Analytical Methods

Verifying the geographical origin of beef: The application of multi-element isotope and trace element analysis

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Abstract

Beef samples originating from the major cattle producing regions of the world (Europe, USA, South America, Australia and New Zealand) have been analysed using IRMS and ICP-MS. C and N isotope composition of the beef defatted dry mass and H and O isotope composition of the corresponding lipid fractions were determined. It was observed that intensive maize and/or C_4 pasture feeding, during cattle production, gave rise to significant differences in the ^{13}C content of beef produced in Brazil and the USA versus British beef fed predominantly on C_3 pasture and fodder. The mean $\delta^2H‰$ and $\delta^{18}O‰$ values of beef lipid correlated well with the latitude of production regions and the relationship between the H and O isotopic contents were found to parallel the Meteoric Water Line. These findings support the hypothesis that the systematic global variations in the ^2H and ^{18}O content of precipitation are transferred through drinking water and feed into beef lipid. Multi-element concentrations determined in the beef were combined with the stable isotope data and submitted to multivariate analysis. Six key variables ($\delta^{13}C‰$ (defatted dry mass), Sr, Fe, $\delta^2H‰$ (lipid), Rb and Se) were identified by canonical discriminant analysis as providing the maximum discrimination between beef samples on the basis of the broad geographical areas (Europe, South America and Australasia). It was concluded that the methodology in its current state can be used to provide reliable origin information, but this is dependent upon the countries under investigation.
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1. Introduction

As a result of concerns relating to bovine spongiform encephalopathy (BSE), Human variant Creutzfeldt-Jakob disease (CJD) and the impact on the internal market, the European Commission established far reaching legislation concerning the labelling of beef. The introduction of pan-European compulsory beef labelling rules, from the 1st September 2000 onwards was designed to provide consumers with correct, complete and transparent information to enable them to make an informed choice on the type and origin of beef they purchased (Council Regulation (EC) No. 2772/1999). As a consequence of this legislation it is reasonable to suggest that there should be analytical methods in place that can verify the information provided on origin labels describing where an animal has been reared.

In addition, there are a number of consumer driven forces for reliable analytical methods to verify the provenance of the food we eat and there is growing enthusiasm amongst consumers for high quality food with a clear regional identity. The European Union Protected Food Names Schemes came into force in 1992 (Council Regulation (EEC) No. 2081/92) and offers an independent inspection and labelling system for the protection of food names on a geographical basis (e.g. Protected Designation of Origin Orkney beef and Protected Geographical Indication

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Welsh beef). Manufacturers of Protected foods usually charge a premium for their produce due to increased production costs and consequently economic incentives exist to replace genuine articles with inferior counterfeit products for financial gain.

The use of multi-isotopic and/or multi-element analyses to provide information on the provenance of foods is gaining wider acceptance. The relevant literature and scientific rationale behind the exploitation of stable isotope and trace element systematics have been recently reviewed (Franke, Gremaud, Hadorn, & Kreuzer, 2005; Hölzl, Horn, Rossmann, & Rummel, 2004; Kelly, Heaton, & Hoogewerff, 2005). However, using these techniques to infer geographical origin is far from trivial. There are confounding factors, such as imported feed, animal movement prior to 'finishing' and metabolic turnover times of animal tissues (Schwagele, 2005) to consider. Nevertheless, there have been a number of specific publications recently relating to the identification of beef geographical origin and production regime using multiple isotope analysis of the bio-elements (H, C, N, O and S), and trace elements, with varying degrees of success (Boner & Förstel, 2004; Franke et al., in press; Hegerding, Seidler, Danneel, Gessler, & Nowak, 2002; Renou et al., 2004; Schmidt, O. et al., 2005) lamb (Piasentier, Valusso, Camin, & Versini, 2003; Sacco, Brescia, Buccolieri, & Jambrenghi, 2005) and pork (González-Martin, González-Pérez, Hernández Méndez, Marqués-Macias, & Sanz-Poveda, 1999). In this paper we report the use of combined multi-element isotope (^{2}H, ^{13}C, ^{15}N and ^{18}O) and trace element analysis to determine the geographical origin of beef.

2. Materials and methods

2.1. Authentic beef muscle samples

Over 200 authentic raw beef samples were obtained from a wide range of geographical locations including major beef producing countries e.g. Brazil, Argentina and Australia. The majority of the samples were obtained in the UK via the Veterinary Medicines Directorate drug residue sampling programme with the agreement of the UK Port Health Authorities. These samples were collected by Port Health Officials as the beef entered the UK. Additional samples were received via food control laboratories in other countries and directly from abattoirs in the UK. Beef samples were frozen at −18 °C directly after collection. Once at the laboratory, all beef samples were stored frozen (−20 °C) until partially thawed before sample preparation. Samples were re-frozen after sub-sampling.

2.2. Sample preparation

Beef samples (∼50 g) were freeze-dried and homogenised using ceramic scissors and/or an agate pestle and mortar. The lipid component from 10 g of the homogenised material was extracted with an iso-propanol/hexane mix-

ture according to the method of Hara and Radin (1978). The beef defatted dry mass (DDM) was separated from the iso-propanol/hexane solvent by vacuum filtration and air-dried overnight in a fume hood. The fat-free dry mass was then re-homogenised using an agate pestle and mortar and stored in a Securitainer® at −20 °C until analysis. The solvent was removed from the extracted lipid under reduced pressure in a water bath at 45 °C. The beef lipid was transferred to a 4 ml vial and residual solvent removed using a flow of nitrogen gas in a Techne® Sample Concentrator set at 30 °C. The lipid was then stored in the dark under nitrogen in a vial at −20 °C until analysis.

