JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLE

pubs.acs.org/JAFC

Multielement Isotope Analysis of Bovine Muscle for Determination of International Geographical Origin of Meat

M. Teresa Osorio,[†] Aidan P. Moloney,[§] Olaf Schmidt,[†] and Frank J. Monahan*[,†]

[†]School of Agriculture, Food Science and Veterinary Medicine, University College Dublin, Belfield, Dublin 4, Ireland

[§]Animal and Grassland Research and Innovation Centre, Teagasc, Grange, Dunsany, County Meath, Ireland

ABSTRACT: Multielemental (C, N, H, S) stable isotope ratio analysis was used as an analytical tool to verify the geographical origin of beef from several European and non-European countries. Beef samples were collected from nine different countries, and the $^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{2}H/^{1}H$, and $^{34}S/^{32}S$ ratios of defatted beef were measured using isotope ratio mass spectrometry (IRMS). There were highly significant differences in the mean isotopic values of the beef from different countries. The results of discriminant analysis showed that the four isotope ratios were significant for the discrimination of geographical origin and that 84.9% of the samples were correctly assigned to the country of origin (82.2% when cross-validated). Beef was also classified according to geographical origin when additional information on different feeding regimens used in Ireland was included, with 85.0% of the samples correctly allocated and 82.9% cross-validated using the isotopic signatures. All of the Irish beef samples verifiable as pasture-fed beef were correctly classified and then cross-validated.

KEYWORDS: beef, authenticity, stable isotope ratio analysis, geographical origin, pasture-fed Irish beef, discriminant analysis

INTRODUCTION

The origin of foods is often associated with food quality and safety and, therefore, food authenticity and traceability are receiving considerable research interest.[1] Moreover, consumers pay a premium for high-quality food products of known and guaranteed origin (e.g., Protected Designation of Origin and Protected Geographical Indication food products).[2] One of the main authenticity criteria related to fresh meat in Europe is geographical origin.[3] The European Union (EU)-wide compulsory beef labeling regulations, effective since September 2000,[4] include a requirement for precise information related to the type and origin of the beef, so that consumers can consider this information when purchasing beef.[2] The authenticity of beef can be ascertained using properties of the beef itself, such as its natural isotopic composition,[5] so that tracing the origin of beef may not depend solely on a paper or computer-based traceability system.

Analytical techniques for authentication have improved greatly in recent years, and research has focused on reliable tests to accurately determine geographical origin, that is, the country or region of origin, of meat. Techniques such as isotope ratio mass spectrometry (IRMS) have been investigated to determine the geographical origin of food products such as milk and milk products, honey, olive oil, and orange juices.[6−10] In general, carbon and nitrogen isotopic compositions of animal products are related to production systems, and not specifically to geographical origin. They can, however, be useful as an indirect indication of geographical provenance if used in combination with other stable isotopes[11] such as hydrogen and oxygen. These latter two elements are especially interesting for the geographical origin of food linked to regional climatic conditions[12] because they are strongly latitude dependent. However, they are also affected by altitude, distance from the sea, total precipitation, and seasonality.[13] Sulfur isotopic compositions are mainly affected by the geology of the area where animal feed is grown (sedimentary or igneous) [6] as well as the proximity to the sea, climatic conditions, and fertilization practices.[14]

The use of multi-isotope analysis to provide information on the dietary and geographical origin of meat has been investigated in beef, pork, and lamb.[2,5,11,15−23] Several of these studies discriminated successfully among beef samples of different geographical origins and concluded that it is possible, with various degrees of certainty, to determine the provenance of beef.

Pasture is used extensively as a ruminant feed in Irish livestock farming due to its high abundance in Ireland. Raising animals at pasture has potential beneficial effects on meat composition, for instance, increasing the polyunsaturated fatty acid/saturated fatty acid ratio and conjugated linoleic acid content while decreasing the n-6/n-3 fatty acid ratio of beef.[24−28] For this reason, although it is not the only production system in Ireland, it is often considered the most desirable and advantageous. Reliable methods for the determination of geographical origin could guarantee the authenticity of Irish beef and, specifically, pasture-fed beef.

Stable isotope discrimination has not been applied previously to distinguish between pasture-fed Irish and non-Irish beef. In this study, our hypothesis was that C, N, H, and S isotopic compositions of beef from different European and non-European countries could be used, first, to authenticate the geographical origin of beef and, second, to authenticate pasture-fed Irish beef with respect to beef from other countries.

MATERIALS AND METHODS

Collection of Beef Samples. A total of 166 beef samples were obtained from a wide range of geographical locations including European and non-European countries. Raw frozen beef samples from Italy ($n = 18$), Austria ($n = 6$), the United Kingdom ($n = 20$), France ($n = 4$), Spain ($n = 7$), and Germany ($n = 6$) were obtained from personal

Received: October 15, 2010
Revised: February 11, 2011
Accepted: February 16, 2011
Published: March 10, 2011

ACS Publications © 2011 American Chemical Society

dx.doi.org/10.1021/jf1040433 | J. Agric. Food Chem. 2011, 59, 3285–3294

contacts of the authors. Beef samples from the United States ($n = 22$), 12 of which were marketed as pasture-fed (hereafter "U.S., retail pasture"), were obtained via IdentiGEN Inc. (IdentiGEN North America, Inc., Lawrence, KS) from U.S. retail outlets. Beef samples from Brazil ($n = 17$) were sourced through a commercial importer (Dawn Farm Foods Ltd., Naas, Co. Kildare, Ireland) and received at three different times: first batch (January 2007), second batch (February 2007), and third batch (July 2007). Irish beef samples of unknown dietary origin (hereafter "Ireland, retail unknown") were obtained from a local supermarket (Superquinn, Ballinteer, Dublin 16, Ireland) ($n = 8$). Geographical locations within each country were unknown, except for samples from Italy, which came from one farm in Sicily. Additionally, Irish retail pasture-fed beef ($n = 18$) (hereafter "Ireland, retail pasture") was obtained from an organic producer (Omegabeefdirect, Ballymacarbry, Clonmel, Co. Tipperary, Ireland). Forty Irish beef samples, verifiable as pasture-fed ($n = 20$) and concentrate-fed ($n = 20$) (hereafter "Ireland, pasture" and "Ireland, concentrate", respectively), were randomly selected from a controlled feeding trial[29] involving different beef production treatments. Samples were frozen after sampling, transferred to the laboratory at −18 °C, and stored frozen at −20 °C until sample preparation for stable isotope ratio analysis (SIRA).

Sample Preparation. Frozen muscle samples were cut into 1 cm cubes using a ceramic knife and then freeze-dried (Edwards Pirani 501 freeze-dryer, Edwards Ltd., Crawley, U.K.) for 4 days. After freeze-drying, samples were stored at −20 °C in plastic bags until lipid extraction. Total lipid from 3 g of the freeze-dried material was extracted using a 2-isopropanol/hexane mixture (2:3, v/v) according to the method of Radin.[30] The defatted muscle was separated from the solvent mixture by vacuum filtration and air-dried overnight in a container covered with aluminum foil to protect samples from the light. The lipid-free dry samples were stored in Eppendorf vials in a vacuum desiccator at room temperature until weighing for SIRA. An amount of 0.9−1.1 mg of the lipid-free dry muscle was weighed into tin capsules and sealed for C, N, and H isotope analyses. For S isotope analysis, 1.9−2.1 mg of lipid-free dry muscle was weighed, with 4 mg of vanadium pentoxide added, into the ultraclean tin capsules. Replicates were used to test the reliability of the IRMS (every fourth sample was measured in duplicate for C, N, and S isotope analyses, whereas every sample was measured in duplicate for H isotope analyses).

