Appendix 7: Hydrogen equilibration experiment described in full

Background

Determination of accurate δ^2H measurement of organic material is complicated by exchange of hydrogen between ambient water vapour and exchangeable hydrogen of the sample matrix; therefore an equilibration step is required prior to analysing hydrogen.

Materials and Methods

Following a publication by Meier-Augenstein *et al* (2013), 4 different equilibration experiments were performed in comparison to the proposed equilibration with 2 waters of different isotopic signature, to identify the best approach for the hydrogen analysis in this project. Each time the same beef samples (n = 3) were run with NIST bovine muscle 8414 for normalisation/drift correction on the V-SMOW scale and TRACE casein as a quality control standard. The experiments and their results are shown below, and summarised in Table A and Figure A respectively.

H equilibration – method 1

- **No** equilibration performed
- Proof of principle
- Samples analysed straight after being weighed

H equilibration – method 2: currently used at Fera

- ***Sample weighed into tin capsule***
- ***Capsule left open > ambient temperature equilibration, with laboratory moisture for 48h in lab***
- ***Capsule sealed and stored in desiccator till analysis***

H equilibration – method 3: used during the British Beef Origin Project (BBOP)I

- Sample weighed into tin capsule
- Capsule left open > ambient temperature equilibration with laboratory moisture for 96h in lab
- Capsule sealed and stored in desiccator till analysis

H equilibration – method 4: used during the Trace Project

- Sample weighed into tin capsule
- Capsule sealed > ambient temperature equilibration with laboratory moisture for 48h in lab

- Capsule transferred to a freeze-dryer overnight
- Capsule stored in desiccator till analysis

<u>H equilibration – method 5: Equilibration with water vapour of different isotopic compositions, at ambient temperature</u>

- Two identical sets of samples weighed into tin capsules
- One set equilibrated with water depleted in ^{2}H, the other set equilibrated with water enriched in ^{2}H- Equilibration time: 4 days
- Sets of samples transferred to separate desiccators equipped with vacuum pump outlet - Drying time: 7 days
- Samples analysed in 4 hours, to avoid equilibrated and dried samples reabsorbing water

Table A: Overview of equilibration methods.

method	capsule		Equilibration Time [h]	water		Freeze-dryer	Calibration standard	Description
	open	closed		1	2			
1	-	x	0	-	-	-	NIST 8414	Proof of principle
2	x	-	48	-	-	-	NIST 8414	Fera
3	x	-	96	-	-	-	NIST 8414	BBOP1
4	-	x	48	-	-	x	NIST 8414	Trace
5	-	x	1 week	x	x	-	IAEA-PE-foil	Meier-Augenstein

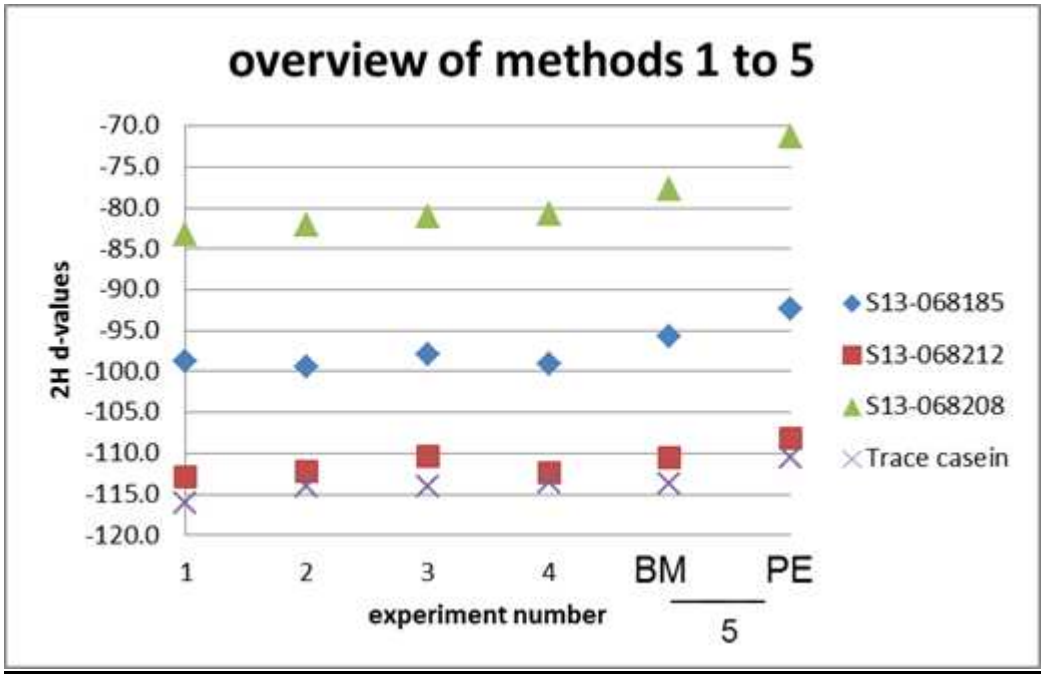

Figure A: Overview of the results for method 1 to 5.

Note: BM = correction vs NIST 8414 Bovine Muscle, PE: correction vs IAEA-CH7 PE foil

Evaluation

The Portion of exchangeable hydrogen of beef protein and standards is around 11.5% (applying Formula 1). Using a matrix matched standard during the equilibration step compensates for exchangeable hydrogen of the sample.

The variation of the δ^2H results falls within range of analytical errors, expected for isotopic work, when comparing the results of the single equilibration (method 2) to the '2 water equilibration' (method 5).

Additionally, beef samples are measured in a similar atmosphere, to where the sampling has taken place. By applying Fera's method (method 2) at both IRMS labs, consistent data can be expected and sample throughput increased, as only one hydrogen batch is to be analysed compared to two hydrogen batches for the approach of equilibration of samples with 2 waters of different isotopic signature (metehod 5). Furthermore, Fera's method is comparable to the one used for projects FA0205, and FA0152, therefore these datasets can be combined.

Formula 1: Molecular exchange fraction

$$f_{Hxch} = \frac{\delta^2 H_{sample,water\,A} - \delta^2 H_{sample,water\,B}}{\delta^2 H_{water\,A} - \delta^2 H_{water\,B}}$$

f_{Hxch} = molecular exchange fraction

$\delta^2_{Hsample,\ water\ A}$ = measured δ^2H value in tray A

$\delta^2_{Hsample,\ water\ B}$ = measured δ^2H value in tray B

$\delta^2_{Hwater\ A}$ = actual δ^2H value for water A

$\delta^2_{Hwater\ B}$ = actual δ^2H value for water B

Reference

W. Meier-Augenstein, K A Hobson, L I Wassenaar, Bioanalysis (2013), Critique: measuring hydrogen stable isotope abundance of proteins to infer origins of wildlife, food and people, 5 (7), 751-767.