

TECHNICAL NOTE**ANTHROPOLOGY**

Sergio Galeano,¹ M.Sc. and Mari Luz García-Lorenzo,² Ph.D.

Bone Mineral Change During Experimental Calcination: An X-ray Diffraction Study

ABSTRACT: The effects of calcination (400–1200°C) on pig bones have been studied using powder X-ray diffraction (XRD) and secondary modifications, such as color change and weight loss. The characterisation by powder XRD confirmed the presence of the crystalline phase of hydroxyapatite, and comparison of the results obtained at different temperatures suggested that at 650°C, all the organic components and carbonate substitutions were completely removed. Accordingly, these samples were white. In addition, the crystallinity degree and the crystallite size progressively increased with the calcination temperature until 650°C, remaining stable until 1200°C. Below 650°C, bone samples presented organic compounds, resulting in background noise in the diffractogram and gray or black color. In addition, impurities in the lattice correspond to low crystallite sizes.

KEYWORDS: forensic science, burned bones, powder X-ray diffraction, crystallite size, hydroxyapatite, calcination

The osseous matrix is formed by organic components, providing flexibility and strength, and inorganic materials, which lend hardness and resistance (1). The mineral matrix is commonly thought of as calcium hydroxyapatite (HA), $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, which has a hexagonal crystallite structure (2,3). Natural HA could contain substitutions of carbonates and other ions (Na, Mg, F, or Sr) into its structure affecting crystallinity (4,5). For this reason, in fresh bones, HA usually is poorly crystallized and showed low structural organization. Different causes, such as thermic exposure, could affect HA structure and crystallinity.

The objective of burned bone research is to provide knowledge of heat-induced changes, and for this aim, different techniques were used to examine different features of induced changes, such as color change, dimensional change (weight loss), mineralogical composition, and crystal size (6,7). Taking into account that the most important changes in bone during calcination involve changes in the skeletal microstructure, the better and more reliable way of addressing these changes is with the powder X-ray diffraction, possibly combined with other types of microscopic approaches (8,9), with a particular focus on the HA mineral phase, which is the main inorganic component of bones.

This study aims to elucidate the changes in pig bones during burning by powder X-ray diffraction, following a range of calcination treatments, between 400°C and 1200°C. In addition, the color change and the weight loss were evaluated as secondary modifications. Pig bones were selected because they are comparable to the human in weight, musculature, and size and are a suitable analogue in forensic experimental analysis.

¹National Police Corps, Homicide Central Command, Avenida Mirador de La Reina 4, Madrid, Spain.

²Department of Petrology and Geochemistry, Faculty of Geology, University Complutense of Madrid, José Antonio Novais 2, 28040 Madrid, Spain.

Received 18 April 2013; and in revised form 21 June 2013; accepted 8 Sept. 2013.

Material and Methods

Sample Preparation

Samples of fresh and immature pig bones (*Sus scrofa*) were selected for this study. Specifically, 13 bone sections of 35 ± 10 g were analyzed, in two steps: the first one consisted of temperature increments of 200°C, from 400°C to 1200°C. According to obtained results in this sequence, a second experiment was designed, from 400°C to 750°C in increments of 50°C, to better understanding changes in this range. The samples were placed on heatproof ceramic trays to aid retrieval after heating. Each sample was placed into the furnace once the temperature had reached 200°C and allowed to heat up to the designated temperature. Preheating at 200°C removes the potential impact of extremely rapid heating as an influence on hard tissue microstructure (10).

The samples used were heat-treated with a heating rate of 10°C/min at selected temperatures for 60 min in air in a Nabertherm L9/L12/P320 muffle furnace. After this time, they were removed from the furnace and allowed to cool naturally in a desiccator before the analytical determinations. The specimens for X-ray investigations were powdered by manual grinding in an agate mortar.

Analytical Methodology

Color sample was determined by Munsell Color Chart under a natural daylight. In addition, a mineralogical analysis of burned samples was made by X-ray diffraction (XRD) analysis using Cu-K α radiation with a PW3040 Philips Diffractometer. Xpowder software was used to analyze the XRD diagrams obtained by the crystalline powder method (11). The powder diffraction file (PDF2) database was used for peak identification, taking into account that the determination of minerals by XRD analysis is not accurate below a limit of 5% of the total weight in a sample.