2.3. Isotopic and trace element reference materials

NBS22 (mineral oil), IAEA-601 (benzoic acid) and USGS 40 (L-glutamic acid) were obtained from the International Atomic Energy Agency, Vienna, Austria. NBS22 has an assigned δ^2H‰ value of −118.5‰ versus Vienna-Standard Mean Ocean Water (V-SMOW) and an assigned δ^{13}C‰ value of −29.7‰ versus Pee Dee Belemnite (PDB). IAEA-601 has a δ^{18}O‰ value of +23.3 versus V-SMOW. The USGS40 has an assigned δ^{13}C‰ value of −26.2‰ versus PDB and an assigned δ^{15}N‰ value of −4.5‰ versus Air. Inductively coupled plasma multi-element standard solution Merck VI CertiPUR® was obtained from VWR, Lutterworth, Leicestershire, UK. The composition and concentration of the Merck VI standard was as described in the accompanying certificate of analysis. Trace element reference material 8414 (bovine muscle powder) was obtained from National Institute of Standards and Technology, Gaithersburg, Maryland, USA. The best estimate values for constituent elements in RM 8414 are detailed in the accompanying certificate.

2.4. Natural-abundance (HCNO) stable isotope analysis

2.4.1. Simultaneous ^{13}C and ^{15}N analysis

Samples of 1 mg of beef fat-free dry mass were weighed into tin capsules. The tin capsules were sealed and placed inside a 'zero-blank' autosampler attached to a Eurovectar ECS4010 elemental analyser (EA). A typical EA combustion/reduction configuration was used to produce carbon dioxide and nitrogen gas for δ^{13}C‰ and δ^{15}N‰ measurement respectively (Vallet, Arendt, Mabon, Naulet, & Martin, 1991).

Samples were analyzed in triplicate and values accepted when precision ($\sigma n - 1$, $n = 3$) was <0.3‰ for δ^{15}N‰ analysis and <0.2‰ for δ^{13}C‰ analysis. Nitrogen and carbon isotope data are reported in conventional δ-notation in units of per mil (‰) with respect to atmospheric nitrogen (air) and Pee Dee Belimnite (PDB), respectively and according to

$$\delta_{ref} = \left(\frac{R_{samp} - R_{ref}}{R_{ref}} \right) \times 1000, \quad (1)$$

where δ_{ref} is the isotope ratio of the sample expressed in delta units (‰, per mil) relative to the reference material.

R_{samp} and R_{ref} are the absolute isotope ratios of the sample and reference material, respectively.

2.4.2. Simultaneous ^2H and ^{18}O analysis

Sufficient beef lipid (1 µl $\approx$ 200 µg H) was dispensed into silver capsules using a Hamilton 5 µl plunger-in-needle syringe. The silver capsule were sealed and placed inside a 'zero-blank' autosampler attached to a Eurovectar High Temperature Generator (HTG) mounted on an ECS4010 elemental analyser (EA). Prior to dropping the capsules the HTG induction coil was energised for 65 s. The current induced in the graphite susceptor raised the localised temperature repeatably to approximately 1500 °C for 20 s. Under these conditions the samples were converted to hydrogen and carbon monoxide which was swept by a flow of helium carrier gas over the lower section of the quartz tube that contained glassy carbon chips maintained at a constant temperature of 900 °C. The pyrolysis gasses then passed through a GC column packed with molecular sieve 5 Å (4 mm i.d., length 2 m) heated to 80 °C, which separated H_2, N_2 and CO.

The GC effluent then flowed into the stable isotope ratio mass spectrometer via a 'Conflo III' interface. Peak jumping between the elution of hydrogen and carbon monoxide permitted the ratio of the isotopologues of both gasses to be determined in one analysis. The test gasses were compared against hydrogen and carbon monoxide reference gasses of known $^2H/^1H$ and $^{18}O/^{16}O$ ratios previously calibrated against accepted International Atomic Energy Agency reference materials.

Measurements were made on four replicate weighings of the same sample of beef lipid. The first of each set of four replicates was discarded due to memory effect between subsequent $\delta^{18}O‰$ analyses. Values were accepted when precision ($\sigma n - 1$, $n = 3$) was $\leqslant 0.4‰$ for $\delta^{18}O‰$ analysis and $\leqslant 3.0‰$ for $\delta^2H‰$ analysis. Oxygen and hydrogen isotope data are reported in conventional δ-notation in units of per mil (‰) with respect to Vienna-Standard Mean Ocean Water and according to Eq. (1) above.

2.5. Trace element analysis

2.5.1. Sample preparation

Freeze-dried beef tissue (0.5 g) was placed in an acid-cleaned 15 cm long quartz tube and digested in 2 ml Ultrapur concentrated nitric at 80 °C for two hours, after closing the tube with a PTFE stopper. One millilitre of Ultrapur hydrogen peroxide solution (31%) was added to the digest and the quartz tube containing the sample placed in a UV-digester for 8 h. A 100 µl aliquot of the UV-digest was then diluted 50-fold with 2% Ultrapur nitric acid containing internal standards (platinum, rhodium and germanium) at 20 ppb (v/v).

2.5.2. Instrumental operation

The test solutions were measured by inductively coupled plasma mass spectrometry (Agilent 7500c ICP-MS). The instrument was calibrated using acid matched multi-element standards (Merck VI). Quality Control consisted of blanks, 10% duplicates and a reference material (NIST8414, bovine muscle) to ascertain detection limits, and accuracy. Normal GLP 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) reference material values within $\pm 40\%$ of certified limits.

2.6. Multivariate analysis of stable isotope and trace element data

The multivariate method chosen, to interrogate the stable isotope and multi-element data, was Canonical Discriminant Analysis (CDA). CDA was applied with the aim of finding a rule that allocated beef samples of unknown origin to the correct group, in this case geographical origin (Rencher, 1992).

