BRITISH BEEF ORIGIN PROJECT II – Improvement of the British beef Isotope Landscape Map (Isoscape) for Scotland and Northern Ireland

FS515009: QUB update for final report to the project officer
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By: contributions from QUB

2.4 Update from QUB

2.4.1 Sample treatment

On arrival of the chilled beef samples, they were stored at -20°C. Subsamples of ~ 40 g were taken, thinly sliced and freeze-dried for more than 30h. The dried sample was turned into a powder using a ball mill, before performing defatting steps with petrol ether/diethyl-ether (2:1), as described by Camin *et al* (2012). The sample preparation was completed with a second freeze-drying step over night.

2.4.2 Stable Nitrogen, Carbon, Sulfur and Hydrogen isotope ratio analysis of beef by EA-IRMS

The stable isotope mass spectrometer (Sercon 20-22 EA-HT IRMS) used for this study was recently installed at QUB, and therefore measurements of HNCS isotope ratios required optimisation. Method development, during the initial stages of this project, was conducted and reported. The results obtained from the stable isotope analysis from aliquots of identical beef samples that have been prepared at both Fera and QUB will be prepared and analysed by both institutes to ensure comparability of data between institutes.

CNS isotope ratio analysis: 1

Initial method development was performed to ensure that simultaneous data acquisition for carbon, nitrogen and sulfur isotope ratios could be performed. The abundance of the element sulfur, in comparison to that of carbon and nitrogen present in the samples, is low. Ultimately, the sample size (4 – 10 mg) and the instrument acquisition parameters were optimised for acquisition of data from sulfur combined with gas dilution to compensate for the higher amounts of carbon and nitrogen. Further test batches to assure consistency of results are pending.

CNS isotope ratio analysis: update 12/11/15

In contrary to the initial results and despite several analysis batches being trialled, stable reproducible data could not be derived from simultaneous N, C & S isotope acquisition at QUB using the Sercon 22-20 IRMS system. Various machine related problems were encountered;

such as a drift in tuning for beam 2, or excess variability in peak amplitude for N. Every effort was made to maintain the sample weights in a suitable range to provide an optimal signal strength for all 3 elements. The large sample size (6-8mg) seemed to influence the consistency, under dilution, of the N data much more so than the C data. It was possible to achieve good data for C and S isotopes using a large sample drop with dilution. It was also possible to achieve good data for N and C using a smaller sample size (~0.7mg) but this did not provide adequate signal strength for S. Using such a large sample size compelled the use of a dilution flow to bring the N and C peaks down into a usable range, but the N peak had a tendency to stray over range. This introduced instability in the drift correction, resulting in unstable unusable data for N. If enough time was available, these problems would have been surmounted, but as the project was already under time pressure following the first extension, a decision was made to send the outstanding CNS analysis to FERA.

H isotope ratio analysis

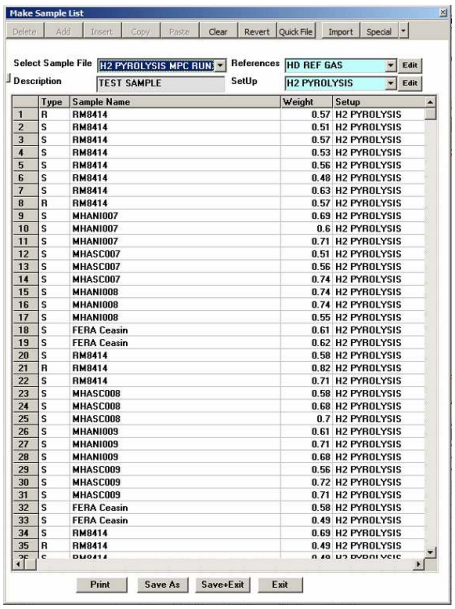
Initial method development was performed for acquisition of hydrogen isotope ratio data. The sample size (0.5 - 0.7 mg) and the instrument acquisition parameters were optimised. Hydrogen data was shown to be consistent and subsequently H isotope ratio analyses will be performed.