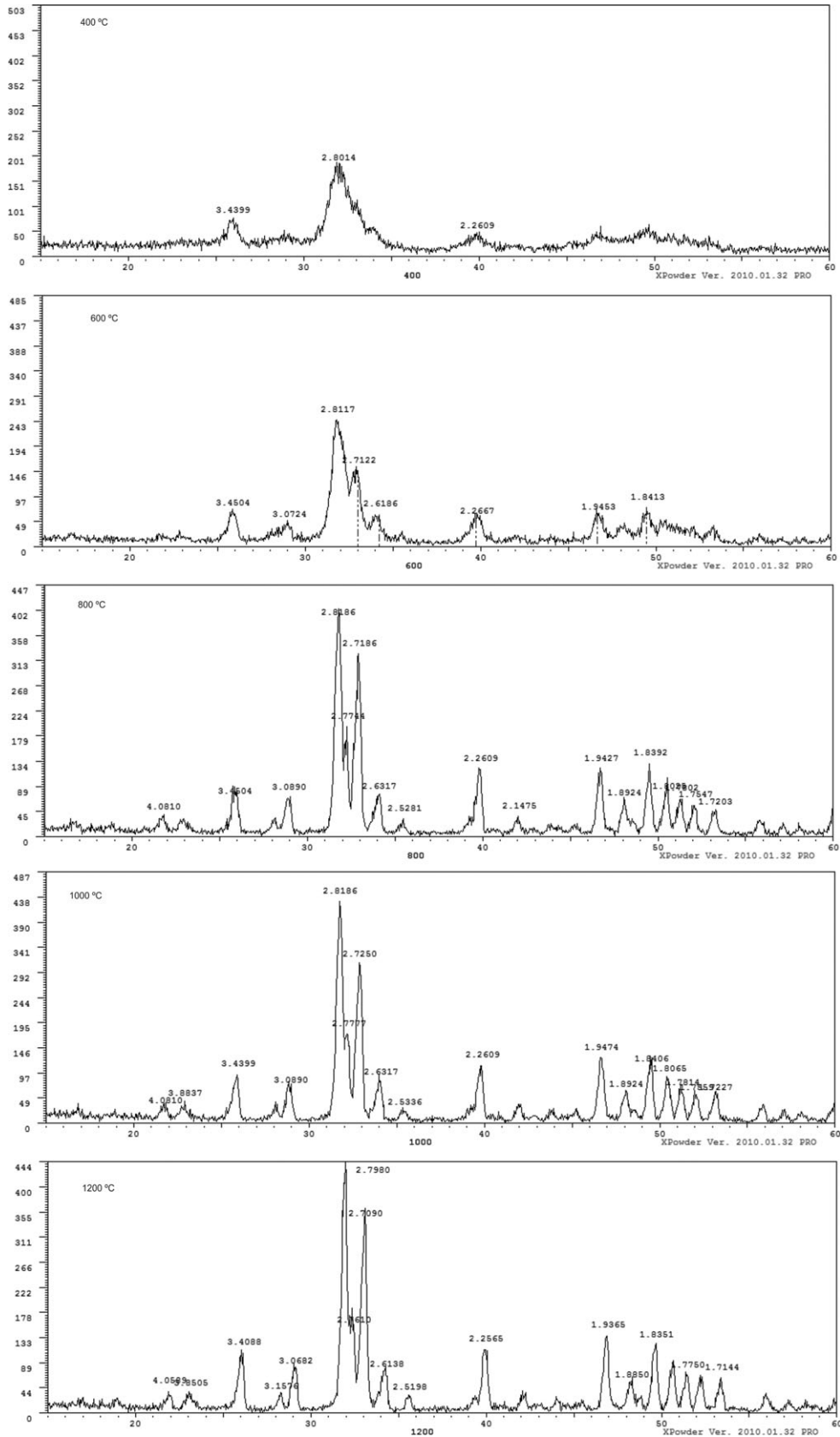


FIG. 1—X-ray diffraction patterns of bones calcined between 400°C and 1200°C.

The Scherrer equation was used to calculate crystal size (τ). In this expression, λ is the wavelength of the radiation, and θ is the Bragg angle and B, the line broadening at half the maximum

intensity (FWHM) of the selected peaks in the diffractogram. The (0 0 2) reflection of the burned samples was used for determining the FWHM data.



