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ASSESSING A MODIFIED CARBONATE DIGESTION PROTOCOL FOR INCREASED CARBON DIOXIDE RECOVERY DURING CREMATED BONE PRETREATMENT

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ABSTRACT. Cremated bones are a commonly preserved material and often found in burial environments where unburned bone may not be preserved. As such direct radiocarbon dating of cremated bone could be essential in determining the chronology of an event. Pretreatment of cremated bone exploits the structural carbonate component of the bone which survives cremation. However, due to the low abundance (ca. 0.1%) of this component, the extraction of an amount of endogenous carbon sufficient for radiocarbon dating may represent a challenge. Here we investigate two modifications to the phosphoric acid digestion protocol used during the preparation of cremated bones at the Oxford Radiocarbon Accelerator Unit (ORAU). The first of these was to use ultrasonication to release evolved CO_2 from the viscous phosphoric acid and cremated bone mixture that is formed during digestion. The second was to double the amount of time during which evolved CO_2 was removed from the reaction vessel by transfer into a cryogenically cooled ampoule. Ultrasonication of the digestion mixture failed to produce a significantly higher carbon yield, while double-time collection resulted in an average 21.5±13.8% increase of C yield without affecting the measured age. Extending the collection time can better enable reliable dating of small (less than 1 g) samples.

KEYWORDS: carbon yield, cremated bone, radiocarbon dating.

INTRODUCTION

Radiocarbon dating of cremated bone is fundamental in resolving the chronology of periods where the primary funerary rite involved cremation which for northern Europe covers the Neolithic to Roman periods (Olsen et al. 2008; De Mulder et al. 2009; Makarowicz et al. 2021). Direct dating of cremated bone is often the only way to accurately determine a burial site chronology. Charcoal is not always present because cremated bones may have been selected from the pyre and placed inside an urn (De Mulder et al. 2009), and even where present, radiocarbon dates on charcoal often reflect an older age than the actual burial time (Olsen et al. 2013). Cremation destroys the collagen normally targeted for dating (Van Strydonck et al. 2005), and until a few decades ago this material was regarded as unsuitable for radiocarbon dating (Lanting et al. 2001; Zazzo and Saliège 2011) despite its important role in archaeology and the abundance of sites where cremated bone is found (Thompson 2015; Gonçalves and Pires 2017).

While collagen in fresh bone is a fibrous mass that accounts for about 30% (Feng 2009) of the weight, the inorganic fraction of bone is a relatively-poorly crystallised mineral of calcium phosphate, called bioapatite. Bioapatite is a form of hydroxy apatite with the formula $(Ca,Mg,Na)_{10-x}[(PO4)_{6-x}(CO3)_x](OH)_{2-x}$ which has the ability to incorporate CO_3^{2-} and other ions from the bloodstream (Neuman and Neuman 1958, Cazalbou et al. 2004). This carbonate is referred to as 'structural carbonate' (Lanting and Brindley 1998) as it substitute to phosphate groups in the reticulum, reducing the degree of crystallinity and enhancing bioapatite reactivity to

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organic molecules (Lebon et al. 2010). The bioapatite of unburnt or charred bone readily incorporates carbonates from the burial environment, which can alter the measured radiocarbon age (Zazzo and Saliège 2011). However, during cremation (>600°C) bioapatite recrystallises, and its crystallinity index (CI) increases as crystal size increases and the number of ionic substitutions (including of carbonate) decreases (Shipman et al. 1984; Minami et al. 2019). This improves resistance to diagenetic alteration allowing the carbonate ion in cremated bone to be exploited to provide radiocarbon dates (Lanting and Brindley 1998; Lanting et al. 2001; Minami et al. 2019).

Methodological work has focused on two challenges facing the radiocarbon dating of cremated bone: contamination from diagenetic processes and the old-wood effect. The potential addition of contaminants during diagenesis is thought to be reduced by the selection of white, dense bone with high CI (Minami et al. 2019) and appropriate pretreatment (Van Strydonck et al. 2009). CI is considered high at values 0.5 to 0.9 if measured by X-ray diffraction analysis (Person 1995), or between 5 and 7 when meausred through attenuated total reflection–Fourier transform infrared spectroscopy (Weiner and Bar-Yosef 1990). Pretreatment is based on Lanting et al. (2001) who employed sodium hypochlorire (NaOCl) as an oxidizing agent to remove organic compounds, followed by an acid leach to remove secondary carbonates. Generally, acetic acid is now preferred over hydrochloric acid, due to its higher ability to dissolve calcite and lower aggressiveness towards bioapatite (Van Strydonck et al. 2009), and the bleach step can be removed as it is regarded as unneccesary (Snoeck and Pellegrini 2015; Rose et al. 2019).