Stable Isotope Ratio Analysis. The isotopic ratios $^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{2}H/^{1}H$, and $^{34}S/^{32}S$ in the freeze-dried muscle samples were determined using an Elemental Analyzer−Isotope Ratio Mass Spectrometer (EA-IRMS) Europa Scientific 20-20 (Sercon Ltd., Crewe, U.K.), equipped with a preparation module for solid and liquid samples (ANCA-SL). In this technique, samples and references are loaded into an autosampler on a Europa Scientific elemental analyzer. Stable isotope ratios are expressed using conventional δ notation in units of per mil (‰) relative to a suitable standard and defined as

$$\delta\ (‰) = [(R_{sample} - R_{reference}) - 1] \times 1000$$

where R_{sample} is the isotope ratio in the sample ($^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{2}H/^{1}H$, $^{34}S/^{32}S$) and $R_{reference}$ is the isotope ratio of the reference material. Results are referenced to Vienna Pee Dee Belemnite (V-PDB) for carbon, atmospheric N_2 for nitrogen, Vienna Canyon Diablo Troilite (V-CDT) for sulfur, and Vienna Standard Mean Ocean Water (V-SMOW) for hydrogen.

The isotopic values were calculated against in-house standards (powdered bovine liver, beet sugar, ammonium sulfate, cane sugar, mineral oil, polyethylene foil, whale baleen, egg shell membrane standard, barium sulfate, and silver sulfide), calibrated and traceable against international isotope reference standards: sucrose IAEA-CH-6 (International Atomic Energy Agency, Vienna, Austria) for $^{13}C/^{12}C$, ammonium sulfate IAEA-N-1 (distributed by IAEA) for $^{15}N/^{14}N$, mineral oil NBS-22 (IAEA) for $^{2}H/^{1}H$, barium sulfate NBS-127, barium sulfate IAEA-SO-5 (IAEA), and silver sulfide IAEA-S-1 (IAEA) for $^{34}S/^{32}S$ measurements.

The precision of the measurements (2 × SD) for freeze-dried muscle samples, as estimated by replicate analysis ($n = 23$) of a powdered bovine liver standard (NBS-1577B, $\delta^{13}C_{V\text{-}PDB} = -21.60‰$, $\delta^{15}N_{Air} = 7.65‰$) analyzed along with the samples was 0.1 and 0.32‰ (2 × SD) for $\delta^{13}C$ and $\delta^{15}N$, respectively. Ammonium sulfate (2 × SD, $n = 11$, 0.36 and 0.3‰ for $\delta^{15}N$), beet sugar (2 × SD, $n = 11$, 0.1‰ for $\delta^{13}C$), and cane sugar (2 × SD, $n = 11$, 0.1‰ for $\delta^{13}C$) were also run as quality control check samples during analysis. For S isotope analysis, IA-R036 (barium sulfate, $\delta^{34}S_{V\text{-}CDT} = 20.74‰$) was used as a reference material and the analytical precision (2 × SD, $n = 16$) was 0.60‰. Working standards IA-R036, IA-R025 (barium sulfate, $\delta^{34}S_{V\text{-}CDT} = 8.53‰$), and IA-R026 (silver sulfide, $\delta^{34}S_{V\text{-}CDT} = 3.96‰$) were used for calibration and correction of the oxygen-18 contribution to the sulfur isotope data. Replicate analysis of IAEA-SO-5 (barium sulfate, $\delta^{34}S_{V\text{-}CDT} = 0.50‰$) and BWB II (whale baleen, $\delta^{34}S_{V\text{-}CDT} = 16.30‰$) run concurrently with the samples gave mean $\delta^{34}S_{V\text{-}CDT} = 0.27‰$ ($n = 8$) and 16.70‰ ($n = 8$), respectively. For H isotope ratio analysis, the analytical precision (2 × SD) was 2‰ when IA-R002 (mineral oil, $n = 42$, $\delta^{2}H_{V\text{-}SMOW} = -111.2‰$) was analyzed along with the preweighed samples and 2.7‰ when IAEA-CH-7 (polyethylene foil, $n = 60$, $\delta^{2}H_{V\text{-}SMOW} = -100.3‰$) was analyzed instead. The sample capsules were comparatively equilibrated with capsules containing the working standard BWB II (whale baleen, nonexchangeable $\delta^{2}H_{V\text{-}SMOW} = -108‰$) and the egg shell membrane standard RSPB EGG (nonexchangeable $\delta^{2}H_{V\text{-}SMOW} = -93.8‰$) for no less than 7 days prior to analysis to allow the exchangeable hydrogen in both samples and working standards to equilibrate fully with moisture in the laboratory air. Replicate analysis of BWB II and RSPB EGG run concurrently with the samples gave a mean $\delta^{2}H_{V\text{-}SMOW} = -103.50‰$ ($n = 20$) and $\delta^{2}H_{V\text{-}SMOW} = -96.23‰$ ($n = 20$), respectively. As the average $\delta^{2}H_{V\text{-}SMOW}$ data obtained for BWB II were within 1 SD of their known nonexchangeable $\delta^{2}H$ values, no correction for exchangeable hydrogen content was applied to the $\delta^{2}H_{V\text{-}SMOW}$ data of muscle samples.

Statistical Analysis. The effect of the country of origin on the stable isotope ratios in beef was tested using a one-way analysis of variance (ANOVA) followed by a Tukey test for post hoc comparisons. Additionally, the existence of differences was verified through representation of variables in box plots. The statistical analysis of data was performed using the PASW version 18.0 for Windows (SPSS, Inc., Chicago, IL).

Multivariate statistical analysis was used to evaluate the possibility of differentiating bovine meat samples according to their geographical origin. Canonical discriminant analysis (CDA) was performed to evaluate whether beef from different countries of origin could be distinguished on the basis of C, N, H, and S isotope ratios and to verify which isotope ratios contribute toward classification. An automatic stepwise elimination was carried out to select the best variables. The procedure generates a set of canonical discriminant functions based on the selected variables that provides the best discrimination between the dietary groups. Those functions can be applied to unknown samples following measurement of the stable isotopic signatures of the determined bioelements. The statistical significance of each discriminant function was evaluated on the basis of the Wilks' λ factor after the function was removed. Results were validated using leave-one-out cross-validation. The success of the discrimination was measured by the proportion of cases correctly classified using this cross-validation. For this purpose, each known sample was used as an "unknown" to validate the model: one sample is removed from the data set and then classified by the functions derived from all samples other than that sample. This process is repeated for each sample.

