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## The application of isotopic and elemental analysis to determine the geographical origin of premium long grain rice

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**Abstract** Rice samples cultivated in the USA, Europe and Basmati regions have been analysed using Isotope Ratio Mass Spectrometry (IRMS) and Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Nine key variables (carbon-13, oxygen-18, boron, holmium, gadolinium, magnesium, rubidium, selenium and tungsten) were identified by canonical discriminant analysis as providing the maximum discrimination between rice samples from these regions. High levels of boron (>2500 ppb) were associated with rice samples from America and notably high levels of holmium were found in rice samples from the state of Arkansas. European rice samples generally contained relatively high levels of magnesium and Indian/Pakistani samples were characterised by relatively low oxygen-18 ( $^{18}\text{O}$ ) abundance.

**Keywords** Rice · Authenticity · Geographical origin · CF-IRMS, ICP-MS

### Introduction

Mislabelling and adulteration is a problem in many areas of the food industry. It threatens the livelihood of honest traders and the rights of the consumer. For most food products the authentic item is distinguished by geographical origin, botanical/cultivar origin or the absence of

adulterants. In the case of rice the determination of authenticity is a more complex issue as it can depend on both geographical origin and cultivar.

Basmati is the name used for a class of rice comprising a few defined varieties grown in the Haryana, Punjab and Uttar Pradesh regions of India and Pakistan. The highly favoured properties of Basmati such as its fragrance and flavour apparently cannot be emulated by growing the seed in other regions. With the increase in the consumer market of premium rice comes the risk that unscrupulous producers will attempt to increase profits by mislabelling inferior rice grown outside these regions. This investigation seeks to address this issue by identifying the key elemental and isotopic variables that could be used to determine the geographical origin of rice.

The trace element composition of rice reflects to some extent that of the soil in which it is grown. Rice growing on soils such as uraniferous black shale, rich in elements such as selenium (Se), molybdenum (Mo) and cadmium (Cd), has been shown to accumulate these elements up to levels that may be of concern to health [1]. Alkali metals, especially rubidium (Rb) and caesium (Cs), being labile in the soil and easily transported into the plant, are good indicators of geographical origin. The concentration of trace elements depends on the topography and soil characteristics. Thus no two countries have identical soil maps. The measurement of the stable isotope ratios of oxygen is also applicable to the characterisation of the geographical origin of rice because of the latitude effects on the fractionation of these isotopes in groundwater [2]. The 'light' isotopomer of water ( $\text{H}_2^{16}\text{O}$ ) evaporates more rapidly at the equator and when this water condenses and falls as rain at higher latitudes and altitudes it is depleted in the heavy isotopomer ( $\text{H}_2^{18}\text{O}$ ).

The oxygen present in rice carbohydrate originates from the water taken up by the plant roots. Transpiration of water through the leaf stomata enriches the water in the heavier isotopomer. Therefore, it is expected that growing regions with relatively low humidity, where the rate of transpiration is higher, result in plant carbohy-

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drate with relatively enriched  $\delta^{18}\text{O}$  values. However, when utilising the  $^{18}\text{O}$  abundance, the topography and local climatic conditions must also be taken into consideration; this data therefore serves to compliment trace element analysis.

The carbon atoms present in the carbohydrate of rice samples are derived from carbon dioxide present in the atmosphere. However, the  $\delta^{13}\text{C}$  value of plant carbohydrate is not the same as atmospheric  $\text{CO}_2$ . This is because of two principal fractionating processes; diffusion of  $\text{CO}_2$ , through the leaf stomata, to the site of assimilation and the carboxylation reaction to fix  $\text{CO}_2$  [3]. These processes discriminate against the heavier isotope ( $^{13}\text{C}$ ) and are dependent on environmental factors such as light intensity, changes in soil water content and relative humidity.

## Materials and methods

### Rice samples

A total of 73 authentic rice samples were obtained from reliable sources such as the International Rice Research Institute in the Phillipines. Of the rice samples, 20 were grown in the USA (Arkansas, Louisiana, Mississippi and Texas), 25 were grown in Europe (French Camargue, Italy and Spain) and 28 were grown in India and Pakistan (Basmati regions).

### Inductively Coupled Plasma – Mass Spectrometry (ICP-MS)

**Sample preparation.** A 0.5-g sample was digested in 5  $\text{cm}^3$  concentrated nitric acid using high pressure microwave digestion. This was diluted to 10  $\text{cm}^3$  with deionised water. Then 1  $\text{cm}^3$  of this solution was diluted with 4  $\text{cm}^3$  of water containing indium as an internal standard and measured by ICP-MS.