Radiocarbon dates on cremated bone can be affected by the "old wood effect" as carbon from fuel used in the pyre is incorporated into the bioapatite structure (Strydonck et al. 2010; Hüls et al. 2010), causing age overestimations of 10–100s years (Van Strydonck et al. 2010; Olsen et al. 2013; Snoeck et al. 2014; Rose et al. 2020). Under wet conditions, environmental carbonate (including from the burning fuel) can substitute for the phosphate ion in apatite, and account for up to 64% of the carbonate in the recrystallised apatite. It may be possible to correct for this old carbon using $\delta^{13}C$, although it is not clear in which proportion isotope fractionation in cremated bone depends on CO^2 incorporation or bioapatite structural change (Hüls et al. 2010).

A third potential challenge when radiocarbon dating cremated bone relates to sample size requirements. The carbonate content of bioapatite is very low, about 4–6% by mass (Zazzo et al. 2009), corresponding to a C content in unburnt bone of around 1% (Minami et al. 2019), which reduces to 0.1wt% carbon during cremation (Lebon et al. 2010). Pretreatment protocols further reduce this carbonate content by removing the least stable, most carbonate rich, bioapatite. Because of its fragility (Pramanik et al. 2012; Strydonck 2016), cremated bone is normally found in small fragments in archaeological contexts (McKinley 1993). The bone can also be non-uniformally burnt (presenting greyish shades), so that only a small portion of a fragment is fully calcined. It is therefore important to ensure that all of the carbonate within calcined bone is extracted and collected for radiocarbon dating.

Once pretreated, carbon dioxide is liberated from cremated bone using phosphoric acid. As phospate ions are dissolved in acid, the acid becomes viscous. Using the protocol employed at the ORAU, it is common that bubbles of gas appear trapped in the acqueous mixture. This paper examines whether the addition of ultrasonication during phosphoric acid digestion or a longer reaction time may produce a higher carbon dioxide yield, thus decreasing the current sample size requirement.

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MATERIALS AND METHODS

Sampling

Samples were selected from fragments of dense, white cremated human bone. These had been identified for radiocarbon measurement as part of the AHRC-funded Project TIME (Project time: Writing new narratives of the past). While some radiocarbon measurements had previously been produced on these cremations on samples of unidentified wood charcoal, measurements on the cremated bone were critical for understanding the history of these sites. Full details of the chronology of these sites are in preparation (Griffiths et al. in prep.).

Pretreatment

Pretreatment of cremated bone at the Oxford Radiocarbon Accelertor Unit (ORAU) currently involves physical cleaning and an acetic acid pre-digestion to remove secondary carbonates, and has been assigned a code of "CB" (Snoeck et al. 2016). This protocol has not included an oxidation step after Snoeck and Pellegrini (2015).

The surface of the cremated bone fragments was removed by air abrasion (0.29 µm aluminium oxide powder), and the bones crushed to small chunks. A 4–5 g sample was pre-digested in acetic acid (1 M; ~ 20 mL; ~ 5 rinses over 24 hr) and rinsed three times in ultrapure water (Millipore MilliQ) before freeze drying (see Figure 1 for this and following steps). The acid leached bone was split in two equal mass replicates each placed in a 50-mL round bottom flask, sealed with a rubber septum and evacuated to $<1\times10^{-3}$ mbar. Phosphoric acid digestion followed the protocol described in Brock et al. (2010). 6 mL 85% H_3PO_4 (Analytical Reagent grade; Fisher Scientific, UK) per 1 g of cremated bone was added to the bone powder, via injection through the septum, and the vessel was placed in a water bath to allow digestion (50° C; 3.5–4.5 hr). After digestion, the first replicate was ultrasonicated in a 38 kHz sonication bath, at room temperature, for either 5, 10 or 30 minutes while the second replicate did not undergo sonication.