RESULTS AND DISCUSSION

Hydrogen Isotopes. $\delta^{2}H$ values varied between countries with values ranging from −130.7 to −94.4‰. $\delta^{2}H$ values of beef

Table 1. Summary of the Stable H, C, N, and S Isotope Composition of Dry Defatted Beef from Different Countries of Origin Given as Mean Values, Standard Deviations of the Mean, and Number of Samples per Country

		δ^2H		δ^{13}C		δ^{15}N		δ^{34}S	
origin	*n*	mean[a]	SD	mean[a]	SD	mean[a]	SD	mean[a]	SD
Austria	6	−122.65 be	3.51	−22.16 ce	2.49	5.29 ce	0.74	1.99 be	2.73
Brazil	17	−106.32 ag	4.29	−10.97 a	0.77	7.21 df	0.81	9.85 ad	1.32
France	4	−108.73 af	7.20	−19.62 bc	2.50	7.21 bcd	1.33	2.68 bce	3.36
Germany	6	−124.44 b	5.01	−23.11 cfe	3.82	9.02 ab	2.32	2.37 be	1.52
Ireland, pasture	10	−122.32 be	2.13	−27.78 d	0.14	9.39 a	0.26	4.69 bc	0.81
Ireland, concentrate	10	−112.40 cfg	3.97	−25.10 de	0.16	6.18 cf	0.45	5.93 cf	0.43
Ireland, retail pasture	18	−113.99 cf	4.55	−27.02 d	0.30	4.99 e	0.54	10.49 a	1.04
Ireland, retail unknown	8	−114.40 def	3.71	−26.84 d	0.40	7.07 dfg	0.94	8.07 adf	1.25
Italy	18	−105.88 ag	5.61	−20.25 bc	1.31	4.97 e	0.55	1.50 e	2.30
Spain	7	−112.88 acf	6.97	−21.71 c	2.30	5.85 ceg	0.84	5.60 cf	1.42
United Kingdom	20	−110.44 acd	4.24	−25.61 df	0.85	7.72 bd	1.07	4.35 bc	1.44
U.S., retail pasture	12	−105.15 ag	9.16	−18.01 b	4.32	6.74 cdfg	1.00	0.69 e	2.00
U.S., retail unknown	10	−104.53 a	4.07	−13.03 a	2.57	6.46 cfg	0.78	−0.40 e	2.26

[a] Within columns, means assigned different letters differ significantly ($P < 0.05$).

showed that there were significant differences between the countries of origin of beef, independent of the production system (Table 1). U.S. retail unknown beef and Italian beef had the highest mean δ^2H values with values of −104.53 and −105.88‰, respectively. The high δ^2H values of beef from Italy were expected because Mediterranean precipitation waters are characterized by a high deuterium excess.[19] However, the high δ^2H values of beef from the United States suggested that samples could have originated in warm regions with dry climate.[19] Alternatively, the high δ^2H values of beef from the United States could be explained by the inclusion of C_4 plant species in the cattle fodder, because it is known that C_4 plant species contain higher amounts of organically bound ^{2}H than C_3 plant species from the same region; however, background information from the U.S. samples was lacking. Beef from Brazil and the United States was significantly different from beef from Austria, Germany, and all Irish samples except Irish concentrate-fed on the basis of the δ^2H values (Table 1). A significant variation in δ^2H means within Ireland due to different production systems was observed. Spanish and Italian samples had a wider range of δ^2H values than any other samples (−104.7 to −123.8‰ and −94.4 to −118.2‰, respectively) when the lowest and highest values were considered, overlapping with all other beef types except beef from Germany (Figure 1a). The wide range of δ^2H values of the Italian samples could be attributed to the different feedstuffs ingested by the animals, with those nominally considered pasture-fed having lower δ^2H values than those considered concentrate-fed. It is known that hydrogen in animal tissues reflects the H isotope ratios of the feed[31] and the water the animal ingested,[2,19] which in turn are influenced by the average H isotopic ratio of precipitation water for a region.[19] On the basis of the annual weighted δ^2H data in the GNIP database from the International Atomic Energy Agency,[32] the δ^2H values of rainwater in the United States and Italy are approximately −70 to −38‰ and −50 to −30‰, respectively, which are higher than those in Germany and Austria (−78 to −54‰). Another factor that influences the δ^2H values is latitude.[2] Thus, countries with higher latitudes and colder climate such as the United Kingdom, Ireland, and Germany had more depleted δ^2H values than countries with warmer climates and lower latitudes such as Brazil and Italy.[2] This deuterium isotopic pattern differed from the findings of Heaton et al.,[2] who reported the lowest δ^2H values for Italy, probably due to the different regional origins of these samples, although these authors measured hydrogen isotopes on beef lipid and their data are thus not directly comparable with ours.

Carbon Isotopes. Analysis of variance showed that there were significant differences in the mean δ^{13}C values of beef according to country of origin (Table 1). Irish beef from different production systems was not significantly different from U.K. beef. This could be explained by the similarity in production systems (based mainly on grass with some cereal concentrate supplementation) between these two countries. Although the box plot (Figure 1b) indicates overlap between some retail pasture-fed samples from the United States and other countries such as Germany, Spain, and Austria, showing that some U.S. δ^{13}C values for pasture-fed beef were similar to those of European beef, the post hoc test indicated significant differences between the mean δ^{13}C values of U.S. retail pasture-fed and beef from those other countries. However, unknown retail beef from the United States was significantly different ($P < 0.001$) from all the other beef except beef from Brazil.

The box plot of the data (Figure 1b) shows that Irish beef had the lowest δ^{13}C values, ranging from −24.90 (pasture-fed Irish beef) to −28.06‰ (concentrate-fed Irish beef), overlapping only with those of Germany and the United Kingdom. Furthermore, beef from Brazil and the United States had the highest δ^{13}C values. For these two non-European countries, a first preliminary separation from Ireland and the United Kingdom is noticeable on the basis of nonoverlap of δ^{13}C values between them. High variability was observed in the U.S. retail pasture-fed samples (from −10.50 to −19.91‰) compared to that in other countries. The differences in the δ^{13}C values of muscle between countries can be explained by the inclusion of different proportions of C_3 and C_4 plant species in the cattle fodder, because the δ^{13}C values of the animal tissues vary according to diet.[33] Consumption of C_3 plants (δ^{13}C values from −35 to −21‰) leads to lower tissue δ^{13}C values than C_4 plants (δ^{13}C values from −14 to −10‰).[2,5,16,18,34,35]

