of isotopic and elemental analysis to determine the geographical origin of premium long grain rice. *European Food and Research Technology*, 214, 72–78.

Kelly S. (2003). Using stable isotope ratio mass spectrometry (IRMS) in food authentication and traceability. In M. Lees (Ed.), *Food Authenticity and Traceability* (pp. 156-183). Cambridge: Woodhead Publishing Limited.

Kelly S., Heaton K. & Hoogewerff J. (2005). Tracing the geographical origin of food: The application of multi-element and multi-isotope analysis. *Trends in Food Science and Technology*, 16, 555–567.

Kelly S. D. & Bateman A. S. (2010). Comparison of mineral concentrations in commercially grown organic and conventional crops − tomatoes (Lycopersicon esculentum) and lettuces (Lactuca sativa). *Food Chemistry*, 119, 738-745.

Kendall C. (1998). Tracing nitrogen sources and cycles in catchments. In C. Kendall & J. J. McDonnell (Eds), *Isotopes Tracers in Catchment Hydrology* (pp. 839). Amsterdam: Elsevier.

Kerstel E. (2007). Isotope ratio infrared spectrometry. In P. A. De Groot (Ed.), *Handbook of Stable Isotope Analytical Techniques* (pp. 759-787). Amsterdam: Elsevier Inc.

Kim S. S., Rhyu M-R., Kim J. M. & Lee S-H. (2003). Authentication of rice using near-infrared reflectance spectroscopy. *Cereal Chemistry*, 80, 346-349.

Kingston H. M. & Walter P. J. (1998). The art and science of microwave sample preparations for trace and ultratrace elemental analysis. In A. Montaser (Ed.), *Inductively Coupled Plasma Mass Spectrometry* (pp. 33-82). Canada: Wiley-VCH, Inc.

Klecka W. R. (1980). *Discriminant Analysis*. California: Sage Publications, Inc.

Komárek M., Vaněk A., Chrastný V., Száková J., Kubová K., Drahota P. & Balík J. (2009). Retention of copper originating from different fungicides in contrasting soil types. *Journal of Hazardous Materials*, 166, 1395-1402.

Korenaga T., Musashi M., Nakashita R. & Suzuki Y. (2010). Statistical analysis of rice samples for compositions of multiple light elements (H, C, N, and O) and their stable isotopes. *Analytical Sciences*, 26, 873-878.

Körner C., Farquhar G. D. & Roksandic Z. (1988). A global survey of carbon isotope discrimination in plants from high altitude. *Oceologia*, 74, 623-632.

Kornexl B. E., Gehre M., Höfling R. & Werner R. A. (1999). On-line $\delta^{18}O$ measurement of organic and inorganic substances. *Rapid Communications in Mass Spectrometry*, 13, 1685-1693.

Kou D. & Mitra S. (2003) Extraction of semivolatile organic compounds from solid matrices. In S. Mitra (Ed.), *Sample Preparation Techniques in Analytical Chemistry* (pp. 139-182). New Jersey: John Wiley & Sons, Inc.

Kropf U., Korošec M., Bertoncelj J., Ogrinc N., Nečemer M., Kump P. & Golob T. (2010). Determination of the geographical origin of Slovenian black locust, lime and chestnut honey. *Food Chemistry*, 121, 839-846.

Lachenmeier D. W. (2007). Assessing the authenticity of absinthe using sensory evaluation and HPTLC analysis of the bitter principle absinthin. *Food Research International*, 40, 167-175.

Lamprecht G. & Blochberger K. (2009). Protocol for isolation of vanillin from ice cream and yoghurt to confirm the vanilla beans origin by ^{13}C-EA-IRMS. *Food Chemistry*, 114, 1130-1134.

Lewis, J. & Hird, S. (2007). Determining the geographical origin of foods: Considerations when designing experimental protocols and choosing analytical approaches. In S. Caroly (Ed.), *The Determination of Chemical Elements in Food: Applications for Atomic and Mass Spectrometry*. New Jersey: John Wiley & Sons Inc.

Li P., Wang X., Zhang T., Zhou D. & He Y. (2008). Effects of several amendments on rice growth and uptake of copper and cadmium from a contaminated soil. *Journal of Environmental Sciences*, 20, 449-455.

Liu J. X., Liao D. Q., Oane R., Estenor L., Yang X. E., Li Z. C. & Bennett J. (2006). Genetic variation in the sensitivity of anther dehiscence to drought stress in rice. *Field Crops Research*, 97, 87-100.