Evolved CO_2 was cryogenically purified with a water trap (–65±3°C; isopropanol and liquid N_2) and collected in an evacuated glass ampoule cooled using liquid N_2. Each vessel was subject to a 3-minute collection (3 × 15 s to move CO_2 into the ampoule, with each 15-s period preceded by 45 s to allow water to condense into the –65°C trap). This was followed by a further identical 3-minute collection step from which any CO_2 was collected into a separate ampoule (the carbonate line allows for the evolved CO_2 to be directed to multiple ampoules). Carbon yield was determined by measuring the CO_2 pressure in each ampoule prior to sealing.

Thus, a total of four measurements, each treated with a different method, was obtained from each specimen. These are listed in Table 1 and are named respectively: Standard (CB protocol), Ultrasonicated (1st 3-minute collection round only), Standard 2nd round (carbon dioxide collected from the second 3-minute collection step of the standard CB protocol), and Ultrasonicated 2nd round (carbon dioxide collected from the second 3-minute collection step of the ultrasoincation protocol).

Graphitization and Radiocarbon Dating

Trapped CO_2 was passed through an elemental analyzer (Carlo-Erba NA 2000) coupled to an isotope-ratio mass spectrometer (Sercon 20/20; recycling process described in Brock et al. 2010) to monitor $\delta^{13}C$. CO_2 was crygoenically collected and 1.8 mg C was graphitized by reaction

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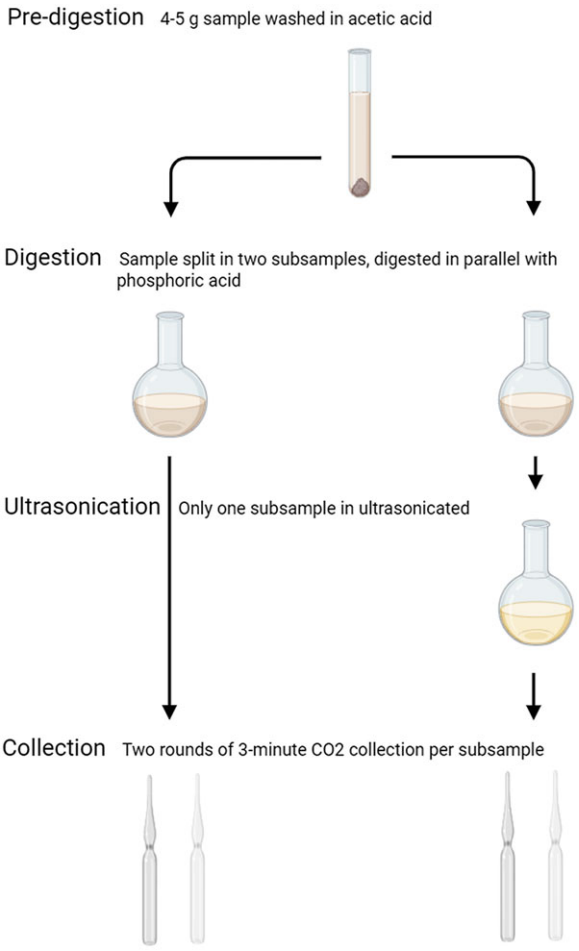

Figure 1 Schematic of the experimental setting and procedure.

with H_2 over iron powder (560°C, 6 hr; Dee and Bronk Ramsey (2000), Bronk Ramsey and Hedges (1997)). Graphite powder was pressed (PSP; Ionplus AG, CH) and radiocarbon determinations made using a MICADAS AMS (Ionplus AG, CH). Radiocarbon dates were calculated following (Stuiver and Polach 1977) using an AMS derived $\delta^{13}C$. Age consistency was tested for each protocol variant against the standard method and compared through a chi-square test (Ward and Wilson 1978) and weighted means calculated, using the R_Combine function in OxCal ver. 4.4 (Bronk Ramsey 1995, 2022). The effect of ultrasonication and extended collection time were assessed by paired t-tests and ANOVA test carried out in Microsoft Excel using an alpha level of 0.05.