16 batches (including repeats) were performed to complete the H isotope analysis. The batch structure was:

Bracket 1
- 6-8 x RM8414- conditioning and drift correction
- Sample (triplicate)
- Sample (triplicate)
- Sample (triplicate)
- FERA Caesin (duplicate)- QC
- RM8414 (triplicate)- drift correction

Bracket 2
- Sample (triplicate)
- Sample (triplicate)
- Sample (triplicate)
- FERA Caesin (duplicate)- QC
- RM8414 (triplicate)- drift correction

..with further brackets up to a maximum of 4 brackets. An example sequence is shown below.



This structure allowed for up to 12 meat samples to be analysed per batch, meaning that 146 meat samples, plus 10 cross laboratory check samples, required 13 sample batches. 2 batches of comparison standards on separate occasions were also run, plus one repeat analysis batch gives the total of 16 analysis batches.

H analysis quality control.

Each analytical batch contained 2 x FERA Caesin in each drift correction bracket. [i.e. 4 brackets = 8 FERA Caesin QCs] The average of the two bracket QCs must pass acceptance criteria to allow results from that bracket to be used. Samples within a failed bracket must be repeated. In total 10 repeats were required (5 samples and 5 cross laboratory checks) out of 169 individual analyses. The FERA Caesin is a matrix matched QC. In total 59 FERA Caesin QCs were run across all of the analytical batches. These had an average delta of 1.64, (within 1SD of the assigned value).

A variety of certified comparison standards were also analysed on two separate occasions.

The results of these runs can be seen below.

On both occasions (Run 1 and Run2) the NBS 22 Oil standard returned a result significantly more enriched than its assigned value. On both occasions a randomly chosen beef sample was analysed and returned a value within 2SD of its first analysed value. In Run 1 all other

standards fell with 2SD of their assigned values, however in Run2 the NIST 1577 Bovine liver strayed to 3SD from the assigned and the IAEA CH7 Polyethylene fell outside range. On hindsight the standards that fell outside range should have been repeated.

Comparison standards Run 1 -06/08/2015								
Count	7							
Sample	**2H**	**StDev**	CV	**reps**		**Assigned value**		
IAEA CH7	-96.92	0.16	0.2%	2	3.38	-100.3	Within 2*SD of assigned value	
FERA Ceasin	-112.6	0.60	0.5%	3	0.40	-113	Within 1*SD of assigned value	
NIST1577b	-132.7	0.50	0.4%	3	3.50	-136.2	Within 2*SD of assigned value	
QUB Ceasin	-105.4	1.80	1.7%	3	0.40	-105	Within 1*SD of assigned value	
FERA Ceasin	-110.52	0.11	0.1%	2	2.48	-113	Within 2*SD of assigned value	
IAEA NBS 22	-109.5	1.30	1.2%	3	7.40	-116.9	outside range	
MHANI050	-102.9	1.50	1.5%	3	2.10	-105.0	Within 2*SD of previous value	
Comparison standards Run 2 -15/09/2015								
Count	7							
Sample	**2H**	**SD**	**CV**	**reps**		**Assigned value**		
IAEA CH7	-91.4	0.8	0.9%	3	8.95	-100.3	outside range	
FERA Ceasin	-110.0	2.4	2.2%	3	3.00	-113	Within 2*SD of assigned value	
NIST1577b	-131.6	1.9	1.5%	3	4.61	-136.2	Within 3*SD of assigned value	
QUB Ceasin	-108.7	1.1	1.0%	3	3.66	-105	Within 2*SD of assigned value	
FERA Ceasin	-113.3	0.4	0.4%	3	0.30	-113	Within 1*SD of assigned value	
IAEA NBS 22	-110.9	1.2	1.1%	3	6.03	-116.9	outside range	
MHASC098	-100.6	2.3	2.3%	3	3.19	-103.8	Within 2*SD of previous value	

2.4.3 Lead isotope ratio analysis by ICP/MS

The method of determination of Pb isotope ratios is routinely performed at QUB and no issues were encountered during the acquisition of this data.