FIG. 2—Calcined bone images at different temperatures.

$$\tau = K\lambda/B\cos\theta$$

Results and Discussion

Experiment 1: 400–1200°C, Increments of 200°C

X-ray diffraction was employed to evaluate the phase purity and the crystallographic structural properties of the mineral component of bone after calcination at the selected temperatures. We report in Fig. 1 a selected portion of the patterns from 15° to 60° in 2 θ from the burning bones at 400°C, 600°C, 800°C, 1000°C, and 1200°C, respectively, and the diffractograms of all samples showed the characteristic pattern of HA. The XRD results also indicate that the relatively broad peaks resultant from heating at 400°C and 600°C reflect a poor crystalline HA, due to the carbonate substitution into the mineral (4). Samples burned at these temperatures were black and grayish white, respectively, according to Munsell Color Charts (Fig. 2) because of the presence of organic substances, which were not completely combusted. When the temperature was 800°C, more intense and sharper peaks were observed, corresponding to an increase in the mineral crystallinity, which is compatible with crystallite growth and with the elimination of carbonate from the lattice, according to the chemical reaction (12):



Above 800°C, no influence on the crystallinity was noticed (Table 1). In addition, the color of the obtained materials at 800°C, 1000°C, and 1200°C turned white, as a consequence of carbon removal. The analysis of the weight loss suggested that it increases when temperature increases, ranging from 76% at 400°C to 92% at 1200°C.

TABLE 1—Details of experimental heating samples in the first calcination phase (400–1200°C).

T (°C)	Color	Weight (g)	Loss (%)	Crystal Size (nm)
400	Black	21.4	72.0	10
600	Grayish white	18.6	79.6	17
800	White	24.4	86.1	26
1000	White	38.3	86.7	26
1200	White	41.3	92.5	26

Experiment 2: 400–750°C, Increments of 50°C

Taking into account that from 800°C to 1200°C, practically, no changes were observed, and the most important changes took place at temperatures below 800°C; a second experience was designed, with temperatures comprised from 400°C to 750°C, with increases of 50°C. Similarly to experiment 1, the diffractograms showed only the characteristic pattern of HA (Fig. 3). Particularly, from 400°C to 550°C, the background noise is high, suggesting the presence of a mixture of heterogeneous, poorly crystalline materials, mainly organic compounds in our case. The background noise decreases as temperature increases, as a consequence of carbon removal. The XRD results also indicate that in this range (400–550°C), broad peaks were detected, as a result of the presence of poor crystalline HA, because of the carbonate substitution into the lattice. The crystallite size ranged from 10 nm to 17 nm, that is, low crystallite size according to the presence of impurities. Color results verify the presence of organic phases not completely combusted, being black at 400°C, olive gray at 450°C, gray at 500°C, and grayish white at 550°C (Table 2).

When temperature was 600°C, organic compounds disappeared, decreasing amorphous phases and then the background noise in the diffractogram. At this temperature, sharp and narrow diffraction peaks of HA were observed and crystal size increased