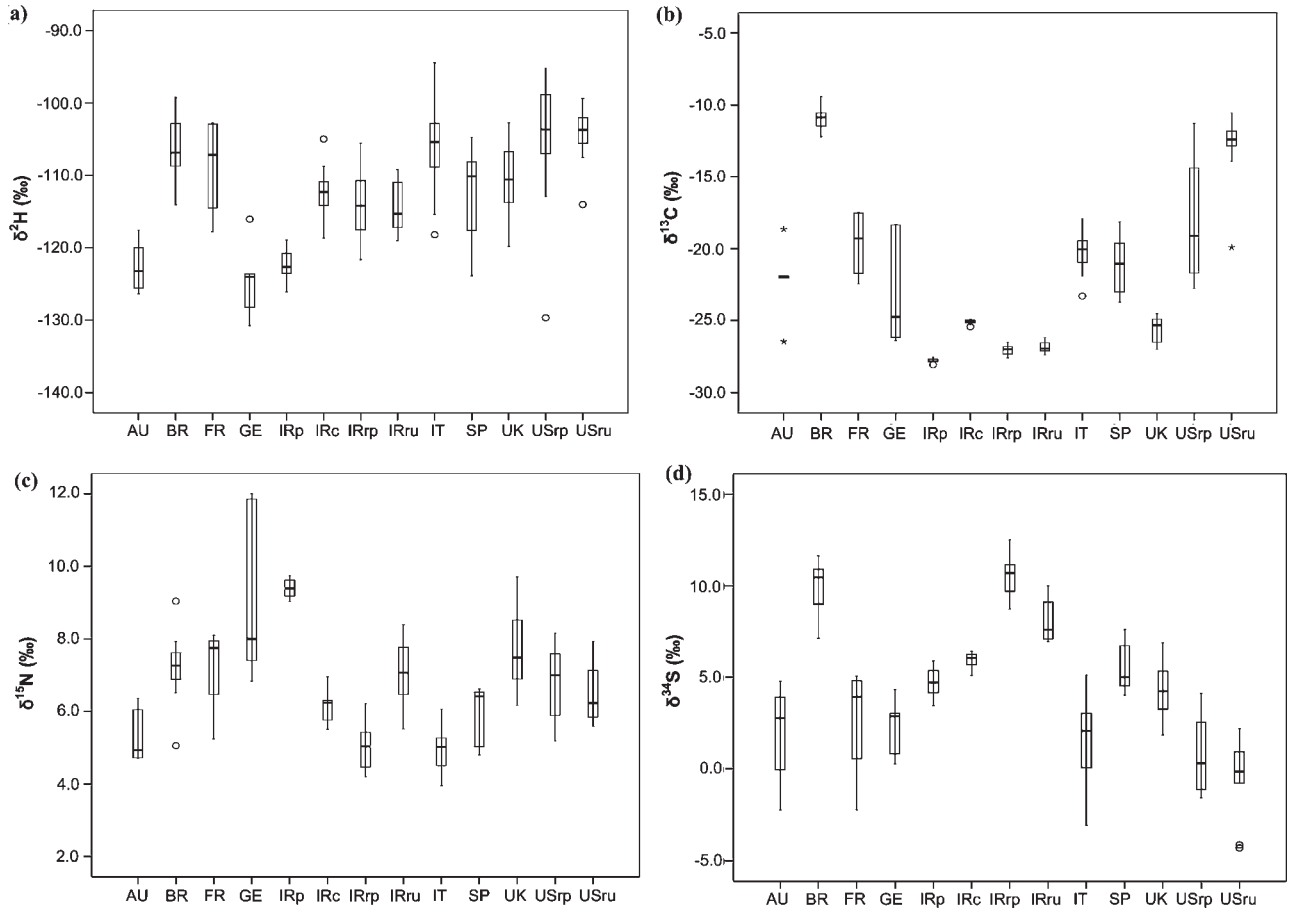

Figure 1. Box plot showing (a) δ^2H, (b) δ^{13}C, (c) δ^{15}N, and (d) δ^{34}S values (‰) obtained from defatted beef produced in different countries and under different production systems. (Line in the center, median; box, 25−75th percentile; whisker, minimum nonoutlier − maximum nonoutlier. Symbols out of the boxplots: outliers, circles; extreme outliers, asterisks). Abbreviations: AU, Austria; BR, Brazil; FR, France; GE, Germany; IRp, Ireland pasture; IRc, Ireland concentrate; IRrp, Ireland retail pasture; IRru, Ireland retail unknown; IT, Italy; SP, Spain; UK, United Kingdom; USrp, U.S. retail pasture; USru, U.S. retail unknown.

Thus, the δ^{13}C values suggest a predominance of C_4 plants (e.g., maize) in the feed of cattle in Brazil and the unknown retail samples from the United States, with a predominance of C_3 dietary ingredients in Ireland and the United Kingdom. On the other hand, the data suggest that both types of plants were used for cattle feeding in Italy, Spain, Austria, France, and Germany (Figure 1b), and δ^{13}C values probably reflect some maize consumption in beef production systems in central and southern Europe.[18] The δ^{13}C values observed in this study were in agreement with those for Italy, Austria, Spain, Germany, the United Kingdom, and Brazil beef reported by Heaton et al.,[2] with those for U.S. beef reported by Bong et al.[22] and with those for Brazil beef reported by Schmidt et al.[18]

Figure 2 is a plot of hydrogen versus carbon stable isotope ratios of beef from different countries. Whereas δ^{13}C values of beef from Brazil and the United States differed markedly from European beef, δ^2H values differed only slightly among European and non-European countries. Schmidt et al.[18] also identified δ^{13}C as a single marker that distinguishes American (Brazil and United States) from European beef. Beef from Ireland and the United Kingdom is grouped into one subset due not only to similar C_3 diet composition but also to the high humidity of those countries compared to central European and non-European countries, which results in high C isotope fractionation during plant biosynthesis.[19] U.S. samples are clustered into two subsets according to δ^{13}C values, but the δ^{13}C data showed that four U.S. samples (3 U.S. retail pasture-fed and 1 U.S. retail unknown) were not consistent with the majority of samples in their respective categories. In the case of the retail pasture-fed samples these anomalies may arise because of the range of feedstuffs permissible under the "grass-fed" claim.[36] Similarly, in the case of U.S. retail unknown samples, in addition to the likelihood of maize being fed,[2,11,18,22] a wide range of C_3 feedstuffs could potentially be fed.

Nitrogen Isotopes. Differences in δ^{15}N values among countries were also significant (Table 1). Post hoc tests indicated a significant variation in δ^{15}N means within Ireland due to different production systems, but not within the United States. Interestingly, cattle from broadly similar production systems but different countries supplied meat with different δ^{15}N values. For instance, δ^{15}N values of pasture-fed beef from Ireland and the United States differed significantly ($P < 0.01$), although they both had a pasture-based feeding regimen. Whereas the beef from Ireland and Germany had the highest mean δ^{15}N values, the beef from Italy had the lowest. Figure 1c shows no overlap between the German samples and those of Austria, Italy, and Spain. Pasture-fed beef produced in Ireland was completely distinguishable on the basis of δ^{15}N values from the beef produced in Ireland under different production systems and

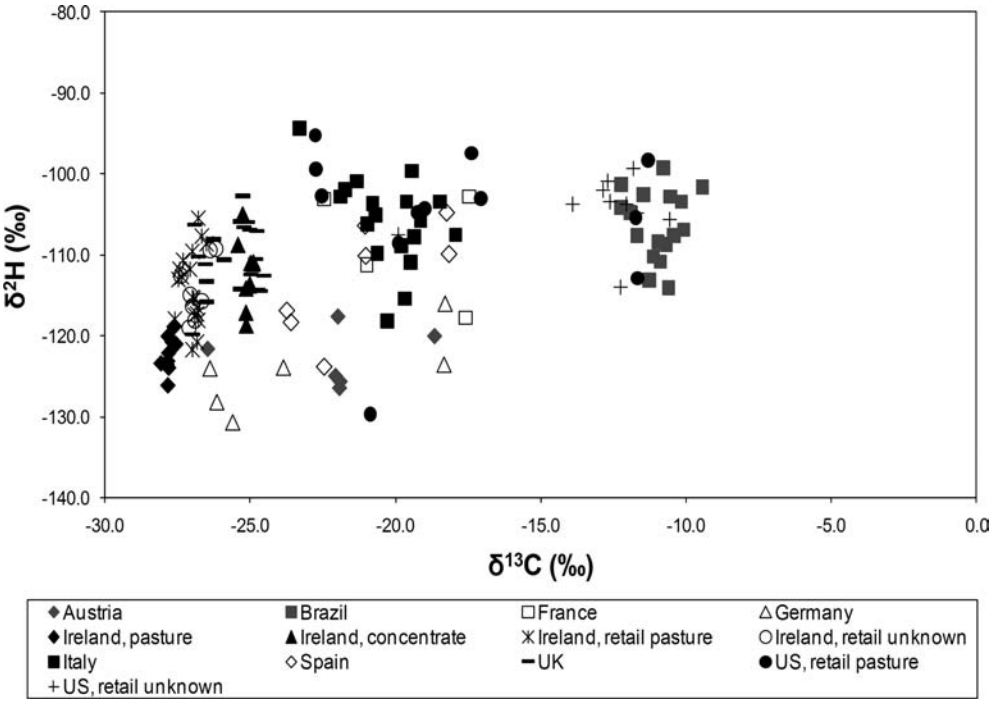

Figure 2. Carbon and hydrogen isotopic ratios of bovine muscle tissue from different countries.

