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Distinguishing colour alteration processes occurred in Late Pleistocene animal remains.

Andrea Perez[1*], Fabio Santaniello[1], Stefano Grimaldi[1], Stefano Gialanella[2]

[1]: *University of Trento, Department of Humanities, Via Tommaso Gar 15, Trento, Italy,* andrea.perez@unitn.it [1], fabio.santaniello@unitn.it[1], stefano.grimaldi@unitn.it [c]
[2]: *University of Trento, Department of Industrial Engineering, Via Sommarive 9, Trento Italy,* stefano.gialanella@unitn.it
*: *Presenting and corresponding author.*

***Abstract* – The study of the usage of fire in a prehistorical group of hominids is extremely important because it outlines and sheds light on their social-economical behaviours. For this reason, studying burned specimens is interesting to identify some of the activities conducted by the inhabitants of that particular the site. Although colour changes associated with material heating may provide a first indication of a pyrotechnological process, the conclusions may sometimes be misleading. With the present paper we propose the study of animal bones from the late Mousterian-Aurignatian archaeological site of Riparo Mochi (Ventimiglia, Italy) with the deliberate aim to identify burned specimens. The approach is based on a combination of FTIR-ATR and SEM-EDXS analyses, providing a comprehensive picture of the molecular and microstructural modifications brought about by the thermal treatments, rather than by the natural diagenesis**

I. INTRODUCTION

Fire is one of the greatest discoveries in the history of humankind, which impacted its evolution pathway considerably. Thanks to fire, our ancestors could defend themselves against animal predators and have access to new edible food. Fire, as a controlled source of heat and light, allowed humans to manipulate raw materials more efficiently and modify the surrounding environment. Ultimately, having control on fire has allowed hominids to spread across the world. Hence, the study of burnt specimens is of great interest for the complete and correct interpretation of archaeological sites. Notably, burned animal bones from prehistorical sites might bring us information about the intentional use of fire: cooking habits, like roasting or marrow boiling, or even the use of bones as fuel. Identifying the thermal alteration process of archaeological specimens turns out to be an important first step forward to assess human cultural-economical behaviour or environmental events that might have occurred in the examined context. To approach the study of thermal alteration of bones it is important to understand the specific physical and chemical properties of this material. The bone is a complex tissue, mainly consisting of three parts: an inorganic fraction (70% bone mineral, a hydrated hydroxyl-depleted carbonated calcium-phosphate phase), a smaller organic fraction (20% mainly collagen), and water (10%) [1]. Each part has its own specific chemical composition and structure and, thereby, would react differently once exposed to increasing temperature. From the reactions occurring at a specific temperature it is in principle possible to identify the heating conditions to which the bones were exposed in the past and to distinguish heated bones from post-depositional altered ones. These data are fundamental to understand fire-related human behaviour and relevant pyrotechnologies [2]. Over the last decades several methodologies and experimental tests for the study of heated bones have been proposed and progressively refined: colour changes, using colorimetry; thermogravimetric analysis (TGA) [3] and scanning electron microscopy (SEM) [4], to identify bone tissue diagenesis; elemental analysis like X-ray fluorescence spectrometry (XRF) [5] and energy dispersive X-ray spectroscopy (EDXS) [6], molecular analysis with Fourier transform infrared (FTIR) [7][8] and Raman spectroscopy [9]; structural analysis made with the X-ray diffraction (XRD) [10]. Several studies [11]-[13] have been focused on the main mineral component of bones, i.e., hydroxyapatite ($Ca_{10}(PO_4)_6OH_x$ – (HA)), which is sensitive to the thermal history of the bone specimens. Fresh bones are made of HA microcrystals embedded in an organic matrix. The small size of the HA crystals, whose coalescence is hindered by the organic matrix, results in a more disordered structure. This is reflected by a lower crystallinity index. The loss of the organic matrix (that might be caused by chemical, biological, thermal and several other processes) triggers the process of recrystallisation of HA: coarser crystalline grains, with a more ordered structure and stable geometry may form