The selection of the most significant variables was performed by forward 'stepwise' analysis. The variables were included in the model one by one, choosing at each step the variable that made the most significant additional contribution to the discrimination (i.e. with the largest F value – the value of F provides a test for the statistical significance of the observed differences among the means of two or more groups). Measured variables were excluded from the model if they were shown to be redundant. On the basis of the selected variables, different independent discriminant functions were computed by canonical discriminant analysis. Their maximum number is equal to either the number of variables or the number of groups (in this case geographical origins) minus one, if the latter is smaller. The statistical significance of each discriminant function was evaluated on the basis of the Wilks' Lambda factor after the function was removed. This parameter ranges from 1.0 (no discriminatory power) to 0.0 (perfect discriminatory power). The separation among groups in the discriminant space was checked by plotting the first and the second functions

Finally, to verify the power and the stability of the model, a 'leave-one-out' cross-validation discriminant analysis was performed. In this test known samples were used as 'unknowns' to validate the model built on the basis of a reduced set of cases. Effectively, one sample was removed from the data set and then classified on the basis of a model constructed from the remainder. This process was then repeated for each sample in turn and the success of the classification re-calculated by comparison with the known origin. The software used to perform the CDA was SPSS version 13.0.

3. Results and discussion

3.1. Evaluation of beef defatted dry mass (DDM) $\delta^{13}C‰$ measurements

The stable C and N isotope composition of the beef DDM and stable H and O isotope composition of the beef

lipid fraction are summarised in Table 1. The Table shows the mean value for each isotope parameter by geographical origin and the sample standard deviation ($\sigma n - 1$) when the number of authentic samples (n) was greater than or equal to three.

The $\delta^{13}C‰$ and $\delta^{15}N‰$ measurements obtained from the DDM of beef samples from England, Ireland, Scotland, Brazil and the USA are shown in Fig. 1. The spatial separation of Brazilian and USA samples from English, Irish and Scottish samples can be seen clearly along the y-axis which displays $\delta^{13}C‰$ values measured from the beef DDM. None of the English, Irish or Scottish beef DDM possessed $\delta^{13}C‰$ values in excess of $-20‰$. These data are in good agreement with the findings of Schmidt, O. et al. (2005), who observed that Irish beef DDM samples possessed $\delta^{13}C‰$ values less than $-20‰$ and the $\delta^{13}C‰$ of beef from other European countries was less than $-17‰$. Furthermore, the majority of Brazilian beef DDM $\delta^{13}C‰$ values measured in this study and those reported by Schmidt, O. et al. (2005) were less negative than $-13‰$. This finding supports the assertion that $\delta^{13}C‰$ may be used as a single discriminatory variable to distinguish between British and Brazilian beef (Schmidt, O. et al., 2005).

It is well established that differences in the $\delta^{13}C‰$ values of cattle tissues, such as hair (Minson, Ludlow, & Troughton, 1975) and dairy products such as milk (Kornexl, Werner, & Rossmann, 1997), butter (Rossmann et al., 2000) and cheese (Camin et al., 2004) are derived from the pro-

portions of C_3 and C_4 plant materials in the diet of the cattle. Extensive C_3 pasture feeding in Northern Europe does not preclude the use of maize (C_4) silage feeding over winter or during 'finishing' prior to slaughter. Furthermore, winter fodder contains high proportions of non-photosynthetic plant components such as cereal grains and this results in relatively higher $\delta^{13}C‰$ values (Schwertl, Auerswald, Schaufele, & Schnyder, 2005). However, it is apparent from our observations that the frequency and duration of maize or grain feeding may give rise to some of the higher $\delta^{13}C‰$ values observed in British beef, but it does not undermine the usefulness of beef DDM $\delta^{13}C‰$ as a simple basis for distinguishing between British and Brazilian beef.

Generally, the ^{13}C content of authentic beef DDM was relatively higher for Central and Southern Europe with mean $\delta^{13}C‰$ values ranging from $-21.24‰$ for Italy to $-22.56‰$ for Denmark. A few individual $\delta^{13}C‰$ values for Austrian beef samples were as high as $-12.8‰$, -14.6 and $-15.2‰$. High $\delta^{13}C‰$ values such as these have been observed previously for Central European beef samples (Boner & Förstel, 2004) and indicate prolonged periods of maize feeding probably in excess of 12 months. It has recently been demonstrated that the skeletal muscle of cattle, switched from a C_3 to C_4-maize silage diet, does not reach equilibrium with the feed $\delta^{13}C‰$ even after 168 days (Bahar, 2006).

Argentinean and Australian beef samples exhibited $\delta^{13}C‰$ values indicative of both exclusive C_3 and exclusive

Table 1
A summary of the stable C and N isotope composition of the beef defatted dry mass (DDM) and stable H and O isotope composition of the beef lipid fraction

| Origin | Beef defatted dry mass | | | | | | Beef lipid | | | | | |
| | $\delta^{13}C‰_{PDB}$ | | | $\delta^{15}N‰_{AIR}$ | | | $\delta^2H‰_{V-SMOW}$ | | | $\delta^{18}O‰_{V-SMOW}$ | | |
	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n
England	−24.34	5.76	78	6.7	0.9	78	−196.9	13.3	78	17.9	1.3	16[a]
Scotland	−25.75	0.57	19	7.1	0.8	19	−208.0	24.8	18	18.2	2.4	18
Ireland	−24.52	1.46	26	6.6	0.7	26	−189.8	19.5	8	18.3	2.8	8[a]
Denmark	−22.56	1.91	4	6.6	1.6	4	−211.3	14.5	4	17.7	1.3	4
France	−22.50	3.12	3	6.1	1.0	3	−216.6	1.6	3	19.0	0.2	3
Germany	−22.53	3.46	28	6.2	1.1	28	n.a	n.a.	n.a.	n.a.	n.a.	n.a.
Austria	−21.51	4.17	25	5.8	1.1	25	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Italy	−21.24	1.86	26	4.7	0.8	26	−220.1	4.7	9	18.3	0.5	9
Spain	−21.48	n.c.	1	5.8	n.c.	1	−176.4	n.c.	1	20.3	n.c.	1
Argentina	−20.79	2.29	10	6.6	1.2	10	−181.6	18.3	10	22.3	2.4	10
Uraguay	−20.28	0.73	3	6.8	1.7	3	−180.0	20.3	3	22.8	0.4	3
Brazil	−11.36	1.50	22	6.7	1.4	22	−183.0	18.0	20	21.4	2.3	20
North America	−11.05	n.c.	2	6.0	n.c.	2	−214.2	n.c.	2	17.9	n.c.	2
Australia	−16.87	4.88	3	6.1	0.1	3	−157.7	16.0	3	23.4	1.9	3
New Zealand	−26.32	0.61	6	5.8	0.8	6	−214.6	9.0	6	19.4	1.6	6
Namibia	−12.46	n.c.	1	7.3	n.c.	1	−151.0	n.c.	1	32.3	n.c.	1
Swaziland	−15.33	n.c.	1	7.3	n.c.	1	−140.5	n.c.	1	26.1	n.c.	1

n.a. = not available.

n.c. = not calculated.