Liu L., Cozzolino D., Cynkar W. U., Gishen M. & Colby C. B. (2006). Geographic classification of Spanish and Australian Tempranillo red wines by visible and near-infrared spectroscopy combined with multivariate analysis. *Journal of Agricultural and Food Chemistry*, 54, 6754-6759.

Luykx D. M. A. M. & van Ruth S. M. (2008). An overview of analytical methods for determining the geographical origin of food products. *Food Chemistry*, 107, 897-911.

Maclean J. L., Dawe D. C., Hardy B. & Hettel, G. P. (2002). *Rice Almanac Source Book for the Most Important Economic Activity on Earth*. (3rd ed.). UK: CABI Publishing, Chapter: Importance of rice, 1-10.

Mannina L., Patumi M., Proietti N., Bassi D. & Segre A. L. (2001). Geographical characterisation of Italian extra virgin olive oils using high-field ^{1}H NMR spectroscopy. *Journal of Agricultural and Food Chemistry*, 49, 2687-2696.

Mar Caja M. D., Preston C., Menzel M., Kempf M. & Schreier P. (2009). Online gas chromatography combustion/pyrolysis-isotope ratio mass spectrometry (HRGC-C/P-IRMS) of (±)-dihydroactinidiolide from tea (Camellia sinensis) and Rooibos tea (Aspalathus linearis). *Journal of Agricultural and Food Chemistry*, 57, 5899-5902.

Marshall J. D., Brooks J. R. & Lajtha K. (2007). Sources of variation in the stable isotopic composition of plants. In R. Michener & K. Lajtha (Eds.), *Stable Isotopes in Ecology and Environmental Science* (pp. 22-60). Australia: Blackwell Publishing Ltd.

Marschner H. (1995). *Mineral Nutrition of Higher Plants*. (2nd ed.). London: Academic Press Limited, Chapter 8, 229-312, and Chapter 13, 483-507.

Martinelli L. A., Moreira M. Z., Ometto J. P., Alcarde A. R., Rizzon L. A., Stange E. & Ehleringer J. R. (2003). Stable carbon isotopic composition of the wine and CO2 bubbles of sparkling wines: detecting C4 sugar additions. *Journal of Agricultural and Food Chemistry*, 51, 2625-2631.

Mauriello G., Molo L., Genovese A. & Ercolini D. (2003). Relationships between flavouring capabilities, bacterial composition, and geographical origin of natural whey cultures used for traditional water-buffalo Mozzarella cheese manufacture. *Journal of Dairy Science*, 86, 486-497.

McMurry J. & Begley T. (2005). *The Organic Chemistry of Biological Pathways*. USA: Roberts and Company Publishers, Chapter 4, 161-220.

Meier-Augenstein W. (2010). *Stable Isotope Forensics*. UK: John Wiley & Sons Ltd, Chapter I, 1-64.

Miller J. B., Pang E., & Bramall L. (1992). Rice: a high or low glycemic index food. *The American Journal of Clinical Nutrition*, 56, 1034-1036.

Montalvan R., Ando A. & Echeverrigaray S. (1998). Use of seed protein polymorphism for discrimination of improvement level and geographic origin of upland rice cultivars. *Genetics and Molecular Biology*, 21, 531–535.

Morales-Rubio, A., Salvador, A., de la Guardia, M. & Ros, R. (1993). Determination of Cd and Ni in rice plants by flame atomic absorption spectrometry. *Atomic Spectroscopy*, 14, 8–12.

Morecroft M. D. & Woodward F. I. (1990). Experimental investigations on the environmental determination of $\delta^{13}C$ at different altitudes. *Journal of Experimental Botany*, 41, 1303-1308.

Moreda-Piñeiro A., Fisher A. & Hill S. J. (2003). The classification of tea according to region of origin using pattern recognition techniques and trace metal data. *Journal of Food Composition and Analysis*, 16, 195-211.

Nakashita R., Miyama K., Yoh M. & Nakatsuka T. (2006). Relationships between altitude and stable isotope compositions of surface waters and plants in the northern Japanese Alps region. Poster presentation at 陸域の生物地球化学. Available at http://wwwsoc.nii.ac.jp/jepsjmo/cd-rom/2006cd-rom/pdf/J238/J238-P009_e.pdf. Accessed on 31 July 2011.

Nhu-Trang T.-T., Casabianca H. & Grenier-Loustalot M.-F. (2006). Deuterium/hydrogen ratio analysis of thymol, carvacrol, γ-terpinene and ρ-cymene in thyme, savory and oregano essential oils by gas chromatography-pyrolysis-isotope ratio mass spectrometry. *Journal of Chromatography A*, 1132, 219-227.

