A NOTE ON DIAGENETIC PARAMETERS FOR BONE REMAINS FROM PEDRA DO ALEXANDRE SITE WITHOUT SAMPLE DESTRUCTION

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Resumo: A diagênese óssea é a alteração pós-deposicional de tecidos calcificados por degradação química. As trajetórias diageneticas têm sido empregadas para avaliar a forma como a diagênese ocorreu no enterramento do osso, usando um conjunto de algumas técnicas como porosimetria, integridade histológica, teor de colágeno e cristalinidade. Foram analisados 8 fragmentos de ossos de três representantes dos enterramentos de cada camada estratigráfica por espectroscopia infravermelha transformada por Fourier (FTIR). Estimamos quatro parâmetros distintos baseados em espectros infravermelhos: índice de cristalinidade (CI), carbono/carbonato (C/C) e taxas de carbonato/fosfato (C/P) e pico de amida 1. Nossos resultados sugerem preservação semelhante para cada camada estratigráfica e a trajetória diagenética pode estar relacionada à hidrólise acelerada de colágeno. Palavras-chaves: ATR-FTIR, Caatinga, índice de cristalinidade, trajetória diagenética.

Abstract: Bone diagenesis is the post-depositional changes of calcified tissues by chemical degradation, and diagenetic trajectories have been employed to evaluate how diagenesis occurred in the burial using a set of a few techniquessuch as porosimetry, histological integrity, collagen content and crystallinity. In our study, 8 bones fragments from three burials representatives of each stratigraphic layer were analyzed by Fourier transformed infrared spectroscopy (FTIR). We estimated four distinct parameters based on infrared spectra: crystallinity index (CI), carbonyl/carbonate (C/C) and carbonate/phosphate (C/P) ratios and amide 1 peak. Our results suggest similar preservation for each stratigraphic layer and the diagenetic trajectory might be related to accelerated collagen hydrolysis. Keywords: ATR-FTIR, Caatinga, crystallinity index, diagenetic trajectory.

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Introduction

Bone diagenesis is the degradation process of organic and inorganic molecules during the formation of the archaeological record along the post-depositional event. The diagenetic trajectories ensure which type of degradation process occurred in the pre-historic burial. Several indices have been used to characterize the diagenetic trajectory: porosimetry, histological integrity, collagen content and crystallinity. Up to now, three trajectories of degradation are identified for prehistoric bones from Well-Preserved (type 1) to the Accelerated Hydrolysis Collagen (type 2), Microbial Attack (type 3), or Catastrophic Dissolution (Type 4), for its estimative several parameters have been employed such as crystallinity index (CI); carbonate/phosphate ratio (C/P); cracking index; percentage of collagen; oxford histological index (OHI) (Smith et al. 2007). The two former parameters are often obtained by Fourier-transforminfrared (FTIR) spectroscopy with destruction of the archaeological samples.

FTIR is a common technique used in archaeology to obtain spectra for diagenetic change estimation, and it is frequently applied as a destructive process with preparation of pellets using powdered bone with KBr. Nowadays it is possible to perform this analysis in a non-destructive way by contact using the attenuated total reflectance (ATR) mode (Hollund et al. 2012, Beasley et al. 2014, Snoeck et al 2014). Also, ATR-FTIR is applied in archaeological research to differentiate burnt and unaltered bone estimating CI, C/C and C/P without sample destruction (Thompson et al. 2011, Snoeck et al. 2014). Therefore, this technique offers a cheap, fast and easy method compared to the previous use KBr-FTIR.

The indices provided by ATR-FTIR is calculated based on spectral intensities of absorbance, one of these is crystallinity (CI) defined as the extent of splitting of phosphate (603 and 565 cm-1) peaks in bone (Termine and Posner 1966), applied by Weiner and Bar-Yosef (1990) as diagenetic parameter to test the chemical condition of archaeological bones, which is often used to characterize diagenetic trajectories (Hedges 2002). The archaeological studies in Brazil has only one example of spectroscopic application to assess the preservation of ancient calcified tissue (Colonese et al. 2014), in which was used another type of non-destructive technique, Raman spectroscopy, to obtain signals of collagen preservation in bones from Brazilian coastal prehistoric populations. ATR-FTIR and Raman-FTIR have been promising techniques to investigate the preservation of biomolecules in archaeological bones; recently, two other studies interpreted the bone diagenesis and collagen preservation respectively in Sudan and France, and Morocco archaeological sites (Dal Sasso et al. 2016,Lebon et al. 2016).

Here, we estimated diagenetic parameters for bone fragments from Pedra do Alexandre site (Martin 2008), that is a Paleoamerican site located in the northeast of Brazil in the semi-arid region of the Caatinga biome with an occupation chronology between 2620 and 9400 years before present (Bueno et al. 2013). The novelty relies on the first estimative of diagenetic trajectory and predictive collagen content for archaeological bones found in Caatinga biome by a non-destructive method of spectroscopy.