The Table shows the mean value for each isotope parameter by geographical origin and the sample standard deviation (SD, $\sigma n - 1$) when the number of authentic samples (n) was greater than or equal to three.

[a] The relatively small number of beef lipid $\delta^{18}O‰$ results for the English and Irish beef samples were due to instrumental problems associated with obtaining acceptable precision for triplicate analyses. If the standard deviation was greater than 0.4‰ the results were excluded.
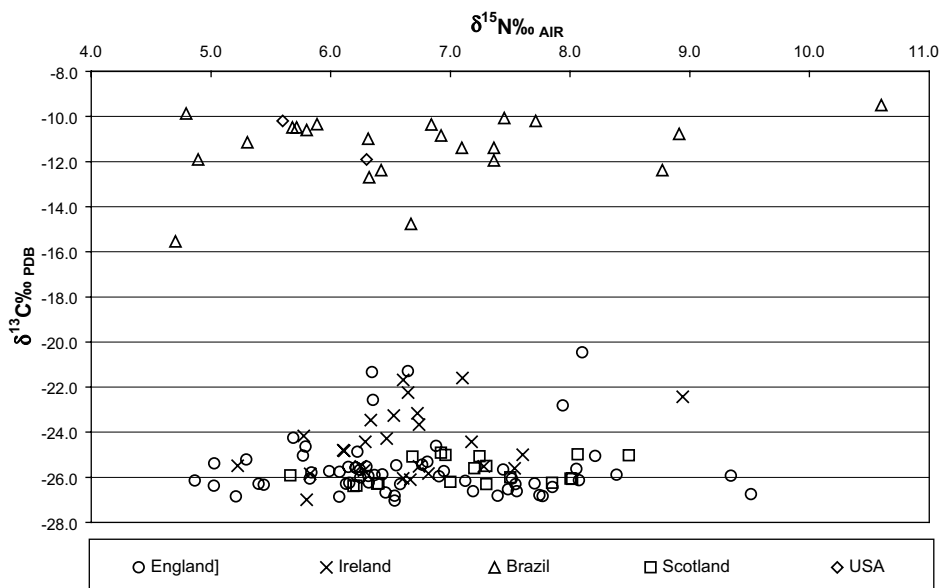
Fig. 1. A cross-plot of $\delta^{15}N‰$ and $\delta^{13}C‰$ values obtained from beef tissue defatted dry mass. The authentic beef samples originated from England, Ireland, Scotland, Brazil and the USA.

C_4 feeding and this may be a result of local feeding practice or an indicator of 'organic' versus conventional feeding regimes. However, no specific sampling of 'organic' beef was undertaken. New Zealand beef $\delta^{13}C‰$ values compare with those of temperate Northern European regions although the New Zealand dataset is relatively small ($n = 6$). Similarly, conclusions regarding the African beef $\delta^{13}C‰$ values must be tentative as they are based on only two samples (Namibia and Swaziland) but appear to indicate the predominance of maize feeding.

3.2. Evaluation of beef defatted dry mass (DDM) $\delta^{15}N‰$ measurements

The mean $\delta^{15}N‰$ values of the authentic beef DDM from the different geographical locations exhibited a relatively small variation from 4.7‰ for Italy to 7.3‰ for Namibia and Swaziland (Table 1). Some individual results for South American and English samples were in excess of 9‰ and may indicate the use of fishmeal or marine products such as seaweed in the cattle feed (Winkler, 1984). Other possible explanations for relatively high $\delta^{15}N‰$ values include the feeding of other animal products to cattle. Increases in trophic level give rise to 'jumps' in $\delta^{15}N‰$ values of around 3‰. However, this practice is forbidden in Europe because of the risks of BSE infection (Delgado & Garcia, 2001). Generally individual beef $\delta^{15}N‰$ results were similar to those reported previously by Boner and Förstel (2004) and Schmidt, O. et al. (2005). In Italy and Spain the beef DDM $\delta^{15}N‰$ values tended to be slightly less positive and may indicate wider use of synthetic nitrogen based fertilisers which may lead to slightly lower bulk $\delta^{15}N‰$ values in crops and cereals (Bateman, Kelly, & Jickells, 2005; Schmidt, H.-L. et al., 2005) that will be

translocated to cattle through feed. Alternatively, foraging of leguminous clover, which directly fixes atmospheric nitrogen, or feeding of leguminous concentrates (e.g. pea and soybean) has been shown to give rise to relatively low $\delta^{15}N‰$ values (Kornexl et al., 1997).

3.3. Evaluation of beef lipid δ^2H and $\delta^{18}O$ isotopic measurements

It has been demonstrated that beef tissue water $\delta^{18}O‰$ analysis is of limited use for geographical origin assignment as controlled experiments revealed that $\delta^{18}O‰$ values were affected by processing and storage conditions (Thiem, Lüpke, and Seifert, 2004). Additionally, measuring the $\delta^{18}O‰$ value of beef DDM proved problematic due to the formation of nitrogen monoxide, under pyrolytic conditions, which has an isotopologue ($^{14}N^{16}O$, m/z 30) that is an isobaric interference with carbon monoxide ($^{12}C^{18}O$, m/z 30). Consequently we opted to measure the deuterium and ^{18}O content of the beef lipid despite the possibility of epoxidation affecting the $\delta^{18}O‰$ results. To reduce the risk of epoxidation, beef lipid samples were stored in the dark at $-20\,°C$ under nitrogen gas until analysis.