Norvell W. A., Wu J., Hopkins D. G. & Welch R. M. (2000). Association of cadmium in durum wheat grain with soil chloride and chelate-extractable soil cadmium. *Soil Science Society of America Journal*, 64, 2162-2168.

Notteghem J.L., Roux-Cuvelier M., André F. & Roumen E. Rice diseases in the Camargue (France). Available at http://ressources.ciheam.org/om/pdf/c15-3/CI011013.pdf. Accessed on 25 July 2011.

Núñez M., Peña R. M., Herrero C. & García-Martín S. (2000). Analysis of some metals in wine by means of capillary electrophoresis. Application to the differentiation of *Ribeira Sacra* Spanish red wines. *Analusis*, 28, 432-437.

O'Brien J. (1992). Authentication and quality assessment of food products. *Trends in Food Science & Technology*, 3, 19-22.

O'Connor G. & Evans E. H. (1999). Fundamental aspects of ICP-MS. In S. J. Hill (Ed.), *Inductively Coupled Plasma Mass Spectrometry and its Applications* (pp. 119-144). UK: Sheffield Academic Press.

O'Leary M. H. (1981). Carbon isotope fractionation in plants. *Phytochemistry*, 20, 553-567.

Ogrinc N., Kosir I. J., Kocjancic M. & Kidric J. (2001) Determination of authenticity, regional origin, and vintage of Slovenian wines using a combination of IRMS and SNIF-NMR analyses. *Journal of Agricultural and Food Chemistry*, 49, 1432-1440.

Oliveira E. (2003). Sample preparation for atomic spectroscopy: evolution and future trends. Journal of the Brazilian Chemical Society, vol. 14. Available at http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0103-50532003000200004. Accessed on 14 March 2011.

Olliver D., Artaud J., Pinatel C., Durbec J. P. & Guérère M. (2003). Triacylglycerol and fatty acid compositions of French virgin olive oils. Characterisation by chemometrics. *Journal of Agricultural and Food Chemistry*, 51, 5723-5731.

Paramás A. M. G., Bárez J. A. G., Garcia-Villanova R. J., Palá T. R., Albajar R. A. & Sánchez J. S. (2000). Geographical discrimination of honeys by using mineral composition and common chemical quality parameters. *Journal of the Science of Food and Agriculture*, 80, 157-165.

Parengam M., Judprason K., Srianujata S., Jittinandana S., Laoharojanaphand S. & Busamongko A. (2010). Study of nutrients and toxic minerals in rice and legumes by instruemental neuron activation analysis and graphite furnace atomic absorption spectrophotometry. *Journal of Food Composition and Analysis*, 23, 340-345.

Peng Y., Liu F. & Ye J. (2006). Quantitative and qualitative analysis of flavonoid markers in *Frucus aurantii* of different geographical origin by capillary electrophoresis with electrochemical detection. *Journal of Chromatography B*, 830, 224-230.

Penza M. & Cassano G. (2004). Chemometric characterisation of Italian wines by thin-film multisensors array and artificial neural networks. *Food Chemistry*, 86, 283-296.

Perez A. L., Smith B. W. & Anderson K. A. (2006). Stable isotope and trace element profiling combined with classification models to differentiate geographic growing origin for three fruits: effects of subregion and variety. *Journal of Agricultural and Food Chemistry*, 54, 4506-4516.

Picque D., Cattenoz T., Corrieu G. & Berger J. L. (2005). Discrimination of red wines according to their geographical origin and vintage year by the use of mid-infrared spectroscopy. *Sciences des Aliments*, 25, 207-220.

Pillonel L., Badertscher R., Bütikofer U., Casey M., Dalla Torre M., Lavanchy P., Meyer J., Tabacchi R. & Bosset J. (2002). Analytical methods for the determination of the geographic origin of Emmentaler cheese. Main framework of the project: chemical, biochemical, microbiological, colour and sensory analyses. *European food Research and Technology*, 215, 260-267.

Pillonel L., Ampuero S., Tabacchi R. & Bosset J. O. (2003). Analytical methods for the determination of the geographic origin of Emmental cheese: volatile compounds by

GC/MS-FID and electronic nose. *European Food Research and Technology*, 216, 179-183.

Povolo M., Contarini G., Mele M. & Secchiari P. (2007). Study on the influence of pasture on volatile fraction of Ewes' dairy products by solid-phase microextraction and gas chromatography-mass spectrometry. *Journal of Dairy Science*, 90, 556-569.

Radovic B. S., Careri M., Mangia A., Musci M., Gerboles M. & Anklam E. (2001). Contribution of dynamic headspace GC-MS analysis of compounds to authenticity testing of honey. *Food Chemistry*, 72, 511-520.