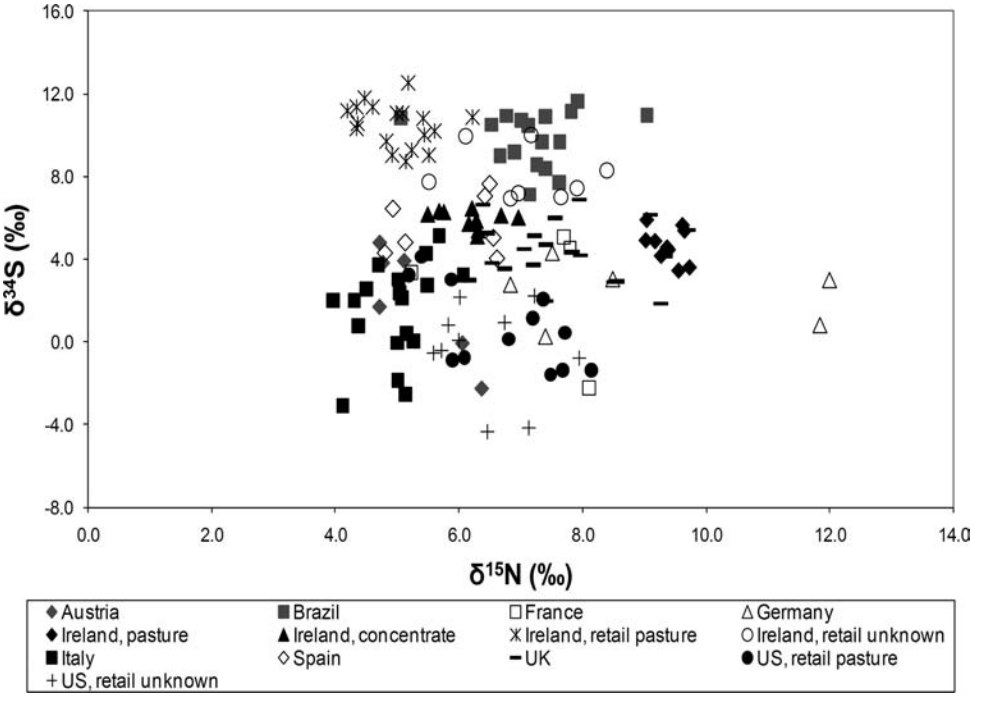

Figure 3. Nitrogen and sulfur isotopic ratios of bovine muscle tissue from different countries.

from the rest of the countries, overlapping only with the U.K. and German samples (Figure 1c). The low δ^{15}N values found in the retail pasture Irish beef, which was produced in an organic farm, are in agreement with the lower δ^{15}N values observed in organic compared to conventional Irish beef.[18] The box plot in Figure 1c also indicates that concentrate-fed Irish beef showed overlapping δ^{15}N values with all of the countries except nonretail pasture-fed beef from Ireland.

The variation of δ^{15}N values could be attributed to the different ^{15}N contents of the local feedstuffs supplied to the animals[37] and regional conditions (climate and soil conditions, agricultural practices, etc.) of different geographical regions.[15] The high δ^{15}N values of Ireland could be due to the application of organic fertilizer, which increases the ^{15}N level of soil and plants [15] or the application of artificial fertilizer and ^{15}N enrichment associated with higher N (preferentially ^{14}N) losses.[18] A

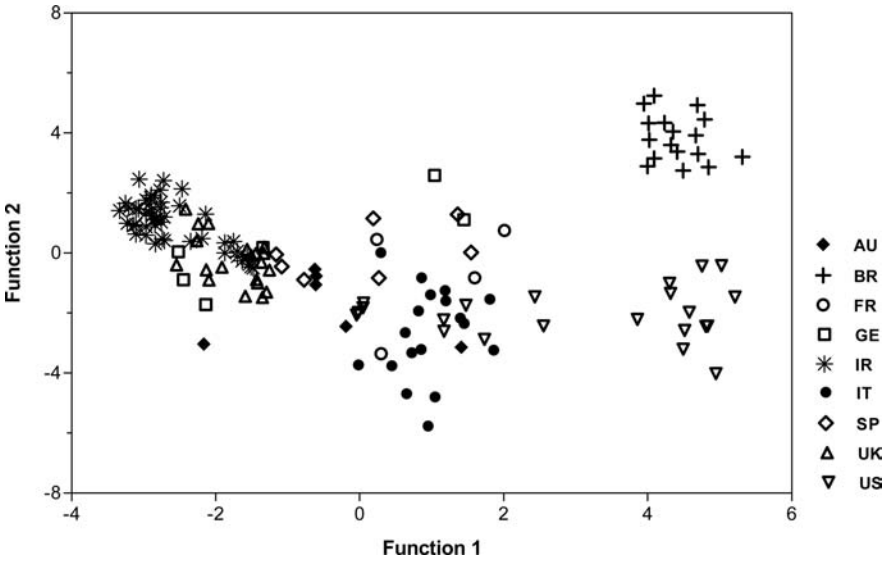

Figure 4. Plot showing the first two discriminant functions derived from bovine muscle samples produced in different countries.

broad δ^{15}N range (from 6.84 to 12.0‰) was observed in German samples (Figure 1c). It is difficult to explain this variability in δ^{15}N values without having information about the feed consumed, although it is known that the German samples were from Bavaria where animals are likely to receive both pasture and cereal-based feedstuffs depending on the season. However, when δ^{15}N values of feed are known, a clear relationship between these values and δ^{15}N values of beef derived from animals consuming the feed has been established.[34,37−40] Beef from Brazil and the United States showed intermediate δ^{15}N values among the beef samples and overlapped with most of the other beef types. The δ^{15}N values observed in this study were very consistent with those of previous authors who reported δ^{15}N values for beef produced in the United States,[2,11,18,22] Brazil,[2] Austria,[2] the United Kingdom,[2] Spain,[2] and Italy,[2] but differed from those of Germany reported by Heaton et al.[2]

Sulfur Isotopes. The δ^{34}S values of the beef samples ranged from −4.3 to +12.5‰. The highest δ^{34}S values were for beef from Ireland (retail pasture) and Brazil, with mean values of 10.49 and 9.85‰, respectively (Table 1). On the other hand, beef from the United States (retail unknown) had the lowest δ^{34}S values, with values from −4.3 to +2.2‰. Overall, beef from Ireland, pooled across production systems, showed a mean δ^{34}S value of 7.82‰, which was higher than in beef from the United States ($P < 0.001$) and other European countries. These δ^{34}S values in Irish beef are in agreement with those observed by Bahar et al.[35] in an earlier survey.

