Thermal Analysis and Infrared Spectroscopy as Complementary Methods for Assessing the State of Preservation of Collagen in Collections of Archaeological Bones

Chloe Pearce[1*], Marianne Odlyha[2]

1* cpearc03@mail.bbk.ac.uk, Birkbeck, University of London, Malet Street, Bloomsbury, London, WC1E 7HX

2 Birkbeck, University of London, Malet Street, Bloomsbury, London, WC1E 7HX

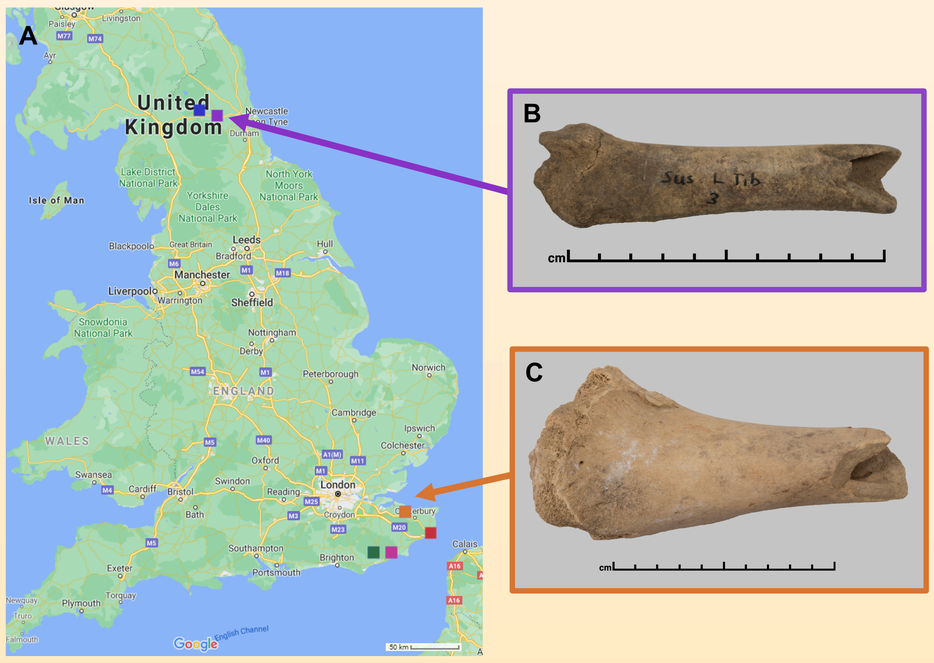

Figure 1. a) Map of UK with points indicating the locations of each site and store, blue = Housesteads Roman Fort (HST), purple = Corbridge Roman Site (CRB), red = Dover Castle, orange = Battle Abbey (BAT), pink = Camber Castle (CAM), green = Maison Dieu (MSD), b) CRB12 from Corbridge, c) BAT4 from Battle Abbey.

Introduction

The English Heritage collections include vast amounts of archaeological bone, including human remains, animal bones and worked bone objects. These objects tell much of our past, including animal husbandry practices, butchery and warfare. Due to its abundance it has also been used as a raw material to produce both functional and decorative objects. A collections audit in 2010 highlighted that over 10% of archaeological bone objects were in poor condition and are therefore at risk of permanent damage. The bones featured within this study were collected from stores in Corbridge Roman Site, located along Hadrian's Wall and Dover Castle on the southern coast of England (Figure 1). The archaeological bones represent five sites dating between the Roman period (80-300 AD) and the Tudor period (1550-1637). The material excavated from Corbridge Roman Site (CRB) and Housesteads Roman Fort (HST) were stored in Corbridge Roman Site. Those excavated from Battle Abbey (BAT), Camber Castle (CAM) and Maison Dieu (MSD) were stored in Dover Castle.