Raspor P., Milek D. M., Polanc J., Možina S. S. & Čadež N. (2006). Yeasts isolated from three varieties of grapes cultivated in different locations of the Dolenjska vine-growing region, Slovenia. *International Journal of Food Microbiology*, 109, 97-102.

Reid L. M., O'Donnell C. P. & Downey G. (2006). Recent technological advances for the determination of food authenticity. *Trends in Food Science & Technology*, 17, 344–353.

Remaud G. S., Martin Y.-L., Martin G. G., Naulet N. & Martin G. J. (1997). Authentication of mustard oils by combined stable isotope analysis (SNIF-NMR and IRMS). *Journal of Agricultural and Food Chemistry*, 45, 1844-1848.

Renou J.-P., Deponge C., Gachon P., Bonnefoy J.-C., Coulon J.-B., Garel J.-P., Vérité R. & Ritz P. (2004). Characterization of animal products according to geographic origin and feeding diet using nuclear magnetic resonance and isotope ratio mass spectrometry: cow milk. *Food Chemistry*, 85, 63-66.

Rezzi S., Axelson D. E., Héberger K., Reniero F., Mariani C. & Guillou C. (2005). Classification of olive oils using high throughput flow ^{1}H NMR fingerprinting with principal component analysis, linear discriminant analysis and probabilistic neural networks. *Analytica Chimica Acta*, 552, 13-24.

Rhodes C. N., Lofthouse J. H., Hird S., Rose P., Reece P., Christy J., Macarthur R. & Brereton P. A. (2010). The use of stable carbon isotopes to authenticate claims that poultry have been corn-fed. *Food Chemistry*, 118, 927-932.

Rice Association. (2004). About rice: varieties. Available at http://www.riceassociation.org.uk/About/varieties.htm. Accessed on 29 January 2011.

Ricegrowers' Association of Australia. About rice. Available at http://www.rga.org.au/. Accessed on 5 February 2011.

Rivero R. C., Hernández P. S., Rodríguez E. M., Martín J. D. & Romero C. D. (2003). Mineral concentrations in cultivars of potatoes. *Food Chemistry*, 83, 247-253.

Rodrigues C., Máguas C. & Prohaska T. (2011). Strontium and oxygen isotope fingerprinting of green coffee beans and its potential to proof authenticity of coffee. *European Food Research and Technology*, 232, 361-373.

Rodrigues C. I., Maia R., Miranda M., Ribeirinho M., Nogueira J. M. F. & Máguas C. (2009). Stable isotope analysis for green coffee bean: a possible method for geographic origin discrimination. *Journal of Food Composition and Analysis*, 22, 463-471.

Rodriquez-Delgado M.-A., Gonzalez-Hernandez G., Conde-Gonzales J.-E. & Perez-Trujillo J.-P. (2002). Principal component analysis of the polyphenol content in young red wines. *Food Chemistry*, 78, 523-532.

Rogers K. M. (2009). Stable isotopes as a tool to differentiate eggs laid by caged, barn, free range and organic hens. *Journal of Agricultural and Food Chemistry*, 57, 4236-4242.

Rossman A., Haberhauer G., Hölzl S., Horn P., Pichlmayer F. & Voerkelius S. (2000). The potential of multielement stable isotope analysis for regional origin assignment of butter. *European Food Research and Technology*, 211, 32-40.

Ruiz del Castillo M. L., Caja M. M., Blanch G. P. & Herriaz M. (2003). Enantiomeric distribution of chiral compounds in orange juices according to their geographical origins. *Journal of Food Protection*, 66, 1448-1454.

Ruoff K., Luginbühl W., Künzli R., Iglesias M. T., Bogdanov S., Bosset J. O., Von Der Ohe K., Von Der Ohe W. & Amado R. (2006). Authentication of the botanical and geographical origin of honey by mid-infrared spectroscopy. *Journal of Agricultural and Food Chemistry*, 54, 6873-6880.

Russo S. & Callegarin A. M. Rice production and research potential in Italy. Available at http://ressources.ciheam.org/om/pdf/c24-2/CI011095.pdf. Accessed on 25 July 2011.

Saad N., Kuramoto D. & Zare R. N. (2009). Compound specific isotope analysis of carbon using cavity ring-down spectrometry. Available at http://www.picarro.com/assets/docs/Picarro_AGU_poster_4_2009_CSIA.pdf. Accessed on 1 March 2011.