The data in Table 1 show that Brazilian beef was isotopically different from beef from Italy, Austria, France, Germany, the United Kingdom, and the United States, with no overlap between δ^{34}S values of beef from Brazil and those countries (Figure 1d). The observed differences in δ^{34}S values of beef between countries in the present study may reflect the use of marine sulfate as a fertilizer or the deposition of sea-spray sulfate as an aerosol over the regions and crops close to the sea.[19] Thus, the most ^{34}S-enriched beef samples were from retail pasture-fed beef produced in a region of Ireland 30−40 km from the sea (Co. Tipperary), differing statistically from all the other countries except Brazil and overlapping only with retail unknown beef produced in Ireland and beef produced in Brazil.

Figure 3 is a plot of sulfur versus nitrogen stable isotope ratios of beef from different countries. Compared to Figure 2, Figure 3 revealed that only the sulfur isotope signature could be used to distinguish beef of Brazil from U.S. beef. The low δ^{34}S values of the beef from the United States could be due to the low ^{34}S content in the soils in the region of production, which appeared to be distant from the sea. Moreover, samples from Ireland (retail pasture) were restricted to a narrow cluster due to their high δ^{34}S and low δ^{15}N values. The variation in δ^{34}S values observed within a single country such as Ireland could be due to the different animal production systems. In general, δ^{34}S values in feedstuffs are affected by the proximity to the sea and the season of production,[14,35] as well as the geology of a region, the latter being an important contributor to δ^{34}S content.[6] Camin et al.[19] observed an apparent correlation between the δ^{34}S and δ^{15}N values of European defatted lamb samples, suggesting that this could be due to the fact that the δ^{34}S and δ^{15}N values of soil compounds (and therefore of plant and animal products) could be correlated, because their isotopic fractionation is influenced by similar factors.[6] However, in the current study, no correlation was observed between the δ^{34}S and δ^{15}N values of beef samples, and this was in agreement with the findings of Rossmann et al.,[6] who did not observe this correlation for milk. The high δ^{34}S values observed in beef from Ireland and the low δ^{34}S values observed in beef from Italy were in agreement with the δ^{34}S values reported by Camin et al.[19] for lamb samples from Ireland and Sicily.

Canonical Discriminant Analysis. Multivariate canonical discriminant analysis was applied to the isotope data considering all of the beef samples grouped by country of origin regardless of the production system. Due to the high number of Irish samples, only 20 random beef samples from the controlled feeding trial (10 pasture-fed, 10 concentrate-fed) were considered for the analysis. The canonical discriminant analysis was performed to classify beef according to country of origin on the basis of stable isotopic signatures (C, N, H, and S) of the muscle. Although the stepwise method was used, all of the variables were relevant for discrimination purposes, and thus no redundant variable was identified. The results suggested four discriminant functions. All of the discriminant functions were statistically significant for the

Table 2. Results of the Classification of Beef Samples from Different European and Non-European Countries on the Basis of Canonical Discriminant Analysis by Means of C, N, H, and S Isotope Ratios[a]

		predicted group membership									
	country	AU	BR	FR	GE	IR	IT	SP	UK	US	total
original count	AU	6	0	0	0	0	0	0	0	0	6
	BR	0	17	0	0	0	0	0	0	0	17
	FR	1	0	1	0	0	1	1	0	0	4
	GE	2	0	0	3	1	0	0	0	0	6
	IR	0	0	0	0	42	0	0	4	0	46
	IT	0	0	0	0	0	16	2	0	0	18
	SP	1	0	0	0	2	0	4	0	0	7
	UK	0	0	0	0	3	0	0	17	0	20
	US	1	0	0	0	0	3	0	0	18	22
original %	AU	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0
	BR	0.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0
	FR	25.0	0.0	25.0	0.0	0.0	25.0	25.0	0.0	0.0	100.0
	GE	33.3	0.0	0.0	50.0	16.7	0.0	0.0	0.0	0.0	100.0
	IR	0.0	0.0	0.0	0.0	91.3	0.0	0.0	8.7	0.0	100.0
	IT	0.0	0.0	0.0	0.0	0.0	88.9	11.1	0.0	0.0	100.0
	SP	14.3	0.0	0.0	0.0	28.6	0.0	57.1	0.0	0.0	100.0
	UK	0.0	0.0	0.0	0.0	15.0	0.0	0.0	85.0	0.0	100.0
	US	4.5	0.0	0.0	0.0	0.0	13.6	0.0	0.0	81.8	100.0
cross-validated count[b]	AU	5	0	0	0	0	1	0	0	0	6
	BR	0	17	0	0	0	0	0	0	0	17
	FR	1	0	0	0	0	1	1	0	1	4
	GE	2	0	1	2	1	0	0	0	0	6
	IR	0	0	0	0	42	0	0	4	0	46
	IT	0	0	0	0	0	16	2	0	0	18
	SP	1	0	0	0	2	0	4	0	0	7
	UK	0	0	0	0	3	0	0	17	0	20
	US	1	0	0	0	0	4	0	0	17	22
cross-validated %	AU	83.3	0.0	0.0	0.0	0.0	16.7	0.0	0.0	0.0	100.0
	BR	0.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0
	FR	25.0	0.0	0.0	0.0	0.0	25.0	25.0	0.0	25.0	100.0
	GE	33.3	0.0	16.7	33.3	16.7	0.0	0.0	0.0	0.0	100.0
	IR	0.0	0.0	0.0	0.0	91.3	0.0	0.0	8.7	0.0	100.0
	IT	0.0	0.0	0.0	0.0	0.0	88.9	11.1	0.0	0.0	100.0
	SP	14.3	0.0	0.0	0.0	28.6	0.0	57.1	0.0	0.0	100.0
	UK	0.0	0.0	0.0	0.0	15.0	0.0	0.0	85.0	0.0	100.0
	US	4.5	0.0	0.0	0.0	0.0	18.2	0.0	0.0	77.3	100.0

[a] 84.9% of original grouped cases were correctly classified, and 82.2% of cross-validated grouped cases were correctly classified. AU, Austria; BR, Brazil; FR, France; GE, Germany; IR, Ireland; IT, Italy; SP, Spain; UK, United Kingdom; US, United States. [b] Cross-validation is done only for those cases in the analysis. In cross-validation, each case is classified by the functions derived from all cases other than that case.

discrimination (Wilk's $\lambda < 0.6$), with the first two being the most significant (Wilks' $\lambda < 0.09$). The first and second discriminant functions accounted for 91.5% of the variance. The first function was mainly correlated with the $\delta^{13}C$ values and explained 58.0% of the variance, whereas the second function was mainly correlated with the $\delta^{34}S$ values. However, $\delta^{2}H$ was the most discriminatory variable for the third function and accounted for 4.8% of the variance. On the other hand, $\delta^{15}N$ values proved to be the largest discriminatory variables for the fourth discriminant function and accounted for 3.7% of the variation. A visualization of the results is shown in Figure 4, a plot of canonical discriminant functions 1 and 2.