**Measurement.** The instrument was calibrated using acid matched multi-element standards. Quality control consisted of blanks, a spiked blank and a reference material (NIST1568 rice flour) to ascertain detection limits, recovery and accuracy, respectively. Normal in-house criteria were applied to assess the quality of the data, these were (1) instrumental drift from beginning to end of batch within  $\pm 20\%$ , (2) recoveries within  $\pm 40\%$ , with at least 75% within  $\pm 20\%$  and (3) reference material values within  $\pm 40\%$  of certified limits. The concentrations of 50 elements were determined simultaneously, including macro (aluminium, calcium, magnesium, phosphorus, sodium and potassium), micro (e.g. manganese, iron, cobalt, nickel, copper and zinc) and trace (e.g. strontium, molybdenum, cadmium, lead and the rare earths).

### Pyrolysis Continuous Flow Isotope Ratio Mass Spectrometry (Py-CF-IRMS)

**Sample preparation.** Rice samples were milled in a coffee grinder to a fine powder. The ground sample was then shaken vigorously for 2 min to homogenise the powder. Then 0.5 mg of the rice powder was weighed into a silver capsule (3.5 $\times$ 5 mm, obtained from EA Scientific, East Grinstead, UK).

**Measurement.** The elemental analyser (Eurovector, Milan, Italy) was connected to the IRMS (PDZ-Europa, Cheshire, UK) via a crimp. Samples were pyrolysed at 1080  $^\circ\text{C}$  in a quartz reactor in the EA as previously described by Werner et al. [4]. Briefly, the pyrolysis gases were swept by a flow of helium carrier gas (grade 5.6, flow rate 100  $\text{cm}^3/\text{min}$ ) through a bed of glassy carbon grit (Sigradur G, HTW, Thierhaupten FRG). Water vapour and carbon

dioxide were removed by a chemical trap (10 mm i.d., length 0.2 m) containing magnesium perchlorate and Carbosorb. The pyrolysis gases then passed through a packed GC column filled with molecular sieve 5A (4 mm i.d., length 1 m) heated to 60  $^\circ\text{C}$ , which separated  $\text{H}_2$ ,  $\text{N}_2$  and  $\text{CO}$ . A portion of the effluent (approximately 0.05  $\text{cm}^3/\text{min}$ ) flowed via the crimp into the ion source of the IRMS. The  $m/z$  30 ( $^{12}\text{C}^{18}\text{O}$ ),  $m/z$  29 ( $^{13}\text{C}^{16}\text{O}$ ) and  $m/z$  28 ( $^{12}\text{C}^{16}\text{O}$ ) ion beams of carbon monoxide entering the source were simultaneously integrated over time.

A beet sugar laboratory reference material with a known  $\delta^{18}\text{O}\text{‰}$  value vs the Vienna Standard Mean Ocean Water (VSMOW) scale was used to calibrate the Py-CF-IRMS measurement of the rice samples. The beet sugar reference material was calibrated using a modified Rittenberg method [5]. The delta value of the rice carbohydrate was calculated relative to the beet sugar laboratory reference material and converted to the VSMOW scale using established formulae [6]:

$$\delta^{18}\text{O per mil} = \left[ \frac{(^{18}\text{O}/^{16}\text{O})_{\text{sample}} - (^{18}\text{O}/^{16}\text{O})_{\text{V-SMOW}}}{(^{18}\text{O}/^{16}\text{O})_{\text{V-SMOW}}} \right] \times 1000$$

where  $[^{18}\text{O}/^{16}\text{O}]_{\text{sample}}$  is the ratio of  $^{18}\text{O}$  to  $^{16}\text{O}$  abundance in a sample,  $[^{18}\text{O}/^{16}\text{O}]_{\text{standard (VSMOW)}}$  is the ratio of  $^{18}\text{O}$  to  $^{16}\text{O}$  abundance in the international standard, VSMOW and  $\delta^{18}\text{O}$  per mil “delta oxygen 18 per mil” is the difference between the ratios of a sample and the standard expressed as per mil (parts per thousand) of the standard ratio.

**Quality control.** A retail beet sugar sample produced by British Sugar was included in each batch of analyses to assess the between batch precision of the pyrolytic  $\delta^{13}\text{C}\text{‰}$  and  $\delta^{18}\text{O}\text{‰}$  measurements. A total of nine triplicate measurements were made over a one-month period. The repeatability of the  $\delta^{18}\text{O}\text{‰}$  measurement ( $\sigma_{n-1}$ ) was 0.5‰, which compares favourably with the precision quoted by Werner et al. [4] for this procedure of 0.8‰.