The scatter-plot shown in Fig. 2 presents an overall indication of how the $\delta^{18}O‰$ values of lipids extracted from authentic beef samples vary with respect to approximate latitude of geographical origin, over 2 years of production. The regression analysis demonstrates that there is a significant degree of correlation ($R^2 = 0.76$) between the country-mean $\delta^{18}O‰$ of the beef lipid (Table 1) and the approximate latitude of the production region.

It is reasonable to assume that the isotopic signatures of plant material and water are both translocated into beef tissue (Hobson, Atwell, and Wassenaar, 1999). In fact,
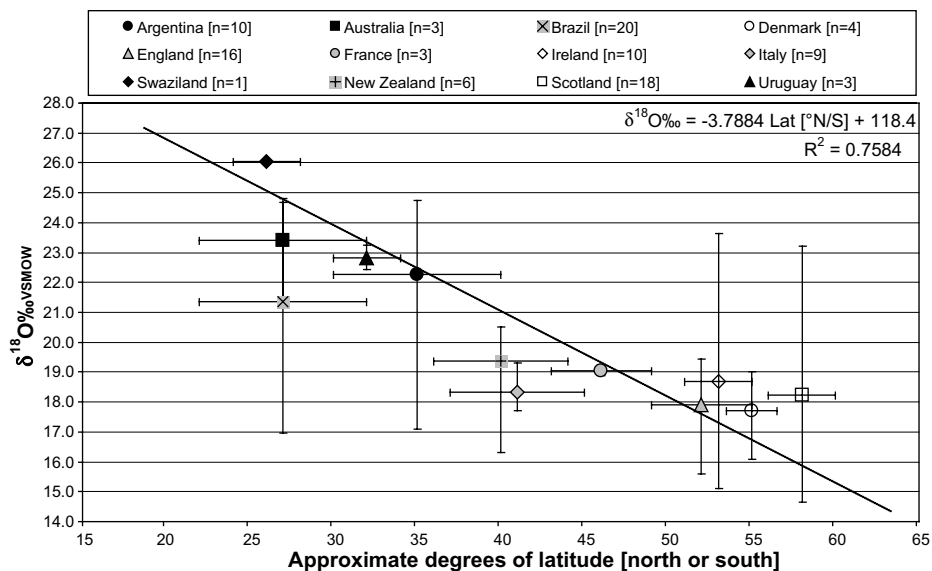
Fig. 2. Overall indication of how beef lipid $\delta^{18}O‰$ values vary with approximate latitude of production region. Y error bars show the maximum and minimum observed $\delta^{18}O‰$ values for that country of origin. X error bars show a range of latitude covering the major beef production areas for that country or origin.

deuterium labelling has recently been used to link hydrogen in dietary carbohydrate and water with those in fatty acids produced by chickens (Zhang, Pionnier, and Buddrus, 2006). Hence, it may be expected that the latitude effects known to influence the ^2H and ^{18}O isotopic values of groundwater taken up by vegetation and ultimately consumed by cattle in a given area should be observed, to some extent, in the $\delta^2H‰$ and $\delta^{18}O‰$ isotopic values of the beef lipid fractions. This is by no means a simple relationship because of the sources of hydrogen and oxygen in feed, water and air (in the case of oxygen). However, the correlation observed in Fig. 2 supports this hypothesis. The country-means of lipid $\delta^{18}O‰$ values were calculated and plotted against the approximate latitude at which the cattle had been reared (in degrees north or south of the equator). This is an approximation as in some cases exact geographical details of rearing have not been available. For example Brazil stretches latitudinally from the equator to around 30°S but it has been assumed for the data presented in Fig. 2 that the Brazilian beef samples originated from cattle that had been reared in that country's major cattle producing region of Matto Grosso do Sul in south west Brazil at a latitude of around 25°S. Nevertheless, the expected trend can clearly be seen in the resulting plot with mean $\delta^{18}O‰$ values increasing with latitudinal proximity to the equator (i.e. higher $\delta^{18}O‰$ values at the lower latitudes nearer the equator). Data obtained from $\delta^2H‰$ analyses also exhibits the expected latitudinal trend in isotopic values ($R^2 = 0.74$, not shown).

Plotting country-means of beef lipid $\delta^2H‰$ and $\delta^{18}O‰$ values (Fig. 3) provides a simpler visual representation of the complete data. It also reinforces the fact noted earlier that the recognised relationships between hydrogen and oxygen isotope ratios in fresh waters on a global scale. Generally, isotopically depleted waters are associated with cold regions and enriched waters are found in warm regions. On average, a 1‰ decrease in average annual $\delta^{18}O‰$ corresponds to a decrease of about 1.1–1.7 °C in the average annual temperature (Dansgaard, 1964). Corresponding variations occur for deuterium, and this covariance is the principal reason for the linear relationship or Global Meteoric Water Line defined by Craig (1961).

This trend can be seen in the $\delta^2H‰$ and $\delta^{18}O‰$ results obtained from beef lipids. At one end of the scale, from higher latitudes with more depleted $\delta^2H‰$ and $\delta^{18}O‰$ values, we observe samples from Scotland, New Zealand, Shetland, England, etc. and at the other end of the scale from warmer climates and lower latitudes, with more enriched $\delta^2H‰$ and $\delta^{18}O‰$ values, we observe samples from Southern Africa, Australia, Brazil and Uruguay.