The classification results are given in Table 2, where the number of samples and percentage of samples correctly classified are shown diagonally. We obtained 84.9% correct classification, whereas with the leave-one-out cross-validation the classification percentage fell slightly to 82.2%. Only beef samples from Brazil were 100% correctly classified and then cross-validated, whereas

Table 3. Results of the Classification of Beef Samples from Different Countries and Production Systems from Ireland on the Basis of Canonical Discriminant Analysis by Means of C, N, H, and S Isotope Ratios[a]

		predicted group membership										
	country	AU	BR	FR	GE	IRc	IRp	IT	SP	UK	US	total
original count	AU	5	0	0	0	0	0	0	0	0	1	6
	BR	0	17	0	0	0	0	0	0	0	0	17
	FR	1	0	1	0	0	0	0	0	1	1	4
	GE	0	0	0	5	0	1	0	0	0	0	6
	IRc	0	0	0	0	20	0	0	0	0	0	20
	IRp	0	0	0	0	0	20	0	0	0	0	20
	IT	0	0	0	0	0	0	16	2	0	0	18
	SP	1	0	0	0	3	0	0	3	0	0	7
	UK	0	0	0	0	4	2	0	0	14	0	20
	US	0	0	0	1	0	0	3	0	0	18	22
original %	AU	83.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	16.7	100.0
	BR	0.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0
	FR	25.0	0.0	25.0	0.0	0.0	0.0	0.0	0.0	25.0	25.0	100.0
	GE	0.0	0.0	0.0	83.3	0.0	16.7	0.0	0.0	0.0	0.0	100.0
	IRc	0.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0	0.0	0.0	100.0
	IRp	0.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0	0.0	100.0
	IT	0.0	0.0	0.0	0.0	0.0	0.0	88.9	11.1	0.0	0.0	100.0
	SP	14.3	0.0	0.0	0.0	42.9	0.0	0.0	42.9	0.0	0.0	100.0
	UK	0.0	0.0	0.0	0.0	20.0	10.0	0.0	0.0	70.0	0.0	100.0
	US	0.0	0.0	0.0	4.5	0.0	0.0	13.6	0.0	0.0	81.8	100.0
cross-validated count[b]	AU	5	0	0	0	0	0	0	0	0	1	6
	BR	0	17	0	0	0	0	0	0	0	0	17
	FR	1	0	0	0	0	0	0	0	1	2	4
	GE	2	0	0	3	0	1	0	0	0	0	6
	IRc	0	0	0	0	20	0	0	0	0	0	20
	IRp	0	0	0	0	0	20	0	0	0	0	20
	IT	0	0	0	0	0	0	16	2	0	0	18
	SP	1	0	0	0	3	0	0	3	0	0	7
	UK	0	0	0	0	4	2	0	0	14	0	20
	US	0	0	0	1	0	0	3	0	0	18	22
cross-validated %	AU	83.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	16.7	100.0
	BR	0.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0
	FR	25.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	25.0	50.0	100.0
	GE	33.3	0.0	0.0	50.0	0.0	16.7	0.0	0.0	0.0	0.0	100.0
	IRc	0.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0	0.0	0.0	100.0
	IRp	0.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0	0.0	100.0
	IT	0.0	0.0	0.0	0.0	0.0	0.0	88.9	11.1	0.0	0.0	100.0
	SP	14.3	0.0	0.0	0.0	42.9	0.0	0.0	42.9	0.0	0.0	100.0
	UK	0.0	0.0	0.0	0.0	20.0	10.0	0.0	0.0	70.0	0.0	100.0
	US	0.0	0.0	0.0	4.5	0.0	0.0	13.6	0.0	0.0	81.8	100.0

[a] 85.0% of original grouped cases correctly classified, and 82.9% of cross-validated grouped cases correctly classified. AU, Austria; BR, Brazil; FR, France; GE, Germany; IRc, Ireland concentrate; IRp, Ireland pasture; IT, Italy; SP, Spain; UK, United Kingdom; US, United States. [b] Cross-validation is done only for those cases in the analysis. In cross-validation, each case is classified by the functions derived from all cases other than that case.

2 of 18 Italian, 4 of 46 Irish, 1 of 6 Austrian, 3 of 7 Spanish, 4 of 4 French, 4 of 6 German, 3 of 20 English, and 5 of 22 U.S. samples were misclassified in the cross-validation (Table 2). The results showed that 91.3% of Irish samples were cross-validated correctly classified and 8.7% of the Irish beef were wrongly classified as U.K. beef. In the case of U.K. samples, 85.0% were correctly assigned, whereas 15.0% were assigned to Ireland. The fact that all of the French samples were classified incorrectly can be attributed to the lower number of samples available for analysis. For the same reason only 57% of Spanish samples and 33% of

German samples were cross-validated correctly classified. A further collection of samples could probably improve this result.

With the aim of studying the possibility of discriminating pasture-fed Irish beef not only from Irish beef produced under different production systems but also from other countries, a second CDA was performed considering Irish samples with known and controlled feeding background (20 beef samples from animals fed barley-concentrate diet and 20 beef samples from animals fed exclusively pasture). In this case, using the four isotope ratios, 85.0% of the individual beef samples were correctly allocated and 82.9% cross-validated. However, all of the Irish beef samples marketed as pasture-fed beef were correctly classified and then cross-validated (Table 3).

Conclusions. Stable isotope ratios of C, N, H, and S of beef have been proven to be good indicators of geographical origin of beef, and they could successfully be used for discriminating beef from distant geographic origins. A model was optimized using the four isotope ratios leading to 82.2% correct classification in the cross-validation. A further approach was tested for discriminating beef produced in Ireland under production systems based exclusively on grass, and it was possible to achieve 100% correct identification for the Irish pasture-fed samples in the cross-validation using the four isotope ratios.

AUTHOR INFORMATION

Corresponding Author

*E-mail: frank.monahan@ucd.ie. Phone: +353 1 7167090. Fax: +353 1 7161147.

Funding Sources

This research was funded by the Irish Department of Agriculture, Fisheries and Food under the Food Institutional Research Measure (FIRM) of the Productive Sector Operational Programme of the 2000−2006 National Development Plan.

ACKNOWLEDGMENT

We acknowledge the personal contacts who helped with the procurement of samples from different countries. We are grateful to Dr. S. Brookes and Dr. I. Begley (Iso-Analytical Ltd., Crewe, Cheshire, U.K.) for isotopic analyses.

REFERENCES

(1) Verbeke, W.; Vackier, I. Profile and effects of consumer involvement in fresh meat. *Meat Sci.* **2004**, *67*, 159–168.

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

(3) Hargin, K. D. Authenticity issues in meat and meat products. *Meat Sci.* **1996**, *43*, S277–S289.

(4) Council Regulation (EC) No. 2772/1999. Providing for the general rules for a compulsory beef labelling system. *Off. J. Eur. Communities* **1999**, *L 334*, 0001−0002.

(5) Boner, M.; Förstel, H. Stable isotope variation as a tool to trace the authenticity of beef. *Anal. Bioanal. Chem.* **2004**, *378*, 301–310.

(6) Rossmann, A.; Kornexl, B. E.; Versini, G.; Pichlmayer, F.; Lamprecht, G. Origin assignment of milk from alpine regions by multielement stable isotope ratio analysis (SIRA). *J. Food Sci. Nutr.* **1998**, *1*, 9–21.

(7) Camin, F.; Larcher, R.; Perini, M.; Bontempo, L.; Bertoldi, D.; Gagliano, G.; Nicolini, G.; Versini, G. Characterisation of authentic Italian extra-virgin olive oils by stable isotope ratios of C, O and H and mineral composition. *Food Chem.* **2010**, *118*, 901–909.

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

(9) Manca, G.; Camin, F.; Coloru, G. C.; Del Caro, A.; Depentori, D.; Franco, M. A.; Versini, G. Characterization of the geographical origin of Pecorino Sardo cheese by casein stable isotope ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) ratios and free amino acid ratios. *J. Agr. Food Chem.* **2001**, *49*, 1404–1409.