Schellenberg A., Chmielus A., Schlicht C., Camin F., Perini M., Bontempo L., Heinrich K., Kelly S. D., Rossmann A., Thomas F., Jamin E. & Horacek M. (2010). Multielement stable isotope ratios (H, C, N, S) of honey from different European regions. *Food Chemistry*, 121, 770-777.

Schmidt O., Quilter J. M., Bahar B., Moloney A. P., Scrimgeour C. M., Begley I. S. & Monahan F. J. (2005) Inferring the origin and dietary history of beef from C, N and S stable isotope ratio analysis. *Food Chemistry*, 91, 545-549.

Schulze E.-D., Beck E. & Muller-Hohenstein K. (2005). *Plant ecology*. Germany: Springer, Chapter 2, 253-296.

Schwartz R. S. & Hecking L. T. (1991). Determination of geographic origin of agricultural products by multivariate analysis of trace element composition. *Journal of Analytical Atomic Spectrometry*, 6, 637-642.

Sefc K. M., Lopes M. S., Mendonça D., Rodrigues Dos Santos M., Laimer Da Câmara Machado M. & Da Câmara Machado A. (2000). Identification of microsatellite loci in olive (*Olea europaea*) and their characterisation in Italian and Iberian olive trees. *Molecular Ecology*, 9, 1171-1173.

Serra F., Guillou C. G., Reniero F., Ballarin L., Cantagallo M. I., Wieser M., Iyer S. S., Héberger K. & Vanhaecke F. (2005). Determination of the geographical origin of green coffee by principal component analysis of carbon, nitrogen and boron stable isotope ratios. *Rapid Communications in Mass Spectrometry*, 19, 2111-2115.

Shaw A. D., Di Camillo A., Vlahov G., Jones A., Bianchi G., Rowland J. & Kell D. B. (1997). Discrimination of the variety and region of origin of extra virgin olive oils using ^{13}C NMR and multivariate calibration with variable reduction. *Analytica Chimica Acta*, 348, 357-374.

Shimoda M., Shibamto T. & Noble A. C. (1993). Evaluation of headspace volatiles of Cabernet Sauvignon wines sampled by an on-column method. *Journal of Agricultural and Food Chemistry*, 41, 1664-1668.

Sillanpää M. (1982). *Micronutrients and the Nutrient Status of Soils: A Global Study*. Finland: Werner Söderström Osakeyhtiö, Chapter 10, 411-416.

Singh V. P. (2000). Basmati rice of India. In R. K. Singh, U. S. Singh, & G. S. Khush (Eds.), *Aromatic Rices* (pp. 135-154). New Delhi: Oxford & IBH Publishing Co. Pvt. Ltd.

Smith B. N., Herath H. M. W. & Chase J. B. (1973). Effect of growth temperature on carbon isotopic ratios in barley, pea and rape. *Plant and Cell Physiology*, 14, 177-182.

Smith B. N., Oliver J. & Mc Millan C. (1976). Influence of carbon source, oxygen concentration, light intensity, and temperature on ^{13}C/^{12}C ratios in plant tissues. *Botanical Gazette*, 137, 99-104.

Sompong R., Siebenhandl-Ehn S., Linsberger-Martin G. & Berghofer E. (2011). Physicochemical and antioxidative properties of red and black rice varieties from Thailand, China and Sri Lanka. *Food Chemistry*, 124, 132-140.

Spitaler R., Araghipour N., Mikoviny T., Wisthaler A., Via J. D. & Märk T. D. (2007). Discrimination of different wine varieties through direct headspace analyses by PTR-MS. 3rd International Conference on Proton Transfer Reaction Mass Spectrometry and Its Application. Available at http://www.ptrms.com/downloads/PTRMS_Conference2007_Web.pdf.!Accessed on 26 March 2011.

Stefanoudaki E., Kotsifaki F. & Koutsaftakis A. (1997). The potential of HPLC triglyceride profiles for the classification of Cretan olive oils. *Food Chemistry*, 60, 425-432.

Stefanoudaki E., Kotsifaki F. & Koutsaftakis A. (2000). Sensory and chemical profiles of three European olive varieties (*Olea europea* L); an approach for the characterisation

and authentication of the extracted oils. *Journal of the Science of Food and Agriculture*, 80, 381-389.

Steine C., Beaucousin F., Siv C. & Pfeiffer G. (2001). Potential of semiconductor sensor arrays for the origin authentication of pure Valencia orange juices. *Journal of Agricultural and Food Chemistry*, 49, 3151-3160.

Sternberg L. D. S. L., Anderson W. T. & Morrison K. (2003). Separating soil and leaf ^{18}O isotopic signals in plant stem cellulose. *Geochimica et Cosmochimica Acta*, 67, 2561-2566.