RESULTS

Ultrasonication

Figure 2 and Supplementary Table 1 show the carbon yield achieved from each modification of the CB protocol. As 3 subsamples failed during collection, yield comparison of all four

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Table 1 Method names and average yields.For each method combination, average values are reported for pre-digested sample mass (Pre-digestion mass (mg)), carbon yield in mg (Carbon yield (mg)), and percent yield in relation to the pre-digested sample mass (Mass/yield (%wt)); relative yield of the second collection round and the total yield in relation to the usual first collection round (Second collection relative yield).

Pre-collection method	Pre-digestion mass (mg)	Collection method	Carbon yield (mg)	Mass/yield (%wt)	Second collection relative yield
Standard	1656.50 ± 241.60	Standard (1st round)	2.71 ± 1.10	0.16 ± 0.10	100.00
		Standard 2nd round	0.59 ± 0.17	0.04 ± 0.01	22.40
		Standard total (1st+2nd round)	3.30 ± 1.15	0.20 ± 0.10	123.00
Ultrasonicated	1644.50 ± 244.00	Ultrasonicated (1st round)	2.69 ± 1.16	0.16 ± 0.10	100.00
		Ultrasonicated 2nd round	0.54 ± 0.13	0.03 ± 0.09	20.50
		Ultrasonicated total (1st+2nd round)	3.22 ± 1.20	0.19 ± 0.10	120.50
All samples (standard & ultrasonicated)	1660.40 ± 253.60	All samples (1st+2nd round)	3.29 ± 1.76	0.20 ± 0.09	121.50

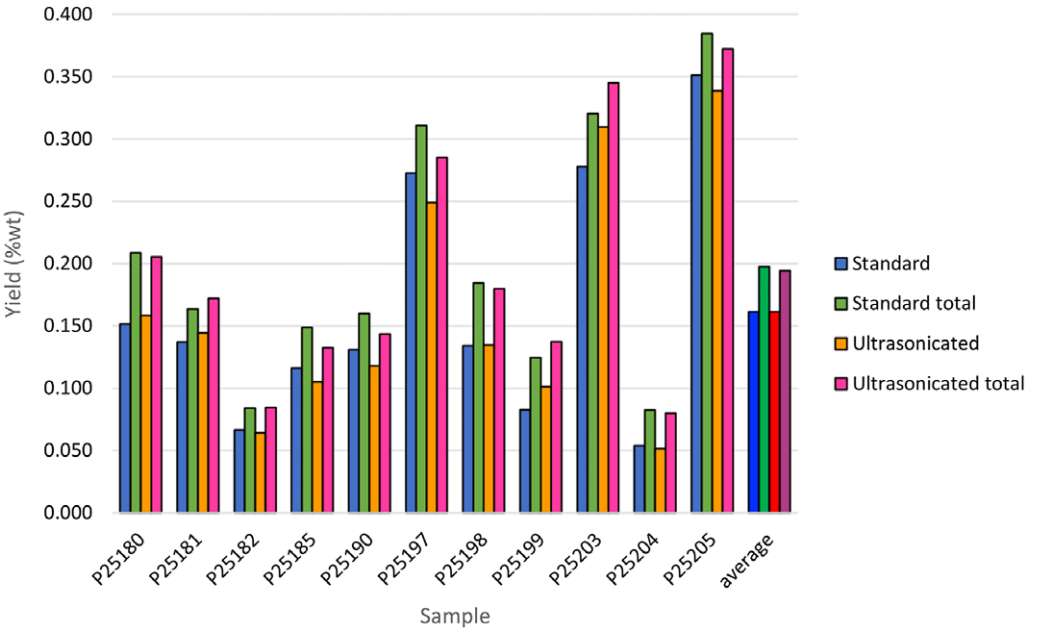

Figure 2 Carbon yield achieved from each modification of the CB protocol. Yield is indicated as %pre-digestion weight (%wt); Standard, Standard total, Ultrasonicated, and Ultrasonicated total yields are shown. Despite inter-sample variability a double-time collection consistently increase the final yield, while ultrasonication does not contribute in a significant way.

replicates was possible for 11 samples only. The mean total C yield (including CO_2 from both the first and second extraction), measured as a percent of the pre-digested mass of cremated bone for the standard treatment (0.198 ± 0.100 %wt) and ultrasonicated samples (0.194 ± 0.099 %wt) are not significantly different ($t(10) = 0.72$, $p = 0.49$) (Supplementary Table 2). The influence of ultrasonication time was also assessed and found to be unimportant (ANOVA $F(2,8) = 0.33$, $p = 0.73$ and $F(2,8) = 0.61$, $p = 0.57$ when comparing total yield and 1st round yield, respectively, see Figure 3 and Supplementary Table 2).