The regression line through the beef lipid $\delta^2H‰$ and $\delta^{18}O‰$ has been plotted in Fig. 3. The correlation coefficient (R^2) was 0.87 and the slope of the regression line was 8.8, which is relatively close to that of the Meteoric Water Line i.e. $\delta^2H‰ = 8\ \delta^{18}O‰ + 10$. However, there is a significant offset of around $-370‰$ when an assumed value for $\delta^{18}O‰ = 0‰$ is substituted into the regression equation for beef lipid $\delta^2H‰$ and $\delta^{18}O‰$. This may be due to the significant depletion that occurs in the ^2H during the bio-synthesis of lipid in mammalian systems and to some extent the relative enrichment of ^{18}O in feed sources containing carbohydrate (Schmidt, Werner, and Rossmann, 2001, 2003). In addition aerial oxygen assimilated during respiration is relatively enriched in ^{18}O compared to groundwater at around $+22‰_{V-SMOW}$.

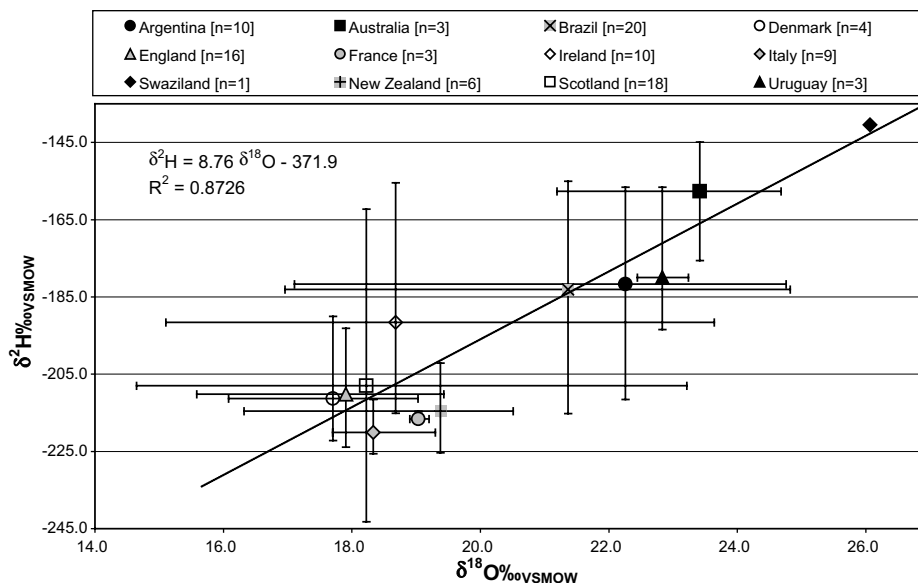Fig. 3. Mean values of beef lipid $\delta^{18}O$‰ and $\delta^{2}H$‰ by country of origin. X and Y error bars show the maximum and minimum observed $\delta^{18}O$‰ and $\delta^{2}H$‰ values, respectively for that country of origin.

3.4. Evaluation of the multivariate analysis of the trace element and stable isotope data

The following elemental concentrations were measured by ICP-MS in addition to the bio-element stable isotope data; sodium, aluminium, potassium, vanadium, chromium, manganese, iron, nickel, copper, rubidium, strontium, molybdenum, caesium and barium. Other elements were measured but are not reported here as the concentrations were at, or below, the detection limit (defined as three times the concentration in the procedural blanks). In this multivariate 'model' the samples were divided into 'broad' groups of European (UK, France, Germany, Austria, Hungary), South America (Argentina, Brazil and Uraguay) and Australasia (Australia and New Zealand). The results of the CDA and cross-validation are shown in Table 2. Original grouped cases (91.2%) were correctly classified and 86.1% of cross-validated grouped cases were correctly classified. Furthermore, it should be noted that 91.7% of cross-validated grouped European cases were correctly classified. The separation between geographical origins of the beef in the discriminant space was checked by plotting the first and the second functions, shown in Fig. 4. These results demonstrate the reliability of the multivariate approach in classifying the origin of beef and identifying those variables responsible for the classification.

The 'structure matrix' of the discriminant functions showed the six variables chosen for the discrimination were, ranked in order of highest correlation first, function 1 – $\delta^{13}C$‰ (DDM), strontium and function 2 – iron, $\delta^{2}H$‰ (lipid), rubidium and selenium. Discriminant function 1 accounted for 70.7% of the total variance and

Table 2
Results of stepwise canonical discriminant analysis obtained from stable isotope and trace element analyses of authentic beef samples

Geo_origin			Predicted group membership			Total
			S. America	Australasia	Europe	
Original	Count	S. America	26	1	5	32
		Australasia	3	5	1	9
		Europe	1	1	94	96
	%	S. America	81.3	3.1	15.6	100.0
		Australasia	33.3	55.6	11.1	100.0
		Europe	1.0	1.0	97.9	100.0
Cross-validated	Count	S. America	25	1	6	32
		Australasia	3	5	1	9
		Europe	5	3	88	96
	%	S. America	78.1	3.1	18.8	100.0
		Australasia	33.3	55.6	11.1	100.0
		Europe	5.2	3.1	91.7	100.0

In this model the samples were divided into 'broad' groups of European (UK, France, Germany, Austria, Hungary), South America (Argentina, Brazil and Uraguay) and Australasia (Australia and New Zealand).
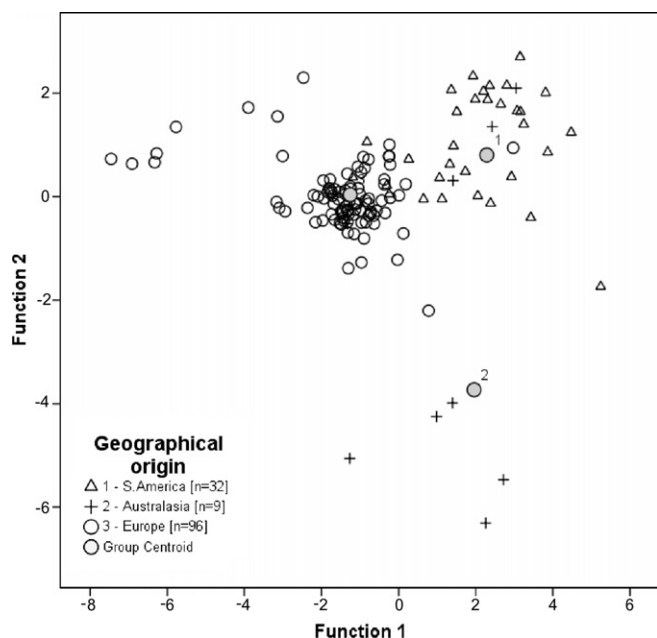
Fig. 4. A cross-plot showing the first two discriminant functions obtained from the stepwise canonical discriminant analysis of authentic beef stable isotope and trace element data. In this model the samples were divided into 'broad' groups of European (UK, France, Germany, Austria, Hungary), South America (Argentina, Brazil and Uraguay) and Australasia (Australia and New Zealand).