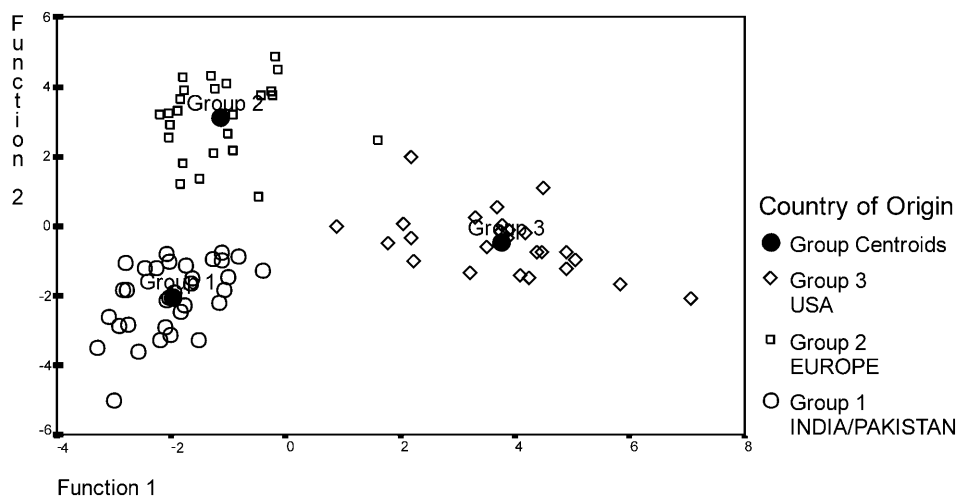
## Results and discussion

The combined isotope and trace element data was subjected to ‘stepwise’ canonical discriminant analysis (CDA) with the Indian/Pakistani, European and USA countries entered as groups 1–3 respectively. This approach was used to find a combination of functions based on the isotope and trace element values that maximised the separation between countries of origin. The ‘stepwise’ approach also selected the minimum number of variables, from the 52 variables in the original data set, required to maximise the separation, therefore reducing the risk of ‘over-fitting’ the data.

The results of the CDA, including the group centroids, are shown in Fig. 1. 100% of the European, American and Indian/Pakistani samples were correctly classified. The following nine variables were selected by the software for the discrimination;  $\delta^{13}\text{C}$ ,  $\delta^{18}\text{O}$ , boron (B), magnesium (Mg), selenium (Se), rubidium (Rb), gadolinium (Gd), holmium (Ho) and tungsten (W). Function 1 (57% of variance) which provided the main separation between the Indian/Pakistani and USA rice was primarily correlated with B,  $\delta^{18}\text{O}$ , W, Ho, Rb and Gd. Function 2 (43% of variance) which provided the discrimination between the Indian/Pakistani and European rice was primarily correlated with  $\delta^{13}\text{C}$ , Mg and Se.

The mean, maximum and minimum values observed for  $\delta^{13}\text{C}$ ,  $\delta^{18}\text{O}$ , boron, magnesium, selenium, rubidium,

**Fig. 1** A graphical representation of the stepwise canonical discriminant analysis of isotopic and multi-element data obtained from the rice samples



**Table 1** Indian and Pakistani rice – the mean values and range for elements selected by CDA as providing the best indication of geographical origin. The oxygen isotopic data is expressed relative to

the international V-SMOW scale and the carbon isotopic data is expressed relative to the international PDB scale. The multi-element data is expressed in parts per billion (ppb)