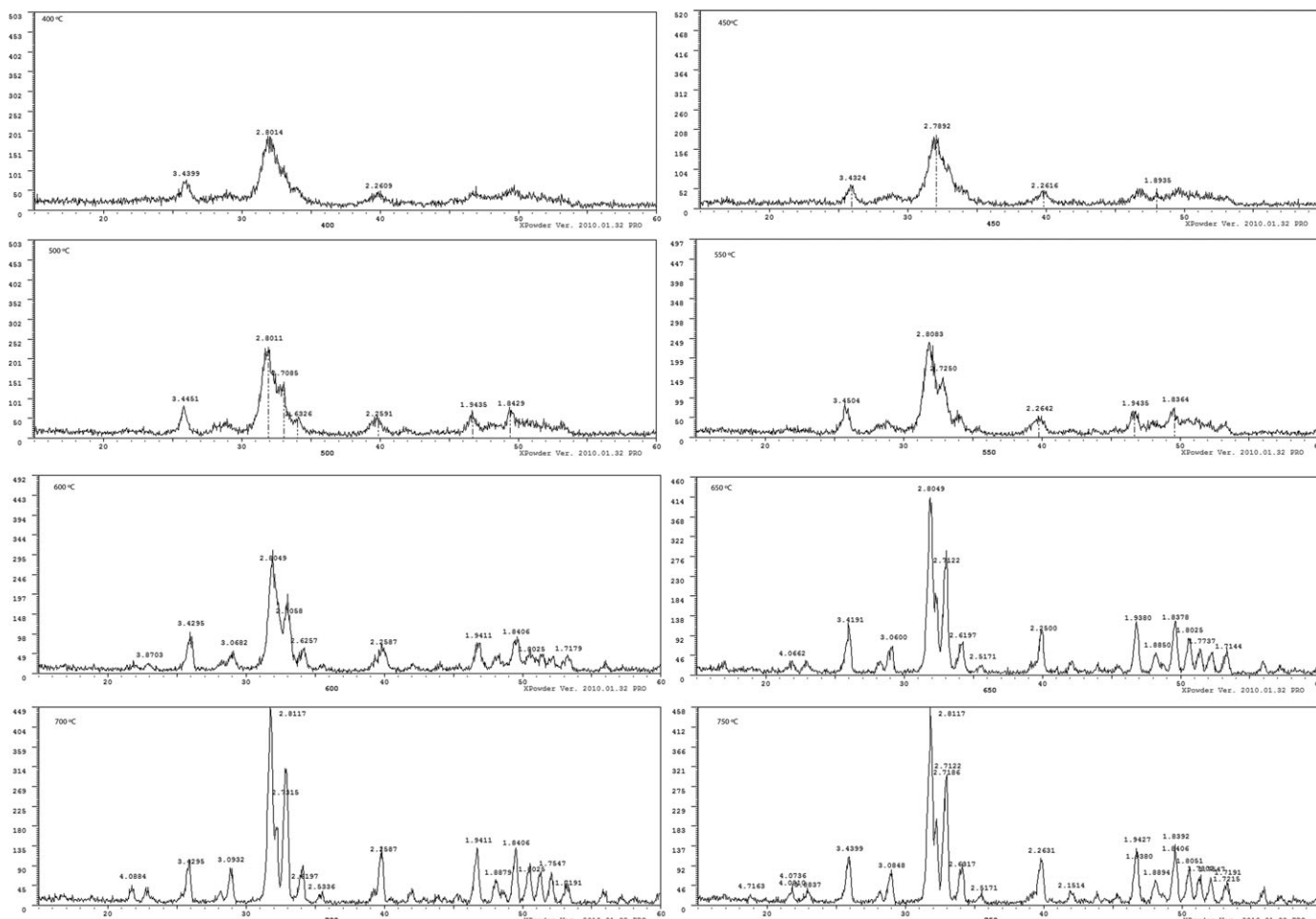


FIG. 3—X-ray diffraction patterns of bones calcined between 400°C and 750°C.

TABLE 2—Details of experimental heating samples in the second calcination phase (400–750°C).

T (°C)	Color	Weight (g)	Loss (%)	Crystal Size (nm)
400	Black	14.8	76.3	10
450	Olive gray	11.3	69.9	13
500	Gray	14.9	77.9	17
550	Grayish white	10.5	81.9	17
600	Grayish white	12.5	84.0	23
650	White	12.9	82.9	28
700	White	13.4	84.3	28
750	White	14.9	83.2	27

until 23 nm, suggesting the elimination of impurities in the lattice. At 650°C, all the organic compounds have disappeared, agreeing with white color of this sample. At this temperature, peaks are narrow and crystallite size was 28 nm, remaining stable at 700°C and 750°C. From 650°C to 750°C, practically, no changes were observed in the crystallite size. In summary, from 650°C to 750°C, narrow peaks of HA were quantified, and impurities, such as carbonates, were not present. In addition, over 650°C, all samples were white (Fig. 2). Weight loss results are also summarized in Table 2 and suggested that the ash percentage diminishes as temperature increases, ranging from 24% at 400°C to 17% at 750°C.

When the average crystallite size evolution for HA as a function of temperature treatment was evaluated, the results obtained

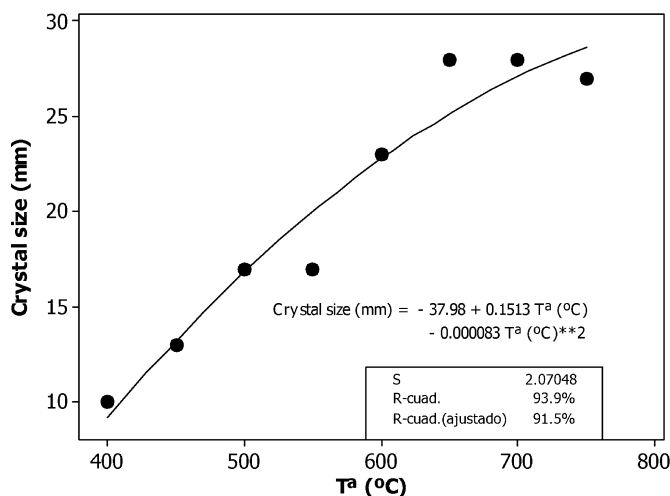


FIG. 4—Average crystallite size evolution for hydroxylapatite as a function of temperature treatment.

suggested that above 750°C, crystallite size remains stable. However, below 750°C, the growth process seems to follow a quadratic pattern until 750°C, and the following equation was fit to the experimental data (Fig. 4):

$$y = -37.98 + 0.1513x - 0.000083x^2$$

where y is the crystal size in nm, and x is the calcinations temperature ($^{\circ}\text{C}$).