[14]. Studying the crystallinity degree of HA would indeed provide information about the ancient heating conditions. Several authors had described the physical alteration of the HA structure caused by heating using SEM observations [15]-[17]. These changes include a coarsening of the HA crystals, accompanied by a complete recrystallisation. The process starts after the loss of water and the combustion of the organic component, occurring between 100°C and 500°C [17]. When the temperature is close to 600°C, a first step of the recrystallisation takes place. The process proceeds stepwise up to a temperature of 1600°C, when all structural features are completely lost due to the melting and subsequent recrystallisation of the mineral on cooling [15][16]. All the specimens analysed in the present study belong to the archaeological site of Riparo Mochi. Riparo Mochi is a rock-shelter site near Ventimiglia (Balzi Rossi, Liguria), in North West Italy, close to the Italy-France border. The site was discovered in 1938 by A.C. Blanc and L. Cardini, (Istituto Italiano di Paleontologia Umana) and it is still under excavation. Riparo Mochi is of great interest to understand the Middle-Upper Palaeolithic transition in North Italy, thanks to its complete stratigraphy. The site presents one of the earliest Mediterranean proto-Aurignacian levels (42.7 – 47.6 ky cal BP), covered by an Aurignacian level with bone-antler tools (37.3 – 36.4ky cal BP). These two levels lay on a thick late Mousterian deposit (44.0 – 41.8 ky cal BP) [18]-[21]. Faunal remains from these three levels have received little attention in the past, and there have been only preliminary analyses [18][22]. Because of this lack of studies, most of the specimens were just taken from the excavation and sorted out by their colouring, in order to recognize their heating alteration degree. However, this preliminary assessment does not actually afford a full and reliable recognition of the main reason for the observed chromatic changes. Indeed, specimens identified as burned or charred might have been coloured by a post-depositional process instead [23]. The main aim of this study is to identify possible alteration agents of the bone specimens and, in particular, whether or not a black-brownish colour corresponds necessarily to a heating event. In this perspective, the analyses have been carried out using FTIR-ATR and SEM-EDXS techniques.

Specimen code	Unit	Colour	Supposed alteration/T
Specimen 1	I	Dark brownish	Burnt / 350°C
Specimen 2	I	Natural white	Calcined / >700°C

Table 1. Analysed specimens

II. MATERIALS AND METHODS

For the analysis we chose two bones fragments, codenamed “1”, Fig. 1, and “2”, Fig. 2 respectively, from the Mousterian unit. The two specimens have markedly different colouring, as specified in Table 1, taken as a first indication of a potential burned and unburned condition [24].

Fig. 1. Specimen 1. Exterior (a) and inner (b) surface.

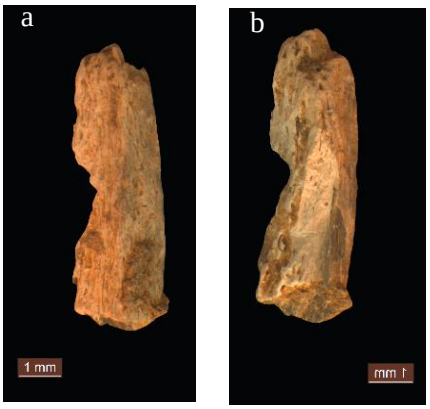

Fig. 2. Specimen 2. Exterior (a) and inner (b) surface.

According to literature studies, we assume that the bone colouration might indicating a thermal diagenesis degree [1][25]. As the fire temperature increases, the chromatic alteration of the bone changes from white natural through yellowish-red to dark reddish-brown at 350°, to greyish and black at temperature from 400° to 600°. At temperatures of 700° and as above, the bone colour turns white, for the complete elimination of any organic residue. However, the same colour might be obtained through different combustion conditions as well as post-depositional processes [23][24]. A millimetric flake of tissue has been removed from both the specimens, to analyse the inner part of the bones, to better identify the effect of potential surface contamination on the analytical results. In this regard, the specimens have been carefully handled using gloves to prevent any contamination.