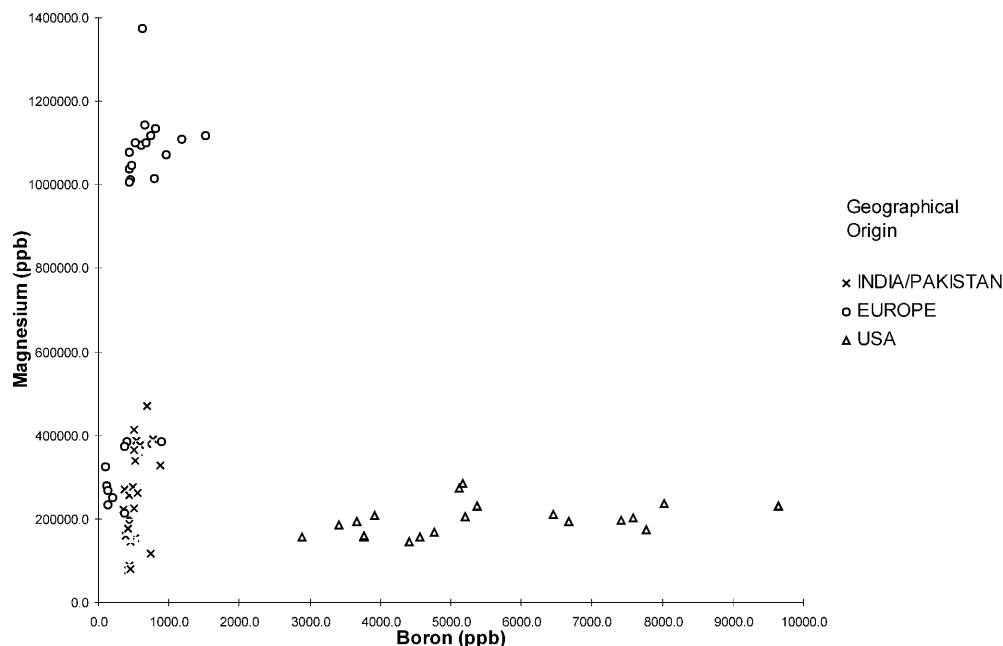
Sample no.	Country	Region	$\delta^{18}\text{O}\text{‰}$	$\delta^{13}\text{C}\text{‰}$	B	Mg	Se	Rb	Gd	Ho	W
1	India	Haryana/UP	23.5	-27.0	687.5	379597.4	173.4	1132.9	0.2	0.0	0.7
2	India	Haryana/UP	22.4	-27.4	575.5	360166.7	152.3	881.6	0.1	0.0	0.5
3	India	Purchased in Madras	21.1	-28.0	445.9	88804.0	69.0	757.0	0.1	0.0	0.9
4	India	Purchased in Madras	23.5	-27.3	516.6	154813.0	211.0	792.0	0.2	0.0	0.7
5	India	Purchased in Madras	24.7	-27.1	441.7	204096.0	161.4	1063.2	0.3	0.0	0.8
6	India	Purchased in Madras	22.9	-27.5	455.2	144113.7	157.9	1165.8	0.3	0.0	0.8
7	India	Purchased in Madras	24.3	-27.2	432.0	186911.0	143.2	1118.1	0.3	0.0	0.5
8	India	Purchased in Madras	23.7	-27.4	384.5	160476.0	175.0	851.0	0.1	0.0	0.3
9	India	Purchased in Madras	21.1	-27.9	428.1	175919.0	157.0	1519.0	0.4	0.1	1.4
10	India	Purchased in Madras	23.5	-27.7	745.6	116238.8	167.0	866.7	0.0	0.0	0.7
11	India	Punjab	23.4	-27.3	772.4	389233.8	115.8	1155.2	0.1	0.0	1.1
12	India	Punjab	22.9	-27.8	547.9	387079.9	219.3	1038.9	0.2	0.0	0.4
13	India	Unknown	23.8	-27.5	369.5	270527.9	232.3	3877.3	0.4	0.1	3.0
14	India	Purchased in Madras	18.2	-28.4	422.7	75697.0	83.0	1418.0	0.1	0.0	1.0
15	India	Haryana	20.1	-27.9	591.6	376761.5	94.2	1613.4	0.2	0.0	0.6
16	India	Haryana	15.4	-28.4	526.9	338748.8	233.1	419.7	0.3	0.0	1.6
17	India	Unknown	19.6	-27.7	541.8	259669.4	151.6	1425.1	0.4	0.1	4.0
18	India	Purchased in Madras	17.3	-28.4	353.5	223430.0	80.0	2169.0	0.6	0.1	0.5
19	India	Unknown	21.5	-29.3	453.5	79309.1	14.5	545.4	0.2	0.0	0.6
20	India	Unknown	24.8	-27.5	542.2	276265.8	38.6	742.1	0.1	0.0	1.7
21	India	Unknown	22.3	-27.6	499.4	226438.1	19.0	377.9	0.1	0.0	1.1
22	India	Unknown	23.1	-28.3	873.8	326688.9	5.3	1028.4	0.4	0.0	1.1
23	India	Unknown	26.1	-26.1	500.1	412154.9	44.5	1164.6	0.2	0.0	2.7
24	Pakistan	Unknown	25.0	-25.8	435.3	255641.8	47.6	1968.5	0.2	0.0	1.8
25	Pakistan	Unknown	24.7	-25.9	512.9	365068.7	24.2	2119.2	0.1	0.0	3.6
26	Pakistan	Unknown	21.7	-26.4	497.6	276520.8	47.1	2773.8	0.0	0.0	2.8
27	Pakistan	Unknown	23.4	-26.6	558.5	261268.1	20.4	2103.0	0.2	0.0	1.2
28	Pakistan	Unknown	21.8	-25.9	695.6	469526.4	22.9	3314.6	0.1	0.0	4.1
MEAN			22.4	-27.4	528.8	258613.1	109.3	1407.2	0.2	0.0	1.4
max			26.1	-25.8	873.8	469526.4	233.1	3877.3	0.6	0.1	4.1
min			15.4	-29.3	353.5	75697.0	5.3	377.9	0.0	0.0	0.3

gadolinium, holmium and tungsten are given in Tables 1, 2 and 3 for India/Pakistan, Europe and the USA respectively.

