**Supplementary tables**

**Supplementary table 1**: Carbon yield achieved from each modification of the CB protocol.

Carbon yields in mg are listed for all samples according to each treatment. Standard = Standard method 1st round only, Standard 2nd round = Standard method 2nd round only, Standard total = Standard method 1st + 2nd round, Ultrasonicated = ultrasonication method 1st round only, Ultrasonicated = ultrasonication method 2nd round only, Ultrasonicated = ultrasonication method 1st + 2nd round. Three samples were excluded from comparisons because of failure during the process of digestion-collection.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Sample | Standard yield (mg) | Standard 2nd round yield (mg) | Standard total yield (mg) | Ultrasonicated yield (mg) | Ultrasonicated 2nd round yield (mg) | Ultrasonicated total yield (mg) |
| P52,180 | 1.83 | 0.69 | 2.52 | 1.85 | 0.55 | 2.40 |
| P52,181 | 2.17 | 0.42 | 2.59 | 2.30 | 0.44 | 2.74 |
| P52,182 | 1.28 | 0.34 | 1.62 | 1.22 | 0.39 | 1.61 |
| P52,185 | 1.98 | 0.56 | 2.54 | 1.77 | 0.46 | 2.23 |
| P52,190 | 2.34 | 0.52 | 2.86 | 2.09 | 0.45 | 2.54 |
| P52,195 | / | / | / | / | / | / |
| P52,197 | 3.99 | 0.56 | 4.55 | 3.65 | 0.53 | 4.18 |
| P52,198 | 2.38 | 0.89 | 3.27 | 2.38 | 0.80 | 3.18 |
| P52,199 | 1.45 | 0.73 | 2.18 | 1.77 | 0.63 | 2.40 |
| P52,203 | 4.51 | 0.69 | 5.20 | 4.98 | 0.57 | 5.55 |
| P52,204 | 0.74 | 0.39 | 1.13 | 0.70 | 0.39 | 1.09 |
| P52,205 | 7.14 | 0.68 | 7.82 | 6.85 | 0.68 | 7.53 |
| P52,206 | / | / | / | / | / | / |
| P52,207 | / | / | / | / | / | / |

**Supplementary table 2:** Mean C yield, measured as % of the pre-digested mass of cremated bone, for the Standard total and the Ultrasonicated total treatments are non-significantly different.

The effect of ultrasonication on C yield was assessed through a two-tailed, paired t-test, accounting for the couples: Standard (1st round only) versus Ultrasonicated (1st round only), and Standard total versus Ultrasonicated total autoduplicates.

Analysis of variance (ANOVA) is calculated to asses differenceses among ultrasonication time groups (5, 10, 30 seconds) for 1st round only (delta 1st round = Ultrasonicated - Standard) and total (delta 1st +2nd round = Ultrasonicated total- Standard total) yield respectively.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Sample | Ultrasonication time (min) | Standard 1st round yield (%wt) | Ultrasonicated 1st round yield (%wt) | Standard total yield (%wt) | Ultrasonicated total yield (%wt) |
| P52,180 | 5 | 0.152 | 0.158 | 0.209 | 0.206 |
| P52,181 | 5 | 0.137 | 0.144 | 0.164 | 0.172 |
| P52,182 | 5 | 0.067 | 0.064 | 0.084 | 0.085 |
| P52,185 | 5 | 0.116 | 0.105 | 0.149 | 0.133 |
| P52,190 | 5 | 0.131 | 0.118 | 0.160 | 0.143 |
| P52,197 | 10 | 0.273 | 0.249 | 0.311 | 0.285 |
| P52,198 | 10 | 0.134 | 0.135 | 0.184 | 0.180 |
| P52,199 | 10 | 0.083 | 0.101 | 0.124 | 0.137 |
| P52,203 | 10 | 0.278 | 0.310 | 0.320 | 0.345 |
| P52,204 | 30 | 0.054 | 0.051 | 0.083 | 0.080 |
| P52,205 | 30 | 0.351 | 0.339 | 0.385 | 0.372 |
| average |  | 0.161 | 0.161 | 0.198 | 0.194 |
| standard deviation | | 0.096 | 0.096 | 0.100 | 0.099 |
|  |  | Standard 1st round vs Ultrasonication 1st round yield (%w) | | Standard total vs Ultrasonication total yield (%w) | |
| paired t test |  | t(10)= 0.002, p= 0.999 | | t(10)= 0.722, p= 0.487 | |
| ANOVA |  | t(2,8)=0.607, p= 0.568 | | t(2,8)=0.325, p= 0.732 | |
| single-tail paired t test |  | t(10)= 0.002, p= 0.499 | | t(10)= 0.722, p= 0.243 | |