A. Bone structure

The structural alteration of the bone tissue has been characterized using a FTIR analysis. The equipment is a BRUKER *Alpha II,* operated in attenuated total reflection (ATR) mode, for which each bone fragment has been positioned onto the measurement diamond plate. It has been analysed the inner side of each flakes only. These analyses have been focused on the quantification of the degree of recrystallization of hydroxyapatite, induced by the thermal treatments, if any, and natural diagenesis. To quantify this alteration, the Splitting Factor (SF) parameter has been evaluated, according to the definition proposed by Weiner & Bar-Yosef [26]. The SF is calculated by summing up the intensities of the 563 cm^{-1} and 603 cm^{-1} IR lines, A and B respectively, and dividing this value by the value of minimum between the two peaks, C- parameter (*Fig. 3*). It has been demonstrated that there is a direct correlation between the crystallinity of HA and SF [14][26][27]. Relatively recent bones have a SF value in a range from 2 to 3; while well mineralized and combusted bones can easily reach a SF value of 7 [28]-[31].

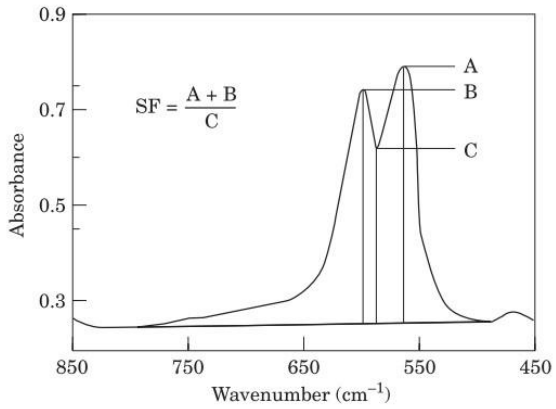

Fig. 3. Splitting factor calculation [26]

B. Chemical composition

To verify if any element from the depositional environment, e.g., soil, redeposited sediments, etc., might had contaminated the specimens, possibly resulting in an alteration of their colour, SEM-EDX measurements have been acquired. This technique measures the elemental composition (B and heavier elements) of the specimens' surface. To do this, specimen is irradiated by an electron beam. The interaction between the sample and the beam produces a backscattered electron deflected by the nuclei of the atoms. Inner shell electrons may be ejected from the atoms: this produces a vacancy in the relevant orbitals. Finally, when an electron from a higher energy level fill up the electron vacancy, a x-ray photon is generated. The EDX technique identify that emitted x-ray which has energy specific to each element [25][32]. Therefore, EDX can be used to evaluate the composition of the bone surface [33] or to analyze thin sections of sediment to recognize possible evidence of the use of fire [34]. An SEM (Model: PHENOM XL) has been used, in low vacuum mode, to avoid sample electrical charging. The microscope is equipped with an EDXS microanalytical system, with a Si_3N_4 window, sensitive to X-ray lines from boron up to uranium.

III. RESULTS

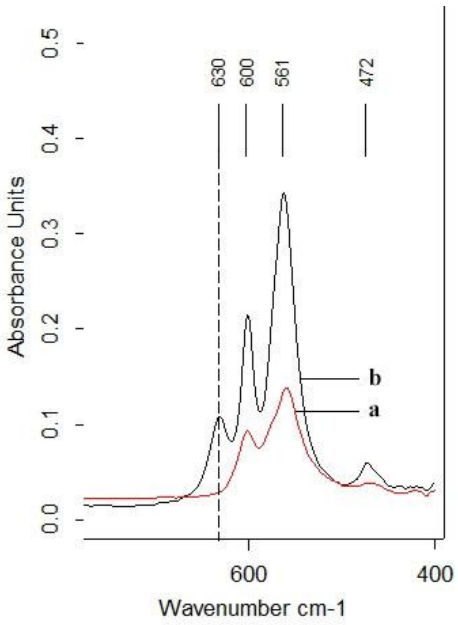

Fig. 4. IR lines for used for the evaluation of the Splitting factor. of Specimen 1 (a) and Specimen 2 (b).