The oxygen isotopic data is expressed relative to the international V-SMOW scale and the carbon isotopic data is expressed relative to the international Pee Dee Belemnite (PDB) scale. The multi-element data is expressed in parts per billion (ppb).

In order to illustrate the contribution of individual elements to the canonical discrimination, a number of X-Y scatter plots were prepared, using those variables with the most significant standardised function coefficients. Figure 2 is a plot of the boron concentration (ppb) vs the magnesium concentration (ppb) for the 73 authentic rice samples. All of the samples cultivated in the USA exhibit relatively high levels of boron (minimum

**Fig. 2** An X-Y scatter plot of the boron and magnesium concentrations (ppb) measured in the rice samples



**Table 2** European rice – the mean values and range for elements selected by CDA as providing the best indication of geographical origin. The oxygen isotopic data is expressed relative to the inter-

national V-SMOW scale and the carbon isotopic data is expressed relative to the international PDB scale. The multi-element data is expressed in parts per billion (ppb)

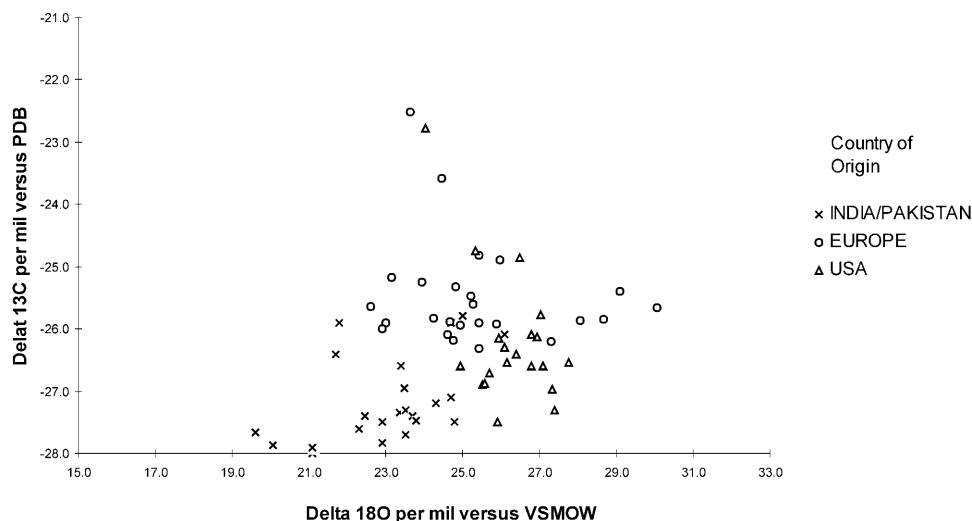
Sample no.	Country	region	$\delta^{18}\text{O}\%$	$\delta^{13}\text{C}\%$	B	Mg	Se	Rb	Gd	Ho	W
29	France	Camargue	24.4	-23.6	406.0	384476.9	6.1	8825.0	0.0	0.0	0.0
30	France	Camargue	23.6	-22.5	897.5	386329.7	58.5	364.6	0.0	0.0	0.2
31	France	Camargue	27.3	-26.2	97.2	323728.5	58.3	169.6	0.0	0.0	1.2
32	Italy	unknown	23.1	-25.2	659.5	1143052.4	23.8	763.0	0.1	0.0	0.4
33	Italy	unknown	25.4	-25.9	121.9	278551.0	44.4	1302.5	0.0	0.0	0.0
34	Italy	unknown	25.9	-25.9	139.8	267967.6	45.9	303.6	0.0	0.0	0.0
35	Italy	unknown	25.4	-26.3	127.7	233574.2	26.3	614.3	0.0	0.0	0.1
36	Italy	unknown	25.3	-25.6	375.6	372345.6	178.3	3624.5	0.3	0.1	2.2
37	Italy	unknown	24.8	-25.3	206.7	252085.0	8.8	390.8	0.1	0.0	0.5
38	Italy	Barragio	25.4	-24.8	451.5	1013084.6	97.4	2881.9	0.1	0.0	0.1
39	Italy	Casalino	24.7	-25.9	606.2	1095491.7	-8.8	2234.2	0.3	0.0	0.3
40	Italy	Casalino	22.6	-25.7	521.7	1101266.6	39.9	1699.5	0.1	0.0	0.3
41	Italy	Ferrara	25.2	-25.5	807.5	1133892.5	131.3	1682.8	0.1	0.0	0.4
42	Italy	Santhia	23.9	-25.2	441.6	1036546.7	15.1	1672.7	0.1	0.0	0.2
43	Italy	Tricerro	24.9	-25.9	441.0	1076864.8	1.4	1079.1	0.1	0.0	0.2
44	Italy	Vercelli	23.0	-25.9	794.4	1015696.4	32.9	282.9	0.1	0.0	0.4
45	Italy	Vercelli	24.2	-25.8	477.3	1046032.2	4.3	1018.5	0.0	0.0	1.4
46	Italy	Vercelli	22.9	-26.0	618.3	1374757.4	59.2	350.6	0.1	0.0	0.3
47	Italy	Vercelli	24.8	-26.2	740.5	1118265.6	113.1	648.5	0.1	0.0	0.1
48	Italy	Vercelli	24.6	-26.1	437.8	1006239.2	32.7	274.4	0.1	0.0	0.2
49	Spain	Seville	26.0	-24.9	1178.5	1108387.7	27.2	561.4	0.4	0.1	0.9
50	Spain	Seville	28.7	-25.8	1513.0	1117532.5	13.4	787.9	0.1	0.0	0.1
51	Spain	Seville	28.1	-25.9	964.1	1072215.2	26.0	895.5	0.2	0.0	0.0
52	Spain	Seville	30.1	-25.7	682.2	1101161.7	132.3	2077.1	0.2	0.0	0.3
53	Spain	unknown	29.1	-25.4	375.0	214265.0	22.0	280.3	0.1	0.0	0.1
MEAN			25.3	-25.5	563.3	810952.4	47.6	1391.4	0.1	0.0	0.4
max			30.1	-22.5	1513.0	1374757.4	178.3	8825.0	0.4	0.1	2.2
min			22.6	-26.3	97.2	214265.0	-8.8	169.6	0.0	0.0	0.0