An accurately weighed aliquot of 100mg of the dried, fat extracted meat powder was digested in concentrated acid and peroxide using a temperature controlled microwave oven, and made up to a known weight with deionised water. The sample batch size performed for this analysis consisted of 48 samples, 2 blanks (empty tubes, only acid) and 2 CRM (RM8414 beef muscle powder) samples. The resultant extraction was analysed for lead isotopes: ^{206}Pb, ^{207}Pb and ^{208}Pb on a 'Thermo iCAP Q' ICP/MS. Calibration, drift and isotopic correction was performed using a certified Pb isotopic standard with the following run structure: Samples, blanks and CRMs were randomly distributed within each run.

Bracket 1

5 x Pb isotope certified standards for drift correction and calibration
Sample 1 (10 analytical replicates)
Sample 2 (10 analytical replicates)

Sample 3 (10 analytical replicates)
Sample 4 (10 analytical replicates)
2 x Pb isotope certified standards for drift correction
Sample 5 (10 analytical replicates)
Bracket 2
Sample 6 (10 analytical replicates)
Sample 7 (10 analytical replicates)
Sample 8 (10 analytical replicates)
2 x Pb isotope certified standards for drift correction
Etc…. Bracket 3,4,5 …

Pb isotopic analysis quality control.

Overall 6 RM8414 QCs were run, two in each of the three analytical batches.

These were tabulated and the recovery of Pb compared to its certified value (0.38mg/Kg) was 71.7% (average 0.272mg/Kg, coefficient of variance cv =17%, n=6). The recovery is a little low but could be considered to be on the acceptable end of the range if this was of primary concern. Accordingly a cv of 17% would be considered high if the empirical concentration was of importance. These certified standards are for isotopic correction and the concentration is only a reference value.

The reproducibility of the corrected isotopic values is of more importance.

For 206/207: The cv was 2.2%

For 208/207: The cv was 1.2%

For 208/206: The cv was 1.2%

All of these are well within the limits of acceptability. Less than 5% is good, approaching 1% or better is optimal.

Tabulated results of the Pb QC samples is shown below.

		CRM Pb		206/207				208/207				208/206		
	RM8414	Certified	0.38											
IGFS code	Sample	Concentration ug/L	mg/Kg	Mass bias corrected IR	IR stdev	IR %RSD		Mass bias corrected IR	IR stdev	IR %RSD		Mass bias corrected IR	IR stdev	IR %RSD
SPb CRM1		1.1304	0.3375	1.1844	0.0056	0.4723		2.4359	0.0094	0.3842		2.2281	0.0110	0.5324
SPb CRM2		0.7294	0.2170	1.1751	0.0038	0.3188		2.4246	0.0121	0.4952		2.2178	0.0120	0.5789
SPb CRM3		1.0959	0.3082	1.1390	0.0046	0.4011		2.3927	0.0148	0.6135		2.1886	0.0123	0.5924
SPb CRM4		1.0302	0.2862	1.1768	0.0060	0.5001		2.4147	0.0068	0.2776		2.2087	0.0102	0.5005
SPb CRM5		0.8610	0.2507	1.1610	0.0035	0.2991		2.4036	0.0087	0.3569		2.1986	0.0134	0.6447
SPb CRM6		0.7861	0.2343	1.1192	0.0058	0.4970		2.3562	0.0114	0.4707		2.1552	0.0088	0.4221
		average	0.2723	1.1592				2.4046				2.1995		
		stdev	0.0463	0.0253				0.0282				0.0258		
		CV	17.0%	2.2%				1.2%				1.2%		
		Recovery	71.7%											
	Canadian beef powder													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