2013 International Nuclear Atlantic Conference - INAC 2013
Recife, PE, Brazil, November 24-29, 2013
ASSOCIAÇÃO BRASILEIRA DE ENERGIA NUCLEAR - ABEN
***ISBN:* 978-85-99141-05-2**

STUDY OF CAPRINE BONES AFTER MOIST AND DRY HEAT PROCESSES BY X-RAY DIFFRACTION

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ABSTRACT

Bone tissue is a biological material composed of hydroxyapatite (HAp) and collagen matrix. The bone X-ray diffraction (XRD) pattern presents characteristics of the hydroxyapatite crystallography planes. This paper presents the characterization by X-ray diffraction of caprine bone powder pattern and the comparison of this pattern with moist or dry heat cooked bone patterns. The parameters chosen to characterize the X-ray diffraction peaks were: angular position (2θ), full width at half maximumt (FWHM), and relative intensity (I_{rel}). The X-ray diffraction patterns were obtained with a Shimadzu XRD-6000 diffractometer. The caprine bone XRD pattern revealed a significant correlation of several crystallographic parameters (lattice data) with hydroxyapatite. The profiles of the three bone types analyzed presented differences. The study showed as small angular displacement (decrease of the 2θ angle) of some peaks was observed after moist and dry heat cooking processes. The characterization of bone tissue aimed to contribute to future analysis in the field of archeology.

1. INTRODUCTION

X-ray diffraction (XRD) is a non-destructive analytical technique, which utilizes an inherent property of the X-ray beam. It is widely used in various fields of science, and

one of the most important tools for structural analysis and characterization of materials used by the industry and research groups around the world. Non-destructive testing such as X-ray diffraction is applied to reveal various aspects of crystalline materials, but its application in biological tissues is growing. Some studies examined the bone tissue [2-12,19] and fossil bone [12-16] by x-ray diffraction, due to the presence of the main crystalline phase, hydroxyapatite (HAp). An investigation by SAXS (Small-Angle X-ray Scattering) provided complementary evidence to that generated by traditional XRD in the characterization of heated bone, and concluded that changes in the crystalline structure that may not be readily apparent otherwise become more clear [16]. Characterization of boiled and unboiled human bones has been realized using X-ray diffraction (XRD) [17]. The results were used to estimate the boiling time in four archaeological samples (Neolithic bones from Malalmuerzo cave, Spain), but boiled and unboiled bones showed the same structural. In this study lattice parameters were not determined. The determination of lattice parameters depends upon the precision of locating the peak profiles in XRD diagrams, but in bioapatite this is difficult because of large peak broadening resulting from the small crystalline size of the phase combined with the high amount of lattice strain [19].

The purpose of this study was to investigate XRD profiles of bones after boiling and roasting process as a contribution for studies and analyzes of archaeological materials. Sambaquis are the Brazilian shell mounds, prehistoric sites of thousand years where animal and human bones are found. The preparation of artifacts made of animal bones and also the food preparation involves cooking in different conditions. Human bones, on the other side, can be accidentally burnt or intentionally burnt in ritual contexts. Distinguishing the cooking/heating patterns of archaeological bones usually involves identifying different morphological characteristics. Otherwise, changes in the cristalographic patterns of apatite and their application to the diagnosing of bones exposed to heat is a new developing field. In the Sernambetiba shell mound, in Rio de Janeiro [20] many bones, animal and humans were found in challenging contextual conditions. The application of the present results and following research to those archaeological remains will be helpful to unveil cultural patters related to funerals, preparation of artifacts and food by the prehistoric human groups who build Sernambetiba sambaqui between 1,500 and 2,000 ybp.

2. MATERIALS AND METHODS

2.1 Equipment

The X-ray diffraction patterns were obtained using a Shimadzu XRD-6000 powder diffractometer, with θ-2θ reflection geometry, X-ray tube target Copper (Cu-K_α radiation) and tungsten filament operated with a voltage of 40 kV and a current of 30 mA, and NaI(Tl) scintillator detector. The slits DS = 1.0º, SS = 1.0º and RS = 0.3 mm and rotational speed was 1.0°/min, with angular step of 0.02º (t = 1.2 s) were also used. The scan angle of the goniometer (2θ) was from 10º to 70º with deviation of ±0,05°. The parameters selected were (Fig. 1): peak positions (2θ), full width half maximum (FWHM), relative intensities (I_{peak1}/I_{peak2}).

2.2 Sample preparation

Three types of caprine bone samples were studied: as received and after moist and dry heat cooking processes. In the process using moist heat, the samples were cooked in water at 100 ºC for 120 min. In the process using dry heat, the samples were placed in a closed oven at 250 ºC for 40 min. After the cooking process the samples were grinded until obtaining a thin powder and placed in the sample holders of the diffractometer. Different parts of the bone were not analyzed separately. Parts from axial, circumferential and transversal bone regions were analyzed together.