Fresh bone contains approximately 25-30% organic material, 85-90% of which is type I collagen[1]. The remaining content is 60-70% carbonated hydroxyapatite (bioapatite), and 8-10% water. These proportions vary between species and location within the body. Type I collagen plays a key role in the mechanical properties of bone, namely its toughness allowing for some flexibility in the tissue while preventing cracking[2]. The degradation of this component is complex and the precise mechanisms vary according to the burial environment[3].

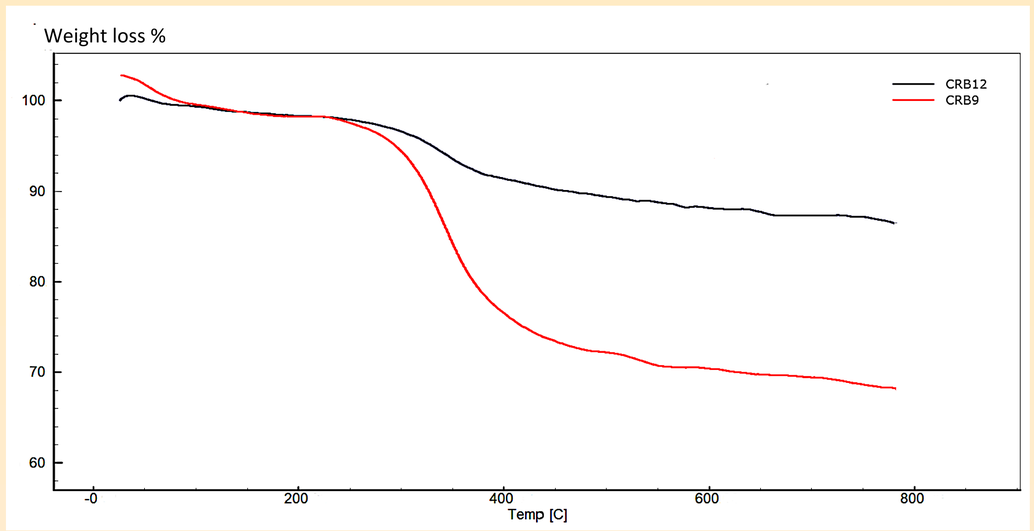

Figure 2. TGA slopes featuring a sample in poor condition, CRB12, and one in good condition, CRB9. CRB12 contains only 9.4% organic material whereas CRB9 demonstrates 26.8% organic material

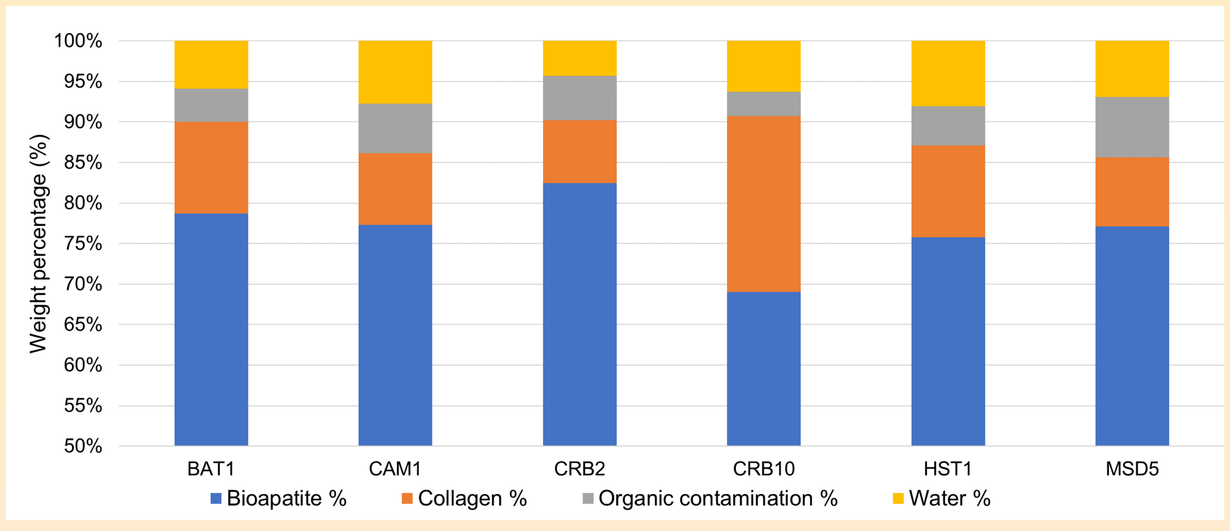

Figure 3. Content percentage of representative samples from each site. 50—100% is displayed here, as 0-50% is bioapatite in all cases.