(10) Rummel, S.; Hoelzl, S.; Horn, P.; Rossmann, A.; Schlicht, C. The combination of stable isotope abundance ratios of H, C, N and S with $^{87}Sr/^{86}Sr$ for geographical origin assignment of orange juices. *Food Chem.* **2008**, *118*, 890–900.

(11) Nakashita, R.; Suzuki, Y.; Akamatsu, F.; Iizumi, Y.; Korenaga, T.; Chikaraishi, Y. Stable carbon, nitrogen, and oxygen isotope analysis as a potential tool for verifying geographical origin of beef. *Anal. Chim. Acta* **2008**, *617*, 148–152.

(12) Kelly, S. D. Using stable isotope ratio mass spectroscopy (IRMS) in food authentication and traceability. In *Food Authenticity and Traceability*; Lees, M., Ed.; Woodhead Publishing: Cambridge, U.K., 2003; pp 156−183.

(13) Rozanski, K.; Araguasaraguas, L.; Gonfiantini, R. Relation between long-term trends of O-18 isotope composition of precipitation and climate. *Science* **1992**, *258*, 981–985.

(14) Krouse, H. R.; Grinenko, V. A. *Stable Isotopes. Natural and Anthropogenic Sulphur in the Environment*; SCOPE 43; Wiley: Chichester, U.K., 1991.

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

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

(17) Renou, J.-P.; Bielicki, G.; Deponge, C.; Micol, D.; Ritz, P. 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 Chem.* **2004**, *86*, 251–256.

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

(19) Camin, F.; Bontempo, L.; Heinrich, K.; Horacek, M.; Kelly, S. D.; Schlicht, C.; Thomas, F.; Monahan, F. J.; Hoogewerff, J.; Rossmann, A. Multi-element (H, C, N, S) stable isotope characteristics of lamb meat from different European regions. *Anal. Bioanal. Chem.* **2007**, *389*, 309–320.

(20) Guo, B. L.; Wei, Y. M.; Pan, J. R.; Li, Y. Stable C and N isotope ratio analysis for regional geographical traceability of cattle in China. *Food Chem.* **2008**, *118*, 915–920.

(21) Perini, M.; Camin, F.; Bontempo, L.; Rossmann, A.; Piasentier, E. Multielement (H, C, N, O, S) stable isotope characteristics of lamb meat from different Italian regions. *Rapid Commun. Mass Spectrom.* **2009**, *23*, 2573–2585.

(22) Bong, Y.-S.; Shin, W.-J.; Lee, A.-R.; Kim, Y.-S.; Kim, K.; Lee, K.-S. Tracing the geographical origin of beefs being circulated in Korean markets based on stable isotopes. *Rapid Commun. Mass Spectrom.* **2010**, *24*, 155–159.

(23) Horacek, M.; Min, J.-S. Discrimination of Korean beef from beef of other origin by stable isotope measurements. *Food Chem.* **2010**, *121*, 517–520.

(24) Enser, M.; Hallett, K. G.; Hewett, B.; Fursey, G. A. J.; Wood, J. D.; Harrington, G. Fatty acid content and composition of UK beef and lamb muscle in relation to production system and implications for human nutrition. *Meat Sci.* **1998**, *49*, 329–341.

(25) Poulson, C. S.; Dhiman, T. R.; Ure, A. L.; Cornforth, D.; Olson, K. C. Conjugated linoleic acid content of beef from cattle fed diets

containing high grain, CLA, or raised on forages. *Livest. Prod. Sci.* **2004**, *91*, 117–128.

(26) Realini, C. E.; Duckett, S. K.; Brito, G. W.; Dalla Rizza, M.; De Mattos, D. Effect of pasture vs. concentrate feeding with or without antioxidants on carcass characteristics, fatty acid composition, and quality of Uruguayan beef. *Meat Sci.* **2004**, *66*, 567–577.

(27) Nuernberg, K.; Dannenberger, D.; Nuernberg, G.; Ender, K.; Voigt, J.; Scollan, N. D.; Wood, J. D.; Nute, G. R.; Richardson, R. I. Effect of a grass-based and a concentrate feeding system on meat quality characteristics and fatty acid composition of longissimus muscle in different cattle breeds. *Livest. Prod. Sci.* **2005**, *94*, 137–147.

(28) Alfaia, C. P. M.; Alves, S. P.; Martins, S. I. V.; Costa, A. S. H.; Fontes, C. M. G. A.; Lemos, J. P. C.; Bessa, R. J. B.; Prates, J. A. M. Effect of the feeding system on intramuscular fatty acids and conjugated linoleic acid isomers of beef cattle, with emphasis on their nutritional value and discriminatory ability. *Food Chem.* **2009**, *114*, 939–946.

(29) Röhrle, F. T.; Moloney, A. P.; Black, A.; Osorio, M. T.; Sweeney, T.; Schmidt, O.; Monahan, F. J. α-Tocopherol steroisomers in beef as an indicator of vitamin E supplementation in cattle diets. *Food Chem.* **2011**, *124*, 935–940.

(30) Radin, N. S. Extraction of tissue lipids with a solvent of low toxicity. *Methods Enzymol.* **1981**, *72*, 5–7.

(31) Harrison, S. M.; Schmidt, O.; Moloney, A. P.; Kelly, S. D.; Rossmann, A.; Schellenberg, A.; Camin, F.; Perini, M.; Hoogewerff, J.; Monahan, F. J. Tissue turnover in ovine muscle and lipids as recorded by multiple (H, C, O, S) stable isotope ratios. *Food Chem.* **2011**, *124*, 291–297.

(32) IAEA, GNIP database 2002, http://www-naweb.iaea.org.

(33) De Niro, M. J.; Epstein, S. Influence of diet on the distribution of carbon isotopes in animals. *Geochim. Cosmochim. Acta* **1978**, *42*, 495–506.

(34) Bahar, B.; Monahan, F. J.; Moloney, A. P.; O'Kiely, P.; Scrimgeour, C. M.; Schmidt, O. Alteration of the carbon and nitrogen stable isotope composition of beef by substitution of grass silage with maize silage. *Rapid Commun. Mass Spectrom.* **2005**, *19*, 1937–1942.

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

(36) U.S. Department of Agriculture (USDA). http://www.usda.gov/wps/portal/usda/usdahome, 2007.

(37) Osorio, M. T.; Moloney, A. P.; Schmidt, O.; Monahan, F. J. Beef authentication and retrospective dietary verification using stable isotope ratio analysis of bovine muscle and tail hair. *J. Agric. Food Chem.* **2011**, doi: 10.1021/jf1040959.

(38) De Niro, M. J.; Epstein, S. Influence of diet on the distribution of nitrogen isotopes in animals. *Geochim. Cosmochim. Acta* **1981**, *45*, 341–351.

(39) Moreno-Rojas, J. M.; Vasta, V.; Lanza, A.; Luciano, G.; Ladroue, V.; Guillou, C.; Priolo, A. Stable isotopes to discriminate lambs fed herbage or concentrate both obtained from C_3 plants. *Rapid Commun. Mass Spectrom.* **2008**, *22*, 3701–3705.

(40) Bahar, B.; Moloney, A. P.; Monahan, F. J.; Harrison, S. M.; Zazzo, A.; Scrimgeour, C. M.; Begley, I. S.; Schmidt, O. Turnover of carbon, nitrogen, and sulfur in bovine longissimus dorsi and psoas major muscles: implications for isotopic authentication of meat. *J. Anim. Sci.* **2009**, *87*, 905–913.