Stürup S. (2004). The use of ICPMS for stable isotope tracer studies in humans: a review. *Analytical and Bioanalytical Chemistry*, 378, 273-282.

SunRice. Fact Sheet. The rice growing and production process. Available at http://www.sunrice.com.au/uploads//documents/education/Rice_Growing_Production_Process.pdf. Accessed on 9 February 2011.

Suzuki Y., Chikaraishi Y., Ogawa N. O., Ohkouchi N. & Korenaga T. (2008). Geographical origin of polished rice based on multiple element and stable isotope analyses. *Food Chemistry*, 109, 470-475.

Suzuki Y., Nakashita R., Akamatsu F. & Korenaga T. (2009). Multiple stable isotope analyses for verifying geographical origin and agricultural practice of Japanese rice samples. *Bunseki Kagaku*, 58, 1053-1058.

Tanchotikul U. & Hsieh T. C.-Y. (1991). An improved method for quantification of 2-acetyl-1-pyrroline, a "popcorn"-like aroma, in aromatic rice by high-resolution gas chromatography/mass spectrometry/selected ion monitoring. *Journal of Agricultural and Food Chemistry*, 39, 944-947.

Tapp H. S., Defernez M. & Kemsley E. K. (2003). FTIR spectroscopy and multivariate analysis can distinguish the geographical origin of extra virgin olive oils. *Journal of Agricultural and Food Chemistry*, 51, 6110–6115.

Thomas F. & Jamin E. (2009). ^{2}H NMR and ^{13}C-IRMS analyses of acetic acid from vinegar, 18O-IRMS analysis of water in vinegar: International collaborative study report. *Analytica Chimica Acta*, 649, 98-105.

Tibet Business Daily. (2010). Additives turn ordinary rice into gourmet Wuchang Rice. Available at http://www.danwei.org/front_page_of_the_day/fake_rice.php. Accessed on 14 April 2012.

Tilda. Facts about Basmati. Available at http://www.tilda.com/#/?section=1&subsection=13. Accessed on 8 February 2011.

Tomás-Barberán F. A., Ferreres F., García-Viguera C. & Tomás-Lorente F. (1993). Flavonoids in honey of different geographical origin. *Zeitschrift für Lebensmittel-Untersuchung und -Forschung*, 196, 38-44.

Ulberth F. (2004). Analytical approaches for food authentication. *Mitteilungen aus Lebensmitteluntersuchung und Hygiene,* 95, 561-572.

USA Rice Federation. The U.S. Rice Industry At-A-Glance. Available at http://www.usarice.com/doclib/188/219/3674.PDF. Accessed on 25 July 2011.

USDA (United States Department of Agriculture). (2010a). Spain's 2010/11 grain crop is higher than last year's drought-afflicted crop, but far below the bumper yields achieved in 2008/09 and 2007/08. Available at http://www.pecad.fas.usda.gov/highlights/2010/07/Spain%20Report/. Accessed on 26 July 2011.

USDA (United States Department of Agriculture). (2010b). Rice at a glance. Available at http://ffas.usda.gov/htp/CP2010/Rice-2010-Final.pdf. Accessed on 26 July 2011.
Vaughan J. G., & Geissler C. A. (1997). *The new oxford book of food plants*. New York: Oxford University Press, (Chapter: Grain crops).

Vela N. P., Olsen L. K. & Caruso J. A. (1993). Elemental speciation with plasma mass spectrometry. *Analytical Chemistry*, 65, 585A-597A.

Vlachos A. & Arvanitoyannis I. S. (2008). A review of rice authenticity/adulteration methods and results. *Critical Reviews in Food Science and Nutrition*, 48, 553–598.

Wang L. C., Cao Y. H., Xing X. P. & Ye J. N. (2005). Fingerprint studies of *Radix Scutellariae* by capillary electrophoresis and high performance liquid chromatography. *Chromatographia*, 62, 283-288.

Waterhouse J. S., Switsur V. R., Barker A. C., Carter A. H. C. & Robertson I. (2002). Oxygen and hydrogen isotope ratios in tree rings: how well do models predict observed values? *Earth and Planetary Science Letters*, 201, 421-430.

Watson D. G., Peyfoon E., Zheng L., Lu D., Seidel V., Johnston B., Parkinson J. A. & Fearnley J. (2006). Application of principal components analysis to ^{1}H-NMR data obtained from Propolis samples of different geographical origin. *Phytochemical Analysis*, 17, 323-331.

Weckerle B., Richling E., Heinrich S. & Schreier P. (2002). Origin assessment of green coffee (Coffea Arabica) by multi-element stable isotope analysis of caffeine. *Analytical and Bioanalytical Chemistry*, 374, 886-890.