Methodology

The thermogravimetric analysis was completed by heating 1.5-2mg samples of powdered bone samples from room temperature to 800°C at a rate of 10°C/min in oxygen in a platinum crucible (60cm^3/min) using the Shimadzu TGA 50 analyser. Data was collected and analysed using TA60 v.1.40 software. Each measurement was repeated three times. Fourier transform infrared spectroscopy (FTIR) was measured between the wavenumbers 4000-550 cm^{-1}, using 32 scans and a resolution of 4cm^{-1} on the Nicolet iS 5 (Thermo Fischer). The data was collected and analysed using GRAMS 8 and OMNIC 9.8. These were also measured three times, and in both cases the average result is presented here.

TGA analysis was used to measure the percentage content of each sample, calculated by percentage weight loss over a temperature range. Weight loss during the first 200°C is water evaporation (both free and bound), between 200-600°C the organic material combustion, and lastly from 600°C onwards is considered the bioapatite content[4]. By subtracting the collagen percentage calculated (see below) from the organic percentage we can estimate the percentage of non-collagenous contaminates that have entered the samples.

Collagen preservation can be identified using the peak height ratio of 1660 and 1630cm^{-1}, both present within the Amide I peak located around 1640cm^{-1}. The wavenumber 1660cm^{-1} indicates intramolecular hydrogen bonding within the triple helix, whereas 1630cm^{-1} indicates these bonds pointed outside of the helix[5]. Therefore, higher ratios indicate high collagen preservation via better structural integrity. Collagen percentage was calculated using the method proposed by Lebon et al[6]; 113.13(AmI/P)+1.69. Where AmI/P is the ratio of the Amide I peak (1640) height to phosphate peak (1010) height.