A. Bone structure

The SF value for the Specimen 1, SF=3.7, indicates a slight alteration of the bone material and seems to exclude any thermal alteration (Fig. 4.a). The SF for the Specimen 2, has been evaluated to be: SF=5.6. Such a value corresponds to a higher degree of alteration than Specimen 1. Moreover, the peak at 630 cm^{-1} indicates an exposition to temperature above 570°C [35] (Koopowitz, 2019) (Fig.4). These two observations confirm that this bone fragment has been exposed to high temperatures for a time interval sufficient to promote a complete recrystallization of HA, as indicated by the relatively high values of SF.

	Region 1	Region 2
	wt. %	wt. %
CaO	60.8	55.9
P_2O_5	37.5	41.8
SO_3	0.9	1.4
SiO_2	0.7	0.8

Table 2. Specimen 1 – Compositional data obtained from two analysed regions.

B. Bone composition

Two regions of the Specimen 1 have been analysed: the results are listed, in oxide concentrations, in table 2. Apart from the main bone components, i.e., calcium and phosphorous, minor sulphur and silicon concentrations have been detected. Their presence can be explained as a contamination from the archaeological sediments. Incidentally, from a microstructural point of view, it is not possible to recognize any significant alteration in the bone tissue of Specimen 1. In fact, the osteons seem to be well preserved (Fig. 5). This is in agreement with the relevant IR results. We expect to see a certain diagenesis degree of the bone tissue of Specimen 2, according to its IR results.

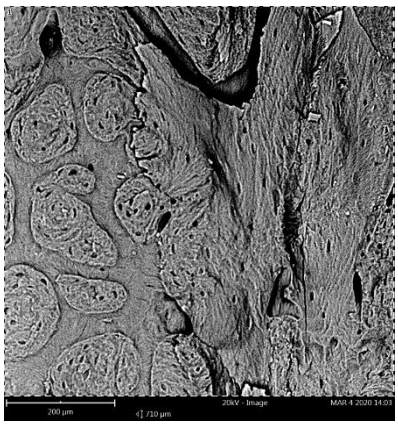

Fig. 5. SEM micrograph showing the microstructure of the Specimen 1.

IV. CONCLUSIONS AND PERSPECTIVES

The heat induced or natural alterations of two archaeological bone specimens have been investigated. Using a combination of different experimental techniques, a better understanding of the processes involved in these alterations have been achieved. In particular, it is possible to discern between anthropic and natural agents. The preliminary sorting of the bone samples according to their surface colours, has been confirmed not to be a reliable approach. Indeed, Specimen 1, tentatively classified as potentially "burnt", on the basis of eye inspection, resulted to be just altered, as concerns its surface colour, by natural ageing, according to the FTIR analysis. Contrarily, it was confirmed that Specimen 2 is actually a burnt bone, exposed to a temperature above 570°C. SEM-EDX analyses confirmed that Specimen 1 exhibits a mostly unaltered microstructure of the bone tissue. Silicon and sulphur, probably come from the archaeological soil. It is interesting to notice how two specimens from the same stratigraphic unit and from the same square show different alteration features, as if one was exposed to high temperature. Future FTIR and SEM-EDX analyses will be carried out on a larger number of bone specimens, in order to improve the statistical reliability of our data. We also plan to carry out an analytical survey on the Riparo Mochis' soil in order to understand if the oxide concentration seen in Specimen 1 its actually due to the archaeological sediment. Moreover, we want to use a colorimeter to establish an objective coloration degree of specimens. Indeed, we think that defining specimen colouration using Munsell chart [36] or by just an arbitrary assignment cannot be taken as an objective method to describe the actual specimens colour.

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