**Appendix A –** Preparation, Calibration, and Analytical Uncertainty for Isotopic Measurements of Hair Keratin

We cleaned segments following the Archaeological Chemistry Laboratory’s protocol (Knudson, et al., 2015). We combed segments parallel, wrapped segments in gauze, and suspended wrapped hair in CH3CH2OH (ethanol) for 24 hours, air-dried, and transected gauze at every two cm (section a was proximal). We chemically cleaned sections with alternating rinses of ultrapure water and 1:2:.0.8 (v/v) ratio of CHCl3(chloroform), CH3OH (methanol), and ultrapure water before air drying at 50° C. We homogenized sections with sterilized surgical scissors and loaded into capsules.

Stable isotope analysis of hair keratin occurred at the Metals, Environmental, and Terrestrial Analytical Laboratory (METAL) at Arizona State University following standard analysis and reporting protocols (Coplen, 1994, Roberts, et al., 2018, Szpak, et al., 2017). Carbon and nitrogen isotopic and elemental compositions were determined using a Thermo Delta Plus Advantage mass spectrometer coupled to a Costech Elemental Analyzer in the Metals, Environmental, and Terrestrial Analytical Laboratory (METAL) at Arizona State University. Stable carbon isotopic compositions are expressed as delta values relative to VPDB (Vienna Peedee belemnite) on a scale normalized such that the δ13C values of NBS 19 calcium carbonate and L-SVEC lithium carbonate are +1.95 ‰ and –46.6 ‰, respectively. Stable nitrogen isotopic compositions are expressed relative to atmospheric nitrogen, which is isotopically homogenous. Our in-house glycine standards (glycine low and glycine high) were characterized using USGS40 and USGS41 reference material (Table A1). Glycine low and glycine high were used as scale anchors in every analytical session (Table A1). Stable isotope ratios are reported in per mil () notation, e.g., *δ*13C = (((13C/12C)/(13C/12Cstandard)) – 1) x 1000 (Coplen, 1994).

**Table A1.** Standard reference materials used to calibrate *δ*13C relative to VPDB and *δ*15N relative to AIR.

|  |  |  |  |
| --- | --- | --- | --- |
| Standard | Material | Accepted *δ*13C  (‰, VPDB) | Accepted *δ*15N  (‰, AIR) |
| USGS40 | L-glutamic acid | –26.39±0.04 | –4.52±0.06 |
| USGS41 | L-glutamic acid | +37.63±0.05 | +47.57±0.11 |
| Glycine low | Glycine | –39.64±0.05 | +1.35±0.11 |
| Glycine high | Glycine | –15.67±0.05 | –51.8±0.18 |

The following internal standards were used for quality control of scale calibration (Table A2). The isotopic compositions reported here for internal standards represent long term averages calibrated to VPDB and AIR.

**Table A2.** In-house standard reference materials used for to monitor internal accuracy and precision.

|  |  |  |  |
| --- | --- | --- | --- |
| Standard | Material | Mean *δ*13C  (‰, VPDB) | Mean *δ*15N  (‰, AIR) |
| Gly mid | Glycine mid | −8.36±0.05 | +27.9±0.11 |
| H Std 2 | Human hair | −16.78±0.05 | +9.11±0.07 |
| TL | Tomato leaves | −26.88±0.06 | +3.96±0.12 |

Table A3 presents the means and standard deviations of the *δ*13C and *δ*15N values for the check and calibration standards as well as the number of standards in each analytical session. Measurement precision (the pooled standard deviation of the check and calibration standards) was ±0.20 ‰ for *δ*13C and ±0.18 ‰ for *δ*15N (*df*=56), calculated from Appendix B following the standard uncertainty calculator in Appendix G of Szpak et al. (2017). Measurement accuracy (bias) was evaluated by comparing the known and measured *δ*13C and *δ*15N values for H Std 2 and tomato leaves and factoring in the long-term uncertainty in these known measurements using the standard uncertainty calculator of Szpak et al. (2017). Measurement bias due to systematic error (accuracy) was determined to be ±0.14 ‰ for *δ*13C and ±0.16 ‰ for *δ*15N.

Table A3. Mean and standard deviation of all check and calibration standards for the analytical sessions containing data presented in this paper.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Session ID** | **Standard** | **n** | ***δ*13C (‰, VPDB)** | | | ***δ*15N (‰, AIR)** | | |
| Session 1 | Glycine low | 6 | -39.64 | ± | 0.11 | +1.42 | ± | 0.06 |
| Session 2 | Glycine low | 6 | -39.40 | ± | 0.49 | +1.41 | ± | 0.02 |
| Session 3 | Glycine low | 6 | -39.66 | ± | 0.06 | +1.35 | ± | 0.03 |
| Session 1 | Glycine high | 5 | +15.67 | ± | 0.08 | +51.81 | ± | 0.06 |
| Session 2 | Glycine high | 6 | +15.67 | ± | 0.14 | +51.81 | ± | 0.05 |
| Session 3 | Glycine high | 6 | +15.66 | ± | 0.04 | +51.81 | ± | 0.13 |
| Session 1 | Glycine mid | 4 | -8.46 | ± | 0.09 | +27.78 | ± | 0.10 |
| Session 2 | Glycine mid | 4 | -8.30 | ± | 0.10 | +27.71 | ± | 0.25 |
| Session 3 | Glycine mid | 4 | -8.37 | ± | 0.04 | +27.81 | ± | 0.09 |
| Session 1 | H Std 2 | 7 | -16.70 | ± | 0.12 | +9.11 | ± | 0.05 |
| Session 2 | H Std 2 | 7 | -16.58 | ± | 0.21 | +9.28 | ± | 0.48 |
| Session 3 | Tomato leaves | 7 | -27.06 | ± | 0.25 | +3.89 | ± | 0.10 |

We analyzed 12.5% of the 40 samples (5/40) as replicates (Table A4). The measurement precision specific to the samples (the pooled standard deviation of all samples analyzed in replicate) was ±.23 ‰ for *δ*13C and ±.12 ‰ *δ*15N (*df*=9).

Table A4. Stable carbon and nitrogen isotopic compositions for all samples analyzed as replicates. Subscripts indicate the analytical session.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Sample ID** | ***δ*13C1** | ***δ*13C2** | ***δ*13C3** | ***δ*15N1** | ***δ*15N2** | ***δ*15N3** |
| ACL 10050a | -17.27 | -17.35 | -17.69 | 8.69 | 8.61 | 8.63 |
| ACL 10030e | -14.07 | -14.36 | -14.61 | 8.95 | 9.00 | 8.94 |
| ACL 10040a | -18.25 | -18.65 | -18.55 | 11.65 | 11.96 | 11.54 |
| ACL 10029a | -14.78 | -14.27 | -14.63 | 6.99 | 7.03 | 6.89 |
| ACL 10033a | -17.12 | -16.98 |  | 10.02 | 10.25 |  |

**Standard Uncertainty**

We estimated standard uncertainty for the *δ*13C and *δ*15N measurements of the samples (*us*) using the spreadsheet calculations presented in Appendix G of Szpak et al. (2017). This was ±0.29 ‰ for *δ*13C and ±0.26 ‰ for *δ*15N.

**References**

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