Second Round Collection

The second 3-minute round of CO_2 collection provides an increase in net yield for each of the 11 samples (Supplementary figure 1). On average, an additional 0.57±0.15 mg C was collected during the second three-minute collection round, corresponding to an increase in yield of 21.5 ±13.8% (Table 1 and Supplementary table 3).

Age Consistency

Thirty-two (8×4) targets were dated, and 4 replicates of 8 specimens were taken in consideration for age consistency evaluation. For each sample, all dates using the three protocol variations overlap with the age obtained using the standard method within 2 standard deviations (Table 2, Figure 4). However, If all four replicates are grouped together, all dates result as identical according to the chi-square test ($df = 3$, T (5%, 7.8), $p>0.05$, see Supplementary Table 4).

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Table 2 Conventional radiocarbon ages of all replicates (Standard 2nd round, Ultrasonicated 1st round, Ultrasonicated 2nd round) are compared against a reference date Standard (1st round only) obtained from the subsample treated with the standard CB method.

Sample	Laboratory code (OxA)	Method	^{14}C date (yr BP)	sigma	Absolute difference (yr)	$\chi 2$ test (df = 1, T (5%) = 3.8)	p value
P52180	42,638	Standard	3710	19	—	—	—
	X-3192-14	Standard 2nd round	3741	22	31 ± 41	1.1	0.22
	X-3190-20	Ultrasonicated	3704	19	6 ± 38	0.0	—
	X-3192-15	Ultrasonicated 2nd round	3744	22	34 ± 41	1.4	0.17
P52181	42,639	Standard	3553	18	—	—	—
	X-3195-17	Standard 2nd round	3554	46	1 ± 64	0.0	—
	X-3190-26	Ultrasonicated	3573	18	20 ± 36	0.6	0.38
	X-3195-18	Ultrasonicated 2nd round	3551	53	2 ± 71	0.0	—
P52185	42,640	Standard	3690	19	—	—	—
	X-3192-16	Standard 2nd round	3709	23	19 ± 42	0.4	0.52
	X-3190-28	Ultrasonicated	3673	19	17 ± 38	0.4	0.52
	X-3192-17	Ultrasonicated 2nd round	3736	24	46 ± 43	2.3	0.08
P52190	42,641	Standard	3633	19	—	—	—
	X-3192-18	Standard 2nd round	3637	23	4 ± 42	2.3	0.08
	X-3190-30	Ultrasonicated	3624	19	9 ± 38	0.1	1.2
	X-3195-19	Ultrasonicated 2nd round	3569	45	64 ± 64	1.7	0.13
P52197	42,642	Standard	3719	19	—	—	—
	X-3200-11	Standard 2nd round	3764	22	45 ± 41	2.4	0.08
	X-3190-33	Ultrasonicated	3697	19	22 ± 38	0.7	0.34
	X-3200-12	Ultrasonicated 2nd round	3717	22	2 ± 41	0.0	—
P52198	42,646	Standard	3663	23	—	—	—
	X-3200-13	Standard 2nd round	3640	20	23 ± 43	0.6	0.38
	X-3190-34	Ultrasonicated	3628	19	35 ± 42	1.4	0.17
	X-3200-14	Ultrasonicated 2nd round	3594	20	69 ± 43	**5.1**	**0.01**
P52199	42,643	Standard	3620	19	—	—	—
	X-3192-20	Standard 2nd round	3687	22	67 ± 41	**5.3**	**0.01**
	X-3190-36	Ultrasonicated	3647	19	27 ± 38	1.0	0.24
	X-3192-24	Ultrasonicated 2nd round	3648	23	28 ± 42	0.9	0.27
P52205	42,725	Standard	4353	20	—	—	—
	X-3200-15	Standard 2nd round	4337	22	16 ± 42	0.3	0.63
	X-3196-36	Ultrasonicated	4310	20	43 ± 40	2.3	0.08
	X-3192-28	Ultrasonicated 2nd round	4335	22	18 ± 42	0.4	0.52