**Supplementary table 3:** Second round increment

The discrepancy between the 1st round-only and the total (1st + 2nd collection rounds) subsample yield was measured through a paired t test. Both results related to mg C and to %wt yield are clearly significant.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Sample | Method | Mass (mg) | 1st round yield (mg) | 1st round yield (%wt) | 2nd round yield (mg) | 2nd round yield (%wt) | Total yield (mg) | Total yield (%wt) | Ultrasonication time (min) | % increase |
| P52,180 | Standard | 1207.31 | 1.83 | 0.15 | 0.69 | 0.057 | 2.52 | 0.21 |  | 137.50 |
| P52,180 | Ultrasonicated | 1167.82 | 1.85 | 0.16 | 0.55 | 0.047 | 2.40 | 0.21 | 5 | 130.38 |
| P52,181 | Standard | 1581.76 | 2.17 | 0.14 | 0.42 | 0.027 | 2.59 | 0.16 |  | 119.71 |
| P52,181 | Ultrasonicated | 1592.17 | 2.30 | 0.14 | 0.44 | 0.028 | 2.74 | 0.17 | 5 | 119.44 |
| P52,182 | Standard | 1924.34 | 1.28 | 0.07 | 0.34 | 0.018 | 1.62 | 0.08 |  | 125.37 |
| P52,182 | Ultrasonicated | 1901.63 | 1.22 | 0.06 | 0.39 | 0.021 | 1.61 | 0.09 | 5 | 132.81 |
| P52,185 | Standard | 1705.95 | 1.98 | 0.12 | 0.56 | 0.033 | 2.54 | 0.15 |  | 128.45 |
| P52,185 | Ultrasonicated | 1682.76 | 1.77 | 0.11 | 0.46 | 0.027 | 2.23 | 0.13 | 5 | 126.67 |
| P52,190 | Standard | 1788.28 | 2.34 | 0.13 | 0.52 | 0.029 | 2.86 | 0.16 |  | 122.14 |
| P52,190 | Ultrasonicated | 1770.52 | 2.09 | 0.12 | 0.45 | 0.025 | 2.54 | 0.14 | 5 | 121.19 |
| P52,195 | Ultrasonicated | 2213.25 | 3.22 | 0.15 | 0.77 | 0.035 | 3.99 | 0.18 | 10 | 124.14 |
| P52,197 | Standard | 1463.92 | 3.99 | 0.27 | 0.56 | 0.038 | 4.55 | 0.31 |  | 113.92 |
| P52,197 | Ultrasonicated | 1466.10 | 3.65 | 0.25 | 0.53 | 0.036 | 4.18 | 0.29 | 10 | 114.46 |
| P52,198 | Standard | 1772.59 | 2.38 | 0.13 | 0.89 | 0.050 | 3.27 | 0.18 |  | 137.31 |
| P52,198 | Ultrasonicated | 1767.40 | 2.38 | 0.14 | 0.80 | 0.045 | 3.18 | 0.18 | 10 | 133.33 |
| P52,199 | Standard | 1751.16 | 1.45 | 0.08 | 0.73 | 0.042 | 2.18 | 0.12 |  | 149.40 |
| P52,199 | Ultrasonicated | 1748.00 | 1.77 | 0.10 | 0.63 | 0.036 | 2.40 | 0.14 | 10 | 135.64 |
| P52,203 | Standard | 1622.88 | 4.51 | 0.28 | 0.69 | 0.043 | 5.20 | 0.32 |  | 115.11 |
| P52,203 | Ultrasonicated | 1608.76 | 4.98 | 0.31 | 0.57 | 0.035 | 5.55 | 0.35 | 10 | 111.29 |
| P52,204 | Standard | 1369.67 | 0.74 | 0.05 | 0.39 | 0.028 | 1.13 | 0.08 |  | 153.70 |
| P52,204 | Ultrasonicated | 1361.73 | 0.70 | 0.05 | 0.39 | 0.029 | 1.09 | 0.08 | 30 | 156.86 |
| P52,205 | Standard | 2033.43 | 7.14 | 0.35 | 0.68 | 0.033 | 7.82 | 0.39 |  | 109.69 |
| P52,205 | Ultrasonicated | 2022.44 | 6.85 | 0.34 | 0.68 | 0.034 | 7.53 | 0.37 | 30 | 109.73 |
| P52,206 | Ultrasonicated | 1498.33 | 1.47 | 0.10 | 0.67 | 0.045 | 2.14 | 0.14 | 30 | 145.92 |
| P52,207 | Standard | 1487.23 | 3.89 | 0.26 | 0.46 | 0.031 | 4.35 | 0.29 |  | 111.45 |
| average |  | 1660.38 | 2.72 | 0.16 | 0.57 | 0.035 | 3.29 | 0.20 |  | 121.48 |
| standard deviation | | 253.63 | 1.70 | 0.09 | 0.15 | 0.009 | 1.76 | 0.09 |  | 13.8 |
|  | 1st round vs total yield (mg) | | | | | 1st round vs total yield (%wt) | | | | |
| paired t-test | t(24)= -19.37, p= 3.70E-16 | | | | | t(24)= -18.63, p= 8.86E-16 | | | | |
| single-tail paired t test | t(24)= -19.37, p= 1.85E-16 | | | | | t(24)= -18.63, p= 4.43E-16 | | | | |