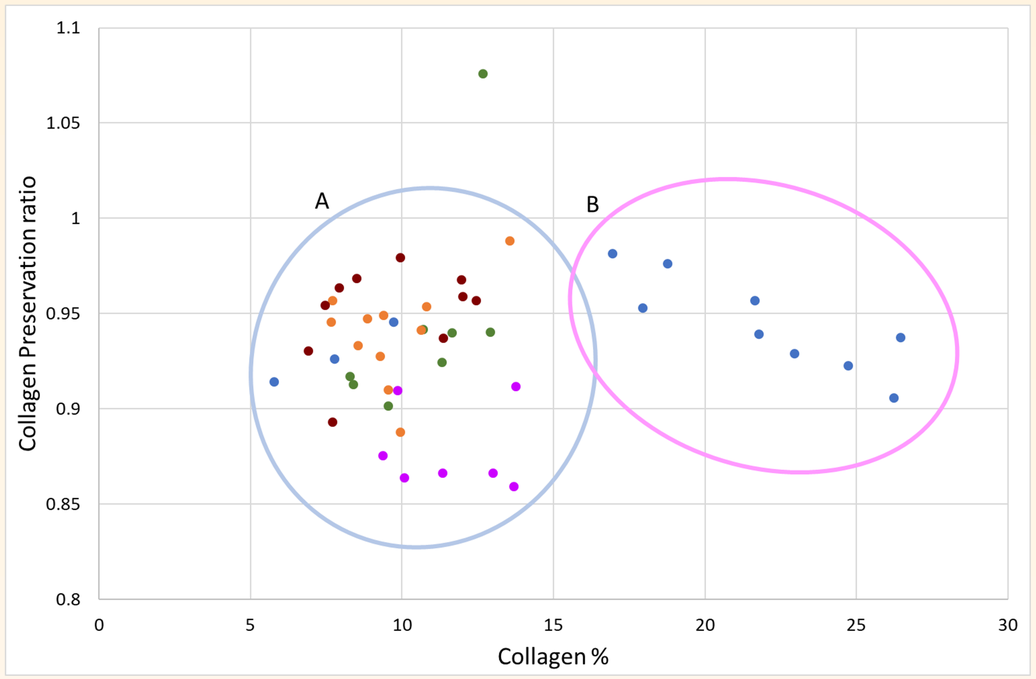

Figure 4. Graph depicting the relationship between collagen degradation and collagen preservation percentage. Green = BAT, orange = CAM, blue = CRB, pink = HST, red = MSD.

Results

Selected TGA results are featured in Figure 2 which demonstrate the diversity of organic preservation within the sample set, CRB12 is in poor condition whereas CRB9 is in exceptionally good. Those featured in Figure 3 should be considered representative of each site. The collagen percentage is generally between 7-12%, with 4-5% of organic contaminants, however a number of samples excavated from CRB demonstrate a significantly higher percentage between 20-25%.

In Figure 4 the relationship between each samples collagen degradation and collagen percentage is explored. The data shows that the samples can be separated into two groups, A and B. Set A includes the most samples. Within this group the samples from HST demonstrate consistently lower collagen preservation while maintaining similar percentage content, while MSD demonstrates higher collagen preservation. Set B includes samples, all from CRB, which have high collagen content, they also demonstrate a collagen preservation ratio consistent with set A (0.9-1.0).

Discussion

The data indicates the majority of the samples, within Figure 4 Set A, are in poor condition, with between 10-15% collagen remaining. It also indicates that most samples have collagen preservation within the range of 0.85-1.0. The data also highlights that there is not linear correlation between collagen content and level of preservation. This is likely a result of bones' complicated degradation process once buried. The mineral component, bioapatite, can act to both accelerate and impede collagen degradation and loss. Bones are also prone to localised preservation, where areas can demonstrate unusually high or low preservation when compared other areas of the sample. However, patterns in preservation are evident. The samples excavated from the south of England (BAT, CAM, MSD) generally demonstrate low collagen content and preservation, although show consistency within this group. However, those excavated along Hadrian's Wall (CRB, HST) are more diverse in condition, demonstrating both good and poor preservation.

Conclusion

In conclusion TGA and FTIR have been used here to give a comprehensive view of preservation in archaeological bone. This includes the quantification of bones organic and inorganic components and assessment of its collagen preservation. The data indicates the majority of the samples are in poor condition, with between 10-15% collagen remaining and a collagen preservation within the range of 0.85-1.0. However, there is no relationship between these analysis. Patterns can be seen according to excavation site, and geographical location. Greater consistency is evident in those excavated in the south of England, whereas the environment of the north of country produces more varied levels of preservation.

References

[1]LOPES, D., MARTINS-CRUZ, C., OLIVEIRA, M. B. & MANO, J. F. 2018. Biomaterials, 185.
[2]CURREY, J. 2004. Journal of Theoretical Biology, 231.
[3]NIELSEN-MARSH, C. M., SMITH, C.I., JANS, .M .M .E., NORD, A., KARS, H., COLLINS, M. J., 2007. Journal of Archaeological Science, 34.
[4]BARAKAT, N. A. M., KHALIL, K. A., SHEIKH, F. A., OMRAN, A. M., GAIHRE, B., KHIL, S. M. & KIM, H. Y. 2008. Materials Science and Engineering: C, 28.
[5]ODLYHA, M., THEODORAKOPOULOS, C., DE GROOT, J., BOZEC, L. & HORTON, M. 2009. e-Preservation Science, .6
[6]LEBON, M., GALLET, R. X., GURLET-BELLOT, L. & ZAZZO, A. 2016. Radiocarbon, 58.