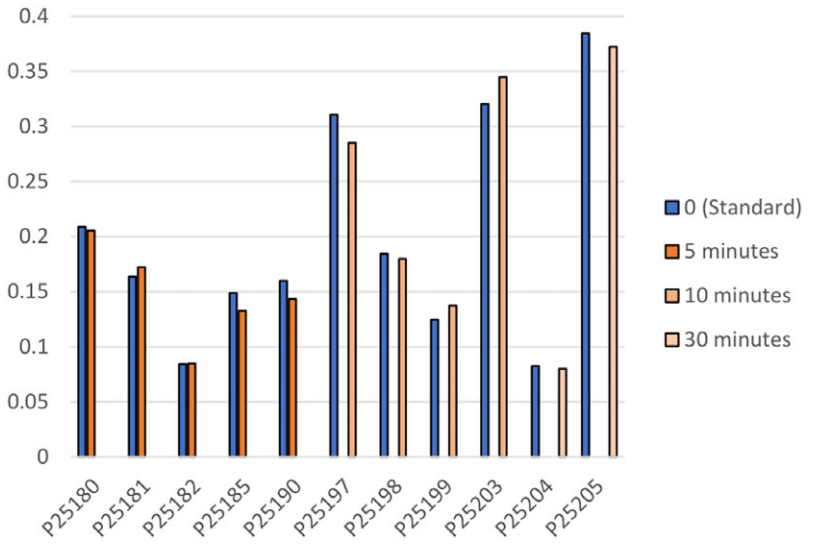

Figure 3 Different times of ultrasonication do not significantly affect the final yield. Mean C yield, measured as % of the pre-digested mass of cremated bone, for the Standard total and the Ultrasonicated total treatments are non-significantly different.

DISCUSSION

To improve carbon yields during the phosphoric acid digestion of cremated bone for radiocarbon dating, we tested two modifications to the existing protocol: ultrasonication and an extension of the time allowed for collection of CO_2 after digestion. Our data show that ultrasonication did not affect the final C yield, regardless of the length of ultrasonication time. However, an additional 3-minute round of CO_2 collection increases the C yield by 21.5±13.8% (Table 1) on average, and up to 56.9% (Supplementary table 2). Yet when the consensus age is considered, all replicate ages are statistically identical.

A sample size of 1.5–2.5 g is required to produce at least 2 mg C obtained with the CB protocol performed at ORAU. This yield accounts for about 0.1–0.2% of the initial sample weight, a value close to the amount of C in cremated bones (Hüls et al. 2010). Unexpectedly, results show no difference in yield between ultrasonicated and non-ultrasonicated replicates. A possible explanation is that CO_2 partial pressure reaches its threshold in the vessel space while still a consistent proportion of CO_3^{2-} is in solution or trapped in bubbles. This proportion is likely to be quite high when the collection starts, as bubbles are forming continuously during the 3 minutes. Another possibility is that the residual carbonate release is not instantaneous after ultrasonication, but needs some time and possibly a second period of exposure to 50°C.

Alternatively, the high viscosity of 85% H_3PO_4 and post-digestion residuals may trap CO_2 bubbles that cannot be released through ultrasonication. To overcome this issue a less viscous digestion medium could be tried out in the future (e.g. 60% H_3PO_4) or a higher digestion temperature used, as used at different laboratories (see for instance Rose et al. 2019).

CONCLUSIONS

Within the present study the ability of ultrasonication to provide higher CO_2 yield by releasing trapped gas was tested on cremated bone fragments by comparing non-ultrasonicated samples