provides the main separation between the European samples and Australasian samples and South American samples (Argentina, Brazil and Uraguay). Function 2 equated to 29.3% of the total variance and provided the main separation of the Australasian samples, from the remaining authentic beef samples.

The identification of $\delta^{13}C‰$ of the DDM as a discriminatory variable confirms the observations made earlier about the relative proportions of C_3 and C_4 food sources in the diet being an indicative geographical proxy permitting differentiation of the majority of European beef samples from other production origins where maize is a major source of cattle diets. Similarly, the previous observations regarding the systematic variation in the deuterium content of the beef lipid fraction were confirmed through the selection of this variable as a geographical discriminator.

Regarding the selection of elemental concentration variables, bio-availability of metals depends on several factors such as pH, humidity, porosity, clay and humic complex, etc. (Kim and Thornton, 1993). The CDA identified rubidium, an element which is easily mobilised in the soil and readily transported into plants, and has previously been identified as a good indicator of geographical identity in rice (Kelly et al., 2002) and recently in chicken and beef (Franke et al., in press). Thus no two countries or regions are likely to have identical soil maps. Hence the range of soils present and bio-availability mean that elemental composition can characterise geographical origin. The concentration of strontium has previously been used to classify the

origin of illicit cocaine seizures (Bermejo-Barrera, Moreda-Pineiro, Moreda-Pineiro, Bermejo-Barrera, and Bermejo-Barrera, 1999) and it is has been used conjunction with calcium concentrations to distinguish between fish spawning (breeding) sites and habitats for Chum salmon (Arai, Hirata, and Takagi, 2007). The single most important dietary source of selenium for humans is beef and a previous study clearly demonstrated that areas with high concentrations of selenium in the soil and forage produced beef containing relatively high levels of selenium (Hintze, Lardy, Marchello, and Finley, 2001).

4. Conclusions

The main objective of the research described here was to apply isotopic and multi-element analyses, in combination with statistical processing, to develop techniques to verify the origin claims made on beef labels. In this respect, the findings in this paper demonstrate that it is possible with varying degrees of certainty to determine the provenance of beef. The techniques described here are at their most useful when applied to specific origin problems such as differentiating Brazilian beef from British beef, which can be achieved at present solely on the basis of the $\delta^{13}C‰$ value of beef muscle defatted dry mass. However, it is far more demanding to answer the general question "What is the origin of this beef sample?" rather than "Is this beef from an animal reared in England?" Consequently, the methodology in its current state can be used to provide reliable origin information, but this is dependent upon the countries under investigation. Furthermore, the use of elemental concentrations requires a deeper understanding and characterisation of the way in which they are transferred from soil and forage into animal tissues and their respective residence times. The use of empirical multivariate models to define origin must be used with great care because the discriminating variables can change each time new data is incorporated into the model. The use of techniques such as CDA does make it more difficult for authenticity tests to be subverted because a larger number of variables can be used simultaneously to define an authentic product. However, a corollary to this is the problem of consistently incorporating techniques such as CDA in Official methods and presenting multivariate information in an accessible form for law enforcement.

Seibersdorf and Quality Meat Scotland. The views expressed in this paper are those of the authors and do not necessarily reflect the views of the UK Food Standards Agency.

References

Arai, T., Hirata, T., & Takagi, Y. (2007). Application of laser ablation ICPMS to trace the environmental history of chum salmon (*Oncorhynchus keta*). *Marine Environmental Research, 63*, 55–66.

Bahar, B. (2006). Development of isotopic and molecular techniques for the authentication of dietary background information in bovine tissues. PhD thesis, University College Dublin, p. 77.

Bateman, A. S., Kelly, S. D., & Jickells, T. D. (2005). Nitrogen isotope relationships between crops and fertilizer: implications for using nitrogen isotope analysis as an indicator of agricultural regime. *Journal of Agriculture and Food Chemistry, 53*, 5760–5765.

Bermejo-Barrera, P., Moreda-Pineiro, A., Moreda-Pineiro, J., Bermejo-Barrera, A., & Bermejo-Barrera, A. M. (1999). A study of illicit cocaine seizure classification by pattern recognition techniques applied to metal data. *Journal of Forensic Sciences, 44*, 270–274.

Boner, M., & Förstel, H. (2004). Stable isotope variation as a tool to trace the authenticity of beef. *Analytical and Bioanalytical Chemistry, 378*, 301–310.

Camin, F., Wietzerbin, K., Blanch Cortes, A., Haberhauer, G., Lees, M., & Versini, G. (2004). Application of multielement stable isotope ratio analysis to the characterization of French, Italian, and Spanish cheeses. *Journal of Agricultural and Food Chemistry, 52*, 6592–6601.

Council Regulation (EEC) No. 2081/92., on the protection of geographical indications and designations of origin for agricultural products and foodstuffs. *Official Journal*, 1992, L208 (Corrigenda: [Official Journal L 27, 30.01.1997 and L 53, 24.02.1998]. Amended by Council Regulation (EC) No. 535/97of 17 March 1997 (Official Journal L 83, 25.03.1997).

Council Regulation (EC) No. 2772/1999., providing for the general rules for a compulsory beef labelling system. *Official Journal 1999*, L334, 0001–0002.