2886.5 ppb). These samples were well differentiated from European samples (maximum 1513.0 ppb) and Indian/Pakistani rice (maximum 873.8 ppb). Relatively high levels of boron are therefore a reliable indicator that a sample of rice has been grown in one of the recognised

rice growing regions in the USA (Arkansas, Louisiana, Mississippi or Texas).

The majority of the European rice samples analysed (64%) contained relatively high levels of magnesium; however, a number of the European rice samples con-

**Fig. 3** An X-Y scatter plot of the  $\delta^{18}\text{O}\text{‰}$  and  $\delta^{13}\text{C}\text{‰}$  values determined for the authentic rice samples



**Table 3** USA rice – the mean values and range for elements selected by CDA as providing the best indication of geographical origin. The oxygen isotopic data is expressed relative to the inter-

national V-SMOW scale and the carbon isotopic data is expressed relative to the international PDB scale. The multi-element data is expressed in parts per billion (ppb)

Sample no.	Country	Region	$\delta^{18}\text{O}\text{‰}$	$\delta^{13}\text{C}\text{‰}$	B	Mg	Se	Rb	Gd	Ho	W
54	USA	Arkansas	26.8	-26.6	7423.1	196684.8	124.6	1849.0	1.8	0.3	0.1
55	USA	Arkansas	27.1	-26.6	6667.4	193288.0	398.6	2661.2	1.9	0.3	0.0
56	USA	Arkansas	26.5	-24.9	6460.6	212144.3	293.2	1517.4	1.8	0.3	0.0
57	USA	Arkansas	27.8	-26.5	7592.5	201437.7	339.5	1899.7	1.8	0.3	0.2
58	USA	Arkansas	26.9	-26.1	9649.4	231956.9	224.4	2697.1	2.0	0.3	0.0
59	USA	Arkansas	26.8	-26.1	7772.2	174370.1	130.6	1260.0	1.5	0.2	0.2
60	USA	Louisiana	24.9	-26.6	5124.6	274294.7	147.4	1414.9	0.1	0.0	0.0
61	USA	Louisiana	25.5	-26.9	5365.6	230964.9	109.1	1576.3	0.1	0.0	0.1
62	USA	Mississippi	25.7	-26.7	3927.1	206831.7	103.9	1486.9	0.3	0.0	0.4
63	USA	Mississippi	26.4	-26.4	4567.2	155779.4	130.8	1656.9	0.1	0.0	0.2
64	USA	Mississippi	24.0	-22.8	3671.8	194310.2	79.8	972.3	0.3	0.0	0.2
65	USA	Mississippi	25.3	-24.7	5160.6	285171.3	103.5	1642.2	0.1	0.0	0.1
66	USA	Mississippi	26.2	-26.5	8028.9	236899.1	99.4	2528.4	0.1	0.0	0.6
67	USA	Mississippi	25.9	-26.2	5208.5	205663.1	63.9	1358.9	0.1	0.0	0.2
68	USA	Texas	25.9	-27.5	3766.4	155963.9	176.5	1885.8	0.1	0.0	0.1
69	USA	Texas	27.4	-27.3	4414.0	146584.7	160.9	4323.4	0.1	0.0	0.1
70	USA	Texas	26.1	-26.3	4759.0	168937.6	198.8	1775.9	0.2	0.0	0.1
71	USA	Texas	27.3	-27.0	3416.9	184778.4	216.3	2493.6	0.1	0.0	0.1
72	USA	Texas	27.0	-25.8	3764.2	158637.5	183.4	2644.3	0.2	0.0	0.0
73	USA	Texas	25.6	-26.9	2886.5	156353.5	105.8	2188.8	0.1	0.0	0.1
MEAN			26.3	-26.2	5481.3	198552.6	169.5	1991.7	0.6	0.1	0.1
max			27.8	-22.8	9649.4	285171.3	398.6	4323.4	2.0	0.3	0.6
min			24.0	-27.5	2886.5	146584.7	63.9	972.3	0.1	0.0	0.0