Material and Methods

The non-destructive method of spectroscopy Fourier transformed infrared by attenuated total reflectance (ATR-FTIR) was used to record reflectance spectra of the bones. A PerkinElmer 400 spectrometer FTIR and FTNIR with ATR universal accessory (UATR) equipped with ZnSe diamond composite crystal was used to record a complete infrared spectrum in the spectral range of 4000 to 400 cm-1, with 4cm-1 resolution and 16 scans. In each sample, three points were measured to calculate a medium value. Considering the non-destructive nature of this work, the intact fragments were pressed against the diamond crystal on the accessory. The requirement to make a fine powder to obtain the spectra was not done due to non-destructive pattern of our analysis.

Origin software was used to perform the graphs and access the frequencies obtained by ATR-FTIR, Microsoft Excel software was used to calculate the diagenetic indexes. The carbonyl carbonate index (C/C) was calculated by 1455 (v3CO3) and 1415 cm-1 (v3CO3) peaks ratio, also the carbonate phosphate index (C/P) was determined by 1415 and 1030 cm-1 ratio (v3CO3 and v3PO4 domain), further crystallinity index was measured by 560 and 600 cm-1 (v4PO4) peaks divided by 590 cm-1 (a review is presented in Table 1); two other new parameters for crystallinity index were used to access the diagenetic alterations 1030/1020 cm-1 and 1060/1075 cm-1 ratios (Lebon et al. 2010; Figure 3). On the other hand, we retrieved the collagen content information by amide 1 (1637 cm-1) peak (Lebon et al. 2016), functional group indicator of type-1 collagen (~1650 cm-1).

Table 1: Review about Crystallinity index (CI), Carbonyl/Carbonate (C/C) and Carbonate/Phosphate (C/P) values of peaks in cm-1. ND = No Data

References	C/P	C/C	CI
Pucéat et al. 2004	1425/1041	1460/1425	605-568
Surovell and Stiner 2001	ND	ND	603-563
Smith et al. 2007	1415/1035	ND	605-567
Reiche et al. 2003	ND	ND	605-565
Patonaiet al. 2012	1428/1042	ND	604-565

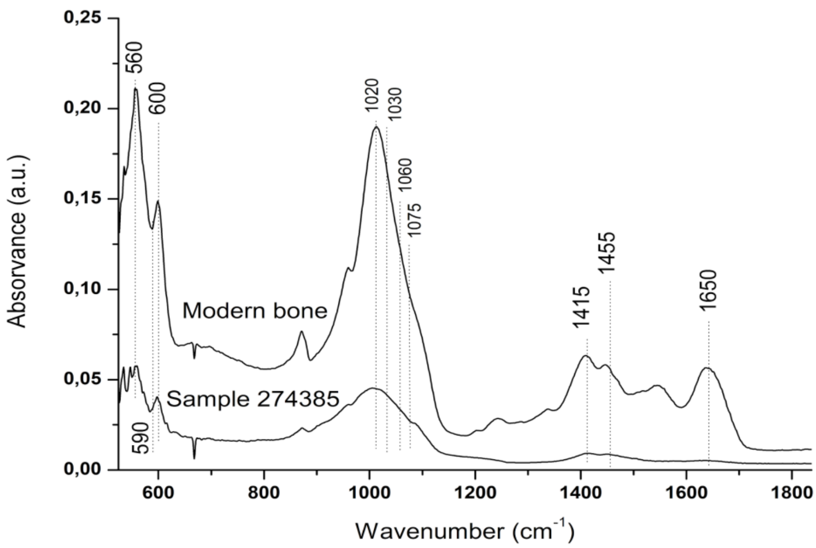

Figure 1: Mid spectra 550 to 2000 cm-1 of one modern and one archaeological sample (274385).

Bone Sample

The bones from three burials of the Paleoamerican site were represented by eight fragments, three are diaphysis of long bones 274385ol, 294380, 284045, cranial bones 274385, 294221, 284329; trabeculae 284343; teeth 274405 (Figure 2).

The sediments were removed mechanically from the bone surface, and the archaeological bones were measured by contact between UATR-ZnSe diamond composite crystal and the sample. A pressure was applied to ensure a great contact without destruction of the archaeological bones fragments; the same procedure was followed for the modern bone.

Figure 2: Fragments from three different burials (prefix 27, 28 and 29). Superior: three diaphysis. Middle: two crania fragments and trabeculae. Inferior: teeth and one femora.

Modern human bone sample was obtained in Department of Anatomy at the Federal University of Pernambuco. The standard place to apply the measurements was the humerus diaphysis. The chosen measurement place followed the advice for specific anatomical areas in bone diagenesis studies (Castro et al. 2010, Thompson et al. 2011,Hollund et al. 2016).

Characterization of the site

The Pedra do Alexandre prehistoric site was studied since the early 1990s (Martin 2008, Queiroz and Carvalho 2008, Figure 3) and has been a frequent chosen field to archaeologists' research about funerary practices in Northeast Brazil. Located in Caatinga biome, exclusive to Brazil semi-arid region characterized by xerophytic and shrub vegetation, the site is in a small elevated rock shelter with a broad view of the vicinity.