3. RESULTS AND DISCUSSIONS

The X-ray diffraction patterns of the bones samples are presented in Fig.1. All the peaks correspond to the well known reflections of crystalline HAp [18]. Two more intense peaks were observed in angular position between 25-26.5º and 30-35º which were identified as *peak 1* and *peak 2*, respectively. The *peak 1* corresponds to reflection (002). The peak 2 contains four characteristics peaks of Hap corresponding to the (211), (112), (300) and (202) respectively (Fig. 2).

The X-ray diffraction profile of bone is diffusive with lower intensity at every peak in most positions. This is due to the low uniformity of the interplanar spacings of the lattice that composes the material [1]. Only the two higher intensity peaks of the bone profiles, *peak 1* and *peak 2*, were chosen for the analysis, because of higher definition (crystallinity). The other peaks presented low intensity and resolution. The background of all the profiles was removed with XRD-6000 V4.1 program. Table 1 shows the parameters of the X-ray diffraction patterns of all the samples analyzed. HAp pattern and its crystallographic planes were obtained at the library of PCSIWIN program and NIST.

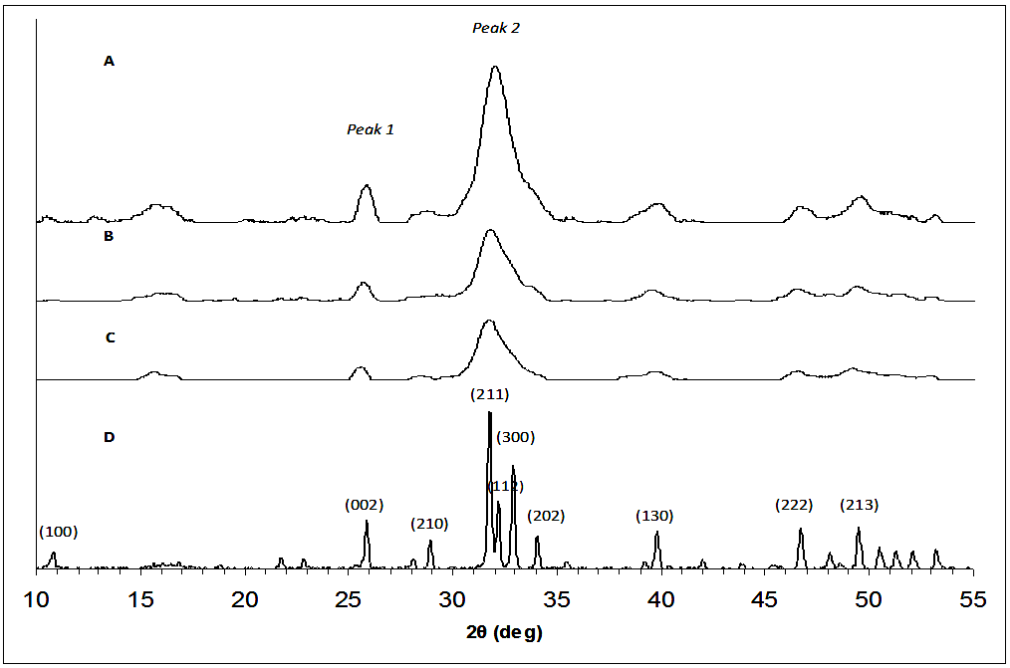

Figure 1: The comparative X-ray diffraction (XRD) patterns of (A) natural caprine bone, (B) caprine bone boiled at 100 °C, (C) caprine bone rosted at 250 °C and hydroxyapatite.