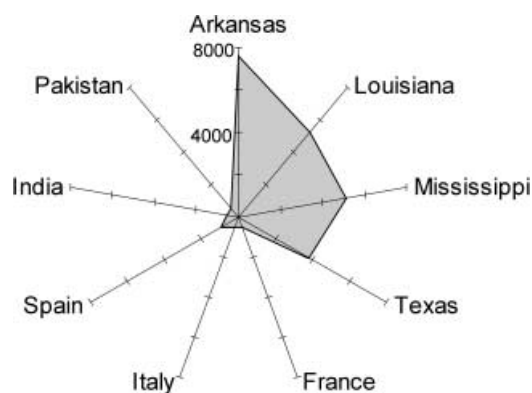
tained relatively low levels of magnesium similar to those found in rice grown in the USA and India/Pakistan. In general a concentration of magnesium above 1,000,000 ppb or 0.1 wt% is a reasonable indicator that a rice has been grown in Europe. However, samples exhibiting lower magnesium levels may still have been cultivated in Europe.

Of the 28 Indian/Pakistani rice samples, 93% contained more than 0.45 ppb tungsten. Of the 25 USA rice samples, 96% contained less than this amount. Similarly, 80% of the 25 European rice samples contained less than 0.45 ppb tungsten. The levels of selenium exhibited by Indian and Pakistani rice samples cover a wide concen-

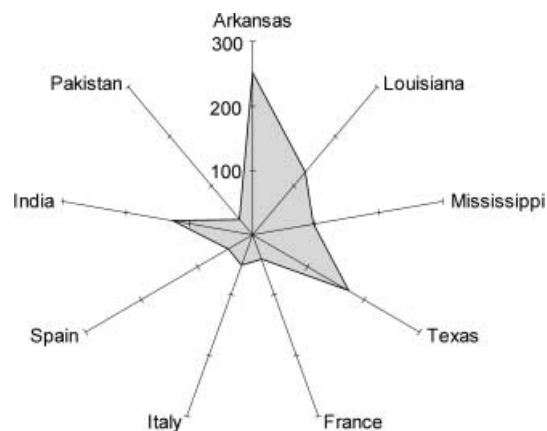
tration range from 0.3 to 4.1 ppb. A significant proportion (72%) of rice samples produced in the USA possess selenium concentrations greater than 110 ppb whereas 84% of European rice samples contain less than 110 ppb selenium.

The effect of the remaining trace elements Rb, Gd and Ho on the geographical separation of rice samples cannot be easily interpreted, but they have a significant effect on the canonical discriminant functions that generate the geographical groupings. It was noted, however, that American rice samples originating from Arkansas exhibited holmium concentrations that were as much as 30 times greater than rice samples from the other grow-