In summary, and considering that there is no sensible difference in the structure of human and pig bones (12), the obtained results could provide valuable information in forensic investigations, particularly when burned human remains are found. In this case, human remains could be subjected to XRD analysis, and then, calcination temperature could be determined by comparing with the obtained results in this study.

Conclusions

According to the results obtained and taking into account the existent similarity between pig and human bones, the present study could be applied in forensic and archeological studies, allowing determining calcination temperatures. Macroscopic examination is useful to gain a general idea of temperature, although more advanced techniques such as powder X-ray diffraction are required to gain a more precise temperature range. The XRD pattern shows the presence of HA in the bone matrix. The results show that the calcination temperature highly conditions the properties of the bone samples. As expected, higher temperatures lead to more pure forms of HA, with higher crystallinity degrees, larger crystallite sizes, and therefore sharper XRD peak profiles. The most significant structural changes occur between 550°C and 650°C . Above this region, the XRD signatures of bones were almost similar. Below 550°C , crystal size was small, confirming the existence of carbonate substitutions and organic compounds, represented as background noise.

Acknowledgments

The authors thank the Department of Agricultural Chemistry, Geology and Pedology, University of Murcia the use of its laboratory, specifically to Feliciana Gázquez for the technical assistance and to Dr. Pérez-Sirvent and Dr. Martínez-Sánchez for helpful discussions concerning the forensic applications.

References

1. Beckett S, Rogers KD, Clement JG. Inter-species variation in bone mineral behavior upon heating. *J Forensic Sci* 2011;56:571–9.
2. Bigi CH, Cojazzi G, Panzavolta S, Ripamonti A, Roveri N, Romanello M, et al. Chemical and structural characterisation of the mineral phase from cortical and trabecular bone. *J Inorg Biochem* 1997;68:45–51.
3. Rogers KD, Daniels P. An x-ray diffraction study of the effects of heat treatment on bone mineral microstructure. *Biomaterials* 2002;23:2577–85.
4. Figueredo M, Fernando A, Martins G, Freitas J, Judas F, Figueredo H. Effect of the calcination temperature on the composition and microstructure of hydroxyapatite derived from human and animal bone. *Ceram Int* 2010;36:2383–93.
5. Ooi CY, Hamdi M, Ramesh S. Properties of hydroxyapatite produced by annealing of bovine bone. *Ceram Int* 2007;33:1171–7.
6. Thompson TJU. Recent advances in the study of burned bone and their implications for forensic anthropology. *Forensic Sci Int* 2004;146S: S2003–205.
7. Ubelaker DH. The forensic evaluation of burned skeletal remains: a synthesis. *Forensic Sci Int* 2009;183:1–5.
8. Holden JL, Phakey PP, Clement JG. Scanning electron microscope observations of heat-treated human bone. *Forensic Sci Int* 1995;74:29–45.
9. Piga G, Thompson TJU, Malgosa A, Enzo S. The potential of x-ray diffraction in the analysis of burned remains from forensic contexts. *J Forensic Sci* 2009;54:534–9.
10. Hiller JC, Thompson TJU, Evison MP, Chamberlain AT, Wess TJ. Bone mineral change during experimental heating: an x-ray scattering investigation. *Biomaterials* 2003;24:5091–7.
11. Martin JD. Using X Powder: a software package for powder x-ray diffraction analysis. (D.L. GR 1001/04. ISBN 84-609-1497-6); <http://www.xpowder.com> (accessed June 11, 2014).
12. Piga G, Malgosa A, Thompson TJU, Enzo S. A new calibration of the XRD technique for the study of archaeological burned human remains. *J Archaeol Sci* 2008;35:2171–8.

Additional information and reprint requests:
 Mari Luz García-Lorenzo, Ph.D.
 Department of Petrology and Geochemistry
 Faculty of Geology
 University Complutense of Madrid
 José Antonio Novais 2
 28040 Madrid
 Spain
 E-mail: mglorenzo@geo.ucm.es