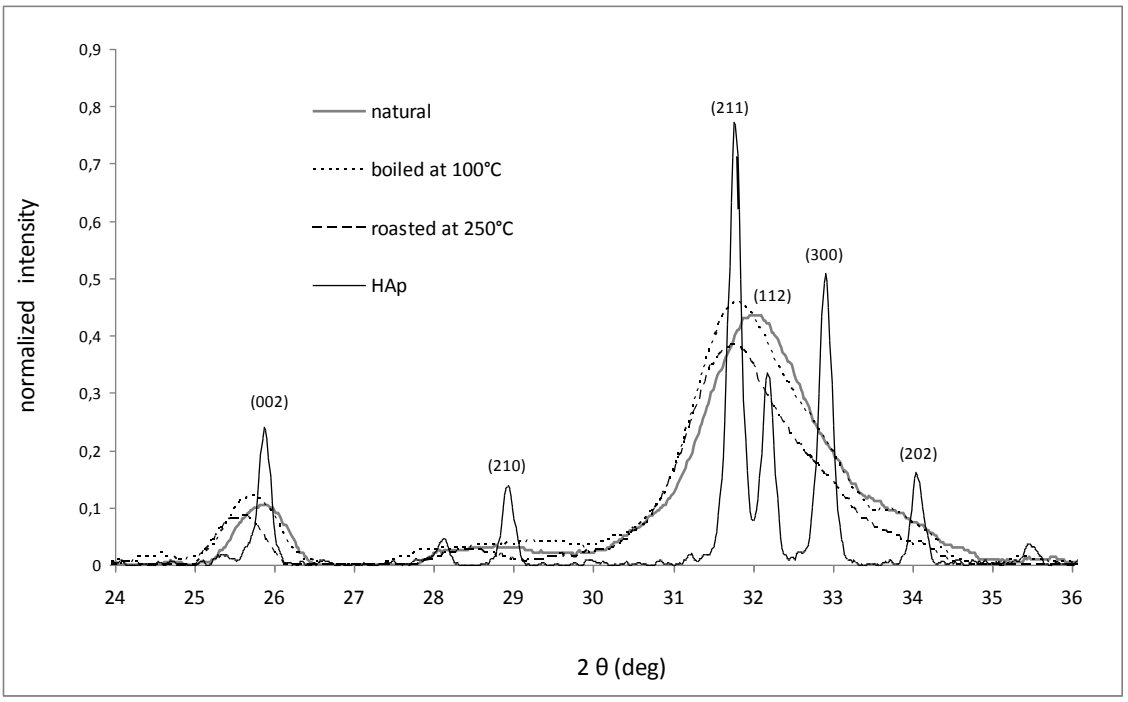

Figure 2: X-ray diffraction pattern of caprine bones as received and after moist and dry heat cooking processes, with an angular scan of 24-36°.

Table 1: X-ray diffraction parameters of bone samples as received, after moist heat cooking and after dry heat cooking.

	natural		boiled		roasted	
	Peak 1	Peak 2	Peak 1	Peak 2	Peak 1	Peak 2
2θ (°)	25.9	32.0	25.7	31.8	25.6	31.7
FWHM (°)	0.6	1.4	0.7	1.3	0.5	1.2
Normalized intensity (count)	0.10	0.43	0.12	0.45	0.08	0.38
I_{peak1}/I_{peak2}	-	0.23	-	0.27	-	0.21

According to Tab.1, in boiled bone there was a small increase in peak intensity and in roasted bone a decrease for the peaks 1 and 2 compared to natural bone profile. This behavior also occurred for the relative intensities (I_{peak1}/I_{peak2}). We observed a decrease in FWHM of roasted bone and thin increase in boiled bone for both peaks also compared to natural bone profile.

Peaks 1 and *2* (Fig. 2) of the bone as received represent the (002) and (211) planes of HAp respectively. It can be observed that in relation to *peaks 1* and *2* (Fig. 1b) there is a displacement of the maximum intensity of the peaks of the bones that suffered a cooking process (moist and dry heat) to a smaller angular position, in the case of *peak 2*, both tend to plane (211).

In the sample that suffered cooking with dry heat, it is possible to observe a small pattern difference in relation to the bone as received. The angular displacement, to smaller values, was higher than that of the boiled bone, indicating a small difference

between the patterns of both cooking processes. As the peaks are very amorphous (wide) it is not possible to describe many details of the structural behavior of the samples. It was possible to observe other peaks of HAp characteristic planes such as: (130). (222) and (213) around the angular positions 40°, 46.7° and 49.5° respectively. Mobasherpouri et al. (2007) informed that there is an intensity increase of the planes (002), (211), (301), (222) and (312) when the samples are treated from 450 to 900 ºC. Kusrini & Sontang (2012) also investigated sintered bovine bone powder at different temperatures ranging from 500 to 1400 °C and the results indicated (211), (300) and (202) reflections corresponding to bovine HAp but its powder X-ray diffraction profile was dependent on sintering temperatures an times.

Miyahara et al. (2007) analyzed that the higher the temperature to which the bone tissue is exposed, the closer that the bone pattern will be to the HAp pattern, although this is more visible from 700 °C. As the objective of the present study was to analyze boiled and roasted bone patterns, calcined bone samples, i.e. submitted to much higher temperatures were not studied. Thus, the analysis of the bone sample patterns cannot be accurately compared to HAp pattern, mainly in relation to lattice parameters, because they present much wider peaks. The determination of lattice parameters depends upon the precision of locating the peak profiles in XRD diagrams, but in bioapatite this is difficult because of large peak broadening [19]. Thus, the analysis was restricted to a comparison among the X-ray diffraction patterns of the analyzed samples.

4. CONCLUSIONS

This study shows a small difference in behavior of caprine bone X-ray diffraction pattern after boiling and roasting processes which can be helpful in future investigations of human and animal archaeological bone specimens. Was possible the analysis of some peaks corresponding to the peaks of hydroxyapatite. Is interesting a study X-ray diffraction characteristic pattern of caprine hydroxiapatite in future, sintering the bones sample at high temperature.

ACKNOWLEDGMENT

The authors acknowledge the funding agencies CNPq, FAPERJ and CAPES for financial support, in special to SAMBAQUIS MGM Project (PRONEX FAPERJ E-26/110.569/2010) and the Unigranrio University.

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