**Supplementary table 4**: Conventional radiocarbon dates of all four replicates combined per each sample.

Weighted mean and the chi-square test are calculated.

If all four replicates are grouped together, all dates result as identical according to the chi-square test (df=3, T (5%) =7.8).

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| OxA No | Sample | Standard 1st round | Standard 2nd round | Ultrasonication 1st round | Ultrasonication 2nd round | weighed mean (BP) | χ2 test | p value |
| 42,638 | P52,180 | 3710±19 | 3741±20 | 3704±19 | 3744±22 | 3722 ±11 | 3.0 | 0.15 |
| 42,639 | P52,181 | 3553±18 | 3554±46 | 3573±18 | 3551±53 | 3562 ±12 | 0.7 | 0.24 |
| 42,640 | P52,185 | 3690±19 | 3709±23 | 3673±19 | 3736±24 | 3698 ±11 | 4.6 | 0.09 |
| 42,641 | P52,190 | 3633±19 | 3637±23 | 3624±19 | 3569±45 | 3627 ±12 | 2.0 | 0.21 |
| 42,642 | P52,197 | 3719±19 | 3764±22 | 3697±19 | 3717±22 | 3722 ±11 | 5.5 | 0.06 |
| 42,646 | P52,198 | 3663±23 | 3640±20 | 3628±19 | 3594±20 | 3629 ±11 | 5.5 | 0.06 |
| 42,643 | P52,199 | 3620±19 | 3687±22 | 3647±19 | 3648±23 | 3648 ±11 | 5.3 | 0.06 |
| 42,725 | P52,205 | 4353±20 | 4337±22 | 4310±20 | 4335±22 | 4334 ±11 | 2.4 | 0.19 |

**Supplementary table 5:** Summary statistics on methods yield.

Collection time extension sensibly affects the yield, while ultrasonication doesn’t.

|  |  |  |
| --- | --- | --- |
| Comparison | Test | p value |
| 1st round vs total yield (mg) | paired t test | 3.698E-16 |
| 1st round vs total yield (%wt) | paired t test | 8.857E-16 |
| Standard vs Ultrasonicated 1st round yield (%wt) | paired t test | 0.999 |
| Standard vs Ultrasonicated 1st+2nd round yield (%wt) | paired t test | 0.487 |
| 1st round 5 vs 10 vs 30 minute Ultrasonication | ANOVA | 0.568 |
| 1st+2nd round 5 vs 10 vs 30 minute Ultrasonication | ANOVA | 0.732 |

**Supplementary figures**

**Supplementary figure 1**: Percent yield increment of a second 3-minute collection round.

An average of an additional 0.57±0.15 mg C was collected during the second three-minute collection round, giving an increase in yield of 21.5%.

Increment is measured as % of C yield (in mg) of the first 3-minute collection round (indicated as relative yield in Table 1).