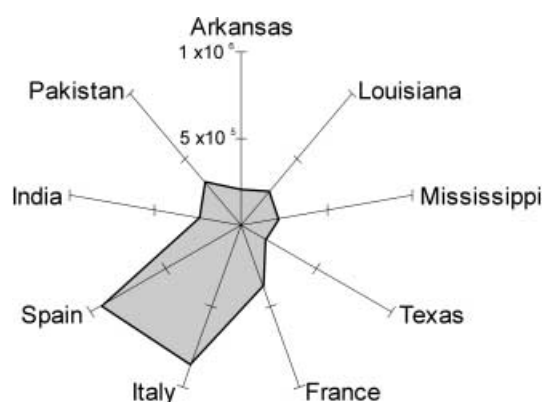




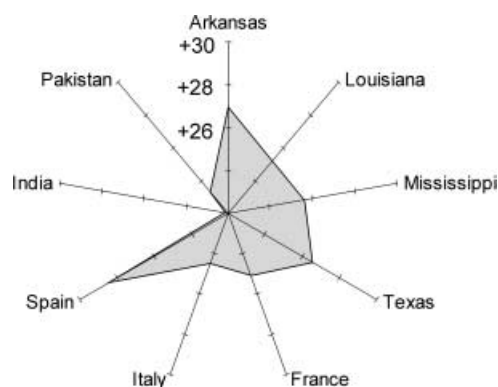
**Fig. 4** A radar plot showing the geographical variation in the mean concentration of boron (ppb) in the rice samples



**Fig. 6** A radar plot showing the geographical variation in the mean concentration of selenium (ppb) in the rice samples



**Fig. 5** A radar plot showing the geographical variation in the mean concentration of magnesium (ppb) in the rice samples



**Fig. 7** A radar plot showing the geographical variation in the mean  $\delta^{18}\text{O}$  value for the rice samples

ing regions in the USA. This also resulted in the elevation of the mean concentration of holmium for the USA. Holmium therefore may provide a USA 'State-specific' marker of geographical origin. However, only six samples have been analysed from this location and further samples would need to be analysed to verify this finding.

The  $\delta^{18}\text{O}\text{‰}$  and  $\delta^{13}\text{C}\text{‰}$  values of rice samples have been plotted as an X-Y scatter graph in Fig. 3. As expected, rice irrigated with water from the Himalayas exhibits relatively low  $\delta^{18}\text{O}\text{‰}$  values due to the depletion in the  $^{18}\text{O}$  isotope associated with precipitation at high altitudes. The mean  $\delta^{18}\text{O}\text{‰}$  value found for Indian and Pakistani rice was +22.3‰. The European rice samples exhibited the next highest set of  $\delta^{18}\text{O}\text{‰}$  values with a mean value of +25.3‰. The rice samples originating from the USA possessed the highest mean  $\delta^{18}\text{O}\text{‰}$  value of +26.3‰.

The Indian and Pakistani rice samples had relatively low  $\delta^{13}\text{C}\text{‰}$  values. The mean  $\delta^{13}\text{C}\text{‰}$  value of this set of samples was -27.4‰. The American rice samples were found to be relatively enriched in  $^{13}\text{C}$  compared to the Indian and Pakistani samples with a mean  $\delta^{13}\text{C}\text{‰}$  value of -26.2‰. The European samples were the most enriched in  $^{13}\text{C}$  and with a mean  $\delta^{13}\text{C}\text{‰}$  value of -25.5‰.

For ease of comparison the geographical variation in the mean concentrations of boron, magnesium and selenium in the rice samplers, presented as radar plots, are shown in Figs. 4, 5 and 6 respectively. Figure 7 shows the geographical variation of  $\delta^{18}\text{O}$  values for the rice samples in the form of a radar plot.

## Conclusions

The values of nine variables –  $\delta^{13}\text{C}$ ,  $\delta^{18}\text{O}$ , boron, magnesium, selenium, rubidium, gadolinium, holmium and tungsten – have been identified, by canonical discriminant analysis, as providing the best information for differentiating between rice grown in Europe, the United States of America and the Basmati regions of India and Pakistan. This has permitted an empirical model to be developed to establish the geographical origin of unknown samples.

The concentration of specific elements such as boron and magnesium can be used exclusively as reasonable indicators of the geographical origin of rice. The role of the other elements in this empirical model is more complex but contributes significantly to the overall discrimination of country of origin.

The stable isotope ratios of oxygen and carbon have been shown to be significantly correlated with the geographical origin of rice samples. However, because the determination of origin is comparative and these variables are altered by seasonal variations, it will always be necessary to have an up-to-date supply of commercially sourced authentic rice samples to assess accurately unknown samples. The trace element composition of rice is less likely to be affected by seasonal variations and in this respect provides a more robust indicator of geographical origin. This set of results clearly indicates that the trace element composition of rice samples due to topographic or soil factors and/or possible input from production processes is a powerful tool for characterisation of the geographical origin of rice. In addition, the measurement of  $\delta^{18}\text{O}\%$  values in rice carbohydrate are applicable to the characterisation of the geographical origin. This is clearly demonstrated by the effect of irrigation with Himalayan waters on the fractionation of these isotopes in rice grown in the Basmati region.

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