White J. W. C. (1988). In P. W. Rundel, J. R. Ehleringer, & K. A. Nagy (Eds.), *Stable Isotopes in Ecological Research*, (pp 142-162). New York: Springer Verlag New York Inc.

White R. E. (2006). *Principles and Practice of Soil Science*. (4th ed.). UK: Blackwell Publishing, Chapter 1, 3-10 and Chapter 5, 81-102.

Williams D. G., Coltrain J. B., Lott M., English N. B., & Ehleringer J. R. (2005). Oxygen isotopes in cellulose identify source water for archaeological maize in the American Southwest. *Journal of Archaeological Science*, 32, 931-939.

Winkler F. J. (1984). In A. Frigerio & H. Milon (Eds.), *Chromatography and Mass Spectrometry in Nutrition Science and Food Safety*, (pp 173-190). Amsterdam, Netherlands: Elsevier Science Publishers BV.

World Rice Statistics (International Rice Research Institute, IRRI). Available at http://irri.org/our-science/targeting-and-policy/world-rice-statistics. Accessed on 25 July 2011.

Yasui A. & Shindoh K. (2000). Determination of the geographic origin of brown-rice with trace-element composition. *Bunseki Kagaku*, 49, 405–410.

Yurtsever Y. & Gat J. R. (1981). Stable isotope hydrology. Atmospheric waters in IAEA technical reports series 210, 103-142.

Zhang C. (2007). *Fundamentals of Environmental Sampling and Analysis*. New Jersey: John Wiley & Sons Inc., Chapter 7, 159-189.

Zhao F. J., McGrath S. P. & Meharg A. A. (2010). Arsenic as a food chain contaminant: mechanisms of plant uptake and metabolism and mitigation strategies. *Annual Review of Plant Biology*, 61, 535-559.

Bibliography

Asfaha D. G., Quétel C. R., Thomas F., Horacek M., Wimmer B., Heiss G., Dekant C., Deters-Itzelsberger P., Hoelzl S., Rummel S., Brach-Papa C., Vab Bocxstaele M., Jamin E., Baxter M., Heinrich K., Kelly S., Bertoldi D., Bontempo L., Camin F., Larcher R., Perini M., Rossmann A., Schellenberg A., Schlicht C., Froeschl H., Hoogewerff J. & Ueckermann H. (2011). Combining isotopic signatures of $n(^{87}Sr)/n(^{86}Sr)$ and light stable elements (C, N, O, S) with multi-elemental profiling for the authentication of provenance of European cereal samples. *Journal of Cereal Science*, 53, 170-177.

Chidthaisong A., Chin K.-J., Valentine D. L. & Tyler S. C. (2002). A comparison of isotope fractionation of carbon and hydrogen from paddy field rice roots and soil bacterial enrichments during CO_2/H_2 methanogenesis. *Geochimica et Cosmochimica Acta*, 66, 983-995.

Ding T. P., Ma G. R., Shui M. X., Wan D. F. & Li R. H. (2005). Silicon isotope study on rice plants from the Zhejiang province, China. *Chemical Geology*, 218, 41-50.

Ding T. P., Tian S. H., Sun L., Wu L. H., Zhou J. X. & Chen Z. Y. (2008). Silicon isotope fractionation between rice plants and nutrient solution and its significance to the study of the silicon cycle. *Geochimica et Cosmochimica Acta*, 72, 5600-5615.

Farquhar G. D., Ehleringer J. R. & Hubick K. T. (1989). Carbon isotope discrimination and photosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology*, 40, 503-537.

Fey A., Claus P. & Conrad R. (2004). Temporal change of ^{13}C-isotope signatures and methanogenic pathways in rice field soil incubated anoxically at different temperatures. *Geochimica et Cosmochimica Acta*, 68, 293-306.

Förstel H. (2007). The natural fingerprint of stable isotopes – use of IRMS to test food authenticity. *Analytical and Bioanalytical Chemistry*, 388, 541-544.

Fu G-F., Song J., Li Y-R., Yue M-K., Xiong J. & Tao L-X. (2010). Alterations of panicle antioxidant metabolism and carbohydrate content and pistil water potential involved in spikelet sterility in rice under water-deficit stress. *Rice Science*, 17, 303-310.

Han G. H., Yoshikoshi T. H., Nagai H., Yamada T., Saito M., Miyata A. & Harazono Y. (2005). Concentration and carbon isotope profiles of CH_4 in paddy rice canopy: isotopic evidence for changes in CH_4 emission pathways upon drainage. *Chemical Geology*, 218, 25-40.