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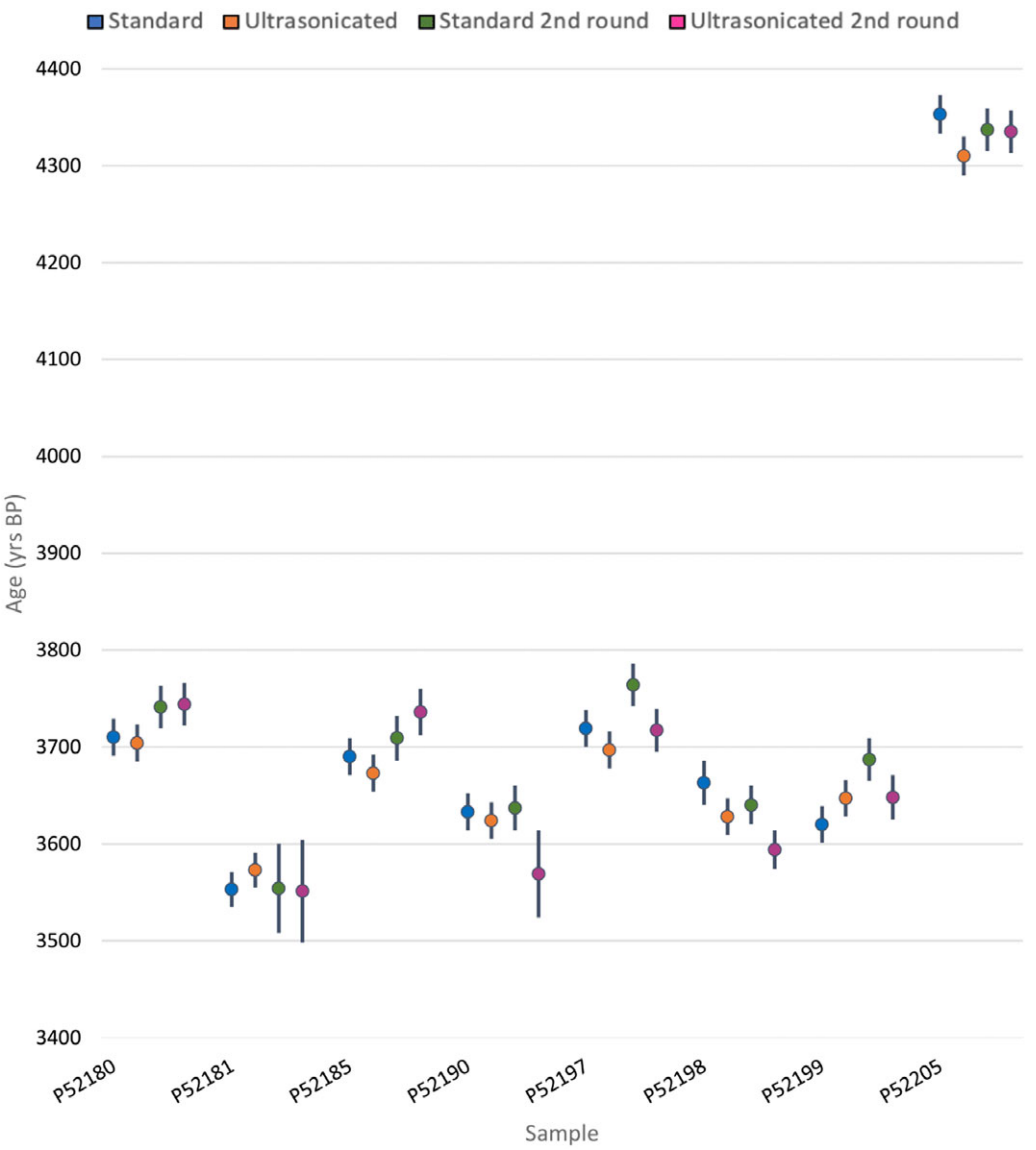

Figure 4 Age consistency. Radiocarbon ages are compared for 8 samples (out of 11 treated) that yielded 4 replicates. Ages are shown within their 1-sigma (black) intervals. For each sample, all dates overlap within 2 standard deviations from the Reference age (Standard method, blue).

with ultrasonicated replicates. Separately, another round of 3-minute collection was performed for which the yield and radiocarbon age were determined and compared among replicates.

Results show that ultrasonication does not affect the final C yield, independently of the length of ultrasonication time. On the contrary, a CO_2 collection time of 6 rather than 3 minutes, increases the C yield by 21.5±13.8% on average, with no impact on date reliability. This enables the starting mass of cremated bone to be reduced by 20%. Our findings are especially relevant

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when dealing with very small or partially incinerated specimens, with black-greyish patches that need to be discarded when sampling, limiting the sample size available.

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SUPPLEMENTARY MATERIALS

To view supplementary material for this article, please visit https://doi.org/10.1017/RDC.2024.97

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