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

Dansgaard, W. (1964). Stable isotopes in precipitation. *Tellus, XVI*, 436–468.

Delgado, A., & Garcia, N. (2001). $\delta^{15}N$ and $\delta^{13}C$ analysis to identify cattle fed on feed containing animal proteins: A safety/quality index in meat, milk and cheese. In *Conference proceedings from sixth international symposium on food authenticity and safety*, 28–30 November, Nantes, France, pp. 1–11.

Franke, B. M., Gremaud, G., Hadorn, R., & Kreuzer, M. (2005). Geographic origin of meat – elements of an analytical approach to its authentication. *European Food Research and Technology, 221*, 493–503.

Franke, B. M., Haldimann, M., Reimann, J., Baumer, B., Gremaud, G., Hadorn, R. Bosset, J. -O., & Kreuzer, M. (in press). Indications for the applicability of element signature analysis for the determination of the geographic origin of dried beef and poultry meat. *European Food Research and Technology*. doi:10.1007/s00217-006-0446-2.

González-Martin, I., González-Pérez, C., Hernández Méndez, J., Marqués-Macias, E., & Sanz-Poveda, F. S. (1999). Use of isotope analysis to characterize meat from Iberian-breed swine. *Meat Science, 52*, 437–441.

Hara, A., & Radin, N. S. (1978). Lipid extraction of tissues with a low-toxicity solvent. *Analytical Biochemistry, 90*, 420–426.

Hegerding, L., Seidler, D., Danneel, H. J., Gessler, A., & Nowak, B. (2002). Oxygen isotope-ratio-analysis for the determination of the origin of beef. *Fleischwirtschaft, 82*, 95–100.

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

Hobson, K. A., Atwell, L., & Wassenaar, L. (1999). Influence of drinking water and diet on the stable hydrogen isotope ratios of animal tissues. *Proceedings of the National Academy of Sciences USA, 96*, 8003–8006.

Hölzl, S., Horn, P., Rossmann, A., & Rummel, S. (2004). Isotope-abundance ratios of light (bio) and heavy (geo) elements in biogenic tissues: methods and applications. *Analytical and Bioanalytical Chemistry, 378*, 270–272.

Kelly, S. D., 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 Research and Technology, 214*, 72–78.

Kelly, S. D., 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.

Kim, K. W., & Thornton, Y. (1993). Influence of Ordovician uraniferous black shales on the trace element composition of soils and food crops, Korea. *Applied Geochemistry Supplement, 2*, 249–255.

Kornexl, B. E., Werner, R., & Rossmann, A. (1997). Measurement of stable isotope abundances in milk and milk ingredients – A possible tool for origin assignment and quality control. *Zeitschrift fur Lebensmittel-Untersuchung und-Forschung A – Food Research and Technology, 20*, 19–24.

Minson, D. J., Ludlow, M. M., & Troughton, J. H. (1975). Differences in natural carbon isotope ratios of milk and hair from cattle grazing tropical and temperate pastures. *Nature (London), 256*, 602.

Piasentier, E., Valusso, R., Camin, F., & Versini, G. (2003). Stable isotope ratio analysis for authentication of lamb meat. *Meat Science, 64*, 239–247.

Rencher, A. C. (1992). Interpretation of canonical discriminant functions, canonical variates, and principal components. *The American Statistician, 46*, 217–225.

Renou, J. P., Bielicki, G., Deponge, C., Gachon, P., Micol, D., & Ritz, P. (2004). Characterization of animal products according to geographic origin and feeding diet using nuclear magnetic resonance and isotope ratio mass spectrometry. Part II: Beef meat. *Food Chemistry, 86*, 251–256.

Rossmann, A., Haberhauer, G., Holzl, 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.

Sacco, D., Brescia, M. A., Buccolieri, A., & Jambrenghi, A. C. (2005). Geographical origin and breed discrimination of Apulian lamb meat samples by means of analytical and spectroscopic determinations. *Meat Science, 71*, 542–548.

Schmidt, H.-L., Rossman, A., Voerkelius, S., Schnitzler, W. H., Georgi, M., Grassmann, J., et al. (2005). Isotope characteristics of vegetables and wheat from conventional and organic production. *Isotopes in Environmental and Health Studies, 41*, 223–228.

Schmidt, H.-L., Werner, R. A., & Eisenreich, W. (2003). Systematics of ^2H patterns in natural compounds and its importance for the elucidation of biosynthetic pathways. *Phytochemistry Reviews, 2*, 61–85.

Schmidt, H.-L., Werner, R. A., & Rossmann, A. (2001). ^{18}O pattern and biosynthesis of natural plant products. *Phytochemistry, 58*, 9–32.

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

Schwagele, F. (2005). Traceability from a European perspective. *Meat Science, 71*, 164–173.

Schwertl, M., Auerswald, K., Schaufele, R., & Schnyder, H. (2005). Carbon and nitrogen stable isotope composition of cattle hair: ecological fingerprints of production systems? *Agriculture, Ecosystems and Environment, 109*, 153–165.

Thiem, I., Lüpke, M., & Seifert, H. (2004). Factors influencing the O-18/O-16-ratio in meat juices. *Isotopes in Environmental and Health Studies, 40*, 191–197.

Vallet, C., Arendt, M., Mabon, F., Naulet, N., & Martin, G. J. (1991). Combination of mass-spectrometry and site-specific NMR isotope

analyses in the characterization of amino-acids. *Journal of the Science of Food and Agriculture, 56*, 167–185.

Winkler, F. J. (1984). In A. Frigerio & H. Milon (Eds.), *Application of natural abundance stable isotope mass spectrometry in food control in Chromatography and Mass Spectrometry in Nutrition Science and Food Safety* (pp. 173–190). Amsterdam, Netherlands: Elsevier Science Publishers BV.

Zhang, B. L., Pionnier, S., & Buddrus, S. (2006). Deuterium NMR study of the origin of hydrogen in fatty acids produced in vivo by chicken. *European Journal of Lipid Science and Technology, 108*, 125–133.