Hu P., Zhao H., Duan Z., Zhang L. & Wu D. (2004). Starch digestibility and the estimated glycemic score of different types of rice differing in amylose contents. *Journal of Cereal Science*, 40, 231-237.

Itani T., Tamaki M., Arai E. & Horino T. (2002). Distribution of amylose, nitrogen, and minerals in rice kernels with various characters. *Journal of Agricultural and Food Chemistry*, 50, 5326-5332.

Joebstl D., Bandoniene D., Meisel T. & Chatzistathis S. (2010). Identification of the geographical origin of pumpkin seed oil by the use of rare earth elements and discriminant analysis. *Food Chemistry*, 123, 1303-1309.

Khush G. S. (1997). Origin, dispersal, cultivation and variation of rice. *Plant Molecular Biology*, 35, 25-34.

Laursen K. H., Hansen T. H., Persson D. P., Schjoerring J. K. & Husted S. (2009). Multi-elemental fingerprinting of plant tissue by semi-quantitative ICP-MS and chemometrics. *Journal of Analytical Atomic Spectrometry*, 24, 1198-1207.

Miura C. & Tanaka F. (2007). Determination of ^{13}C content in starch from paddy rice plants using stable isotope ratio mass spectrometry. *Soil Science and Plant Nutrition*, 53, 606-611.

Rang Z. W., Jagadish S. V. K., Zhou Q. M., Craufurd P. Q. & Heuer S. (2011). Effect of high temperature and water stress on pollen germination and spikelet fertility in rice. *Environmental and Experimental Botany*, 70, 58-65.

Rentschler K. (2003). California's new gold rush. *The New York Times*. Available at http://www.nytimes.com/2003/10/08/dining/california-s-new-gold-rush.html?pagewanted=all&src=pm. Accessed on 9 February 2011.

Seyfferth A. L., Webb S. M., Andrews J. C. & Fendorf S. (2011). Defining the distribution of arsenic species and plant nutrients in rice (*Oryza sativa* L.) from the root to the grain. *Geochimica et Cosmochimica Acta*, 75, 6655-6671.

Shaheen R. & Hood-Nowotny R. C. (2005). Effect of drought and salinity on carbon isotope discrimination in wheat cultivars. *Plant Science*, 168, 901-909.

Shivrain V. K., Burgos N. R., Scott R. C., Gbur Jr. E. E., Estorninos Jr. L. E. & McClelland M. R. (2010). Diversity of weedy red rice (*Oryza sativa* L.) in Arkansas, U.S.A. in relation to weed management. *Crop Protection*, 29, 721-730.

Terwilliger V. J., Betancourt J. L., Leavitt S. W. & Van De Water P. K. (2002). Leaf cellulose δD and $δ^{18}O$ trends with elevation differ in direction among co-occurring, semiarid plant species. *Geochimica et Cosmochimica Acta*, 66, 3887-3900.

Tieszen L. L. (1991). Natural variations in the carbon isotope values of plants: implications for archaeology, ecology, and paleoecology. *Journal of Archaeological Science*, 18, 227-248.

Tsialtas J. T. & Maslaris N. (2006). Leaf carbon isotope discrimination relationships to element content in soil, roots and leaves of sugar beets grown under Mediterranean conditions. *Field Crops Research*, 99, 125-135.

Uchida S., Tagami K., Shang Z. R. & Choi Y. H. (2009). Uptake of radionuclides and stable elements from paddy soil to rice: a review. *Journal of Environmental Radioactivity*, 100, 739-745.

Werner R. A., Kornexl B. E., Roßmann A. & Schmidt H.-L. (1996). On-line determination of $\delta^{18}O$ values of organic substances. *Analytica Chimica Acta*, 319, 159-164.

Yapp C. J. & Epstein S. (1982). Climatic significance of the hydrogen isotope ratios in tree cellulose. *Nature*, 297, 636-639.

Yasui A. & Shindoh K. (2000). Determination of the geographic origin of brown-rice with trace-element composition. *Bunseki Kagaku*, 49, 405–410.

Yurtsever Y. & Gat J. R. (1981). Stable isotope hydrology. Atmospheric waters in IAEA technical reports series 210, 103-142.

Zhang C. (2007). *Fundamentals of Environmental Sampling and Analysis*. New Jersey: John Wiley & Sons Inc., Chapter 7, 159-189.

Zhao F. J., McGrath S. P. & Meharg A. A. (2010). Arsenic as a food chain contaminant: mechanisms of plant uptake and metabolism and mitigation strategies. *Annual Review of Plant Biology*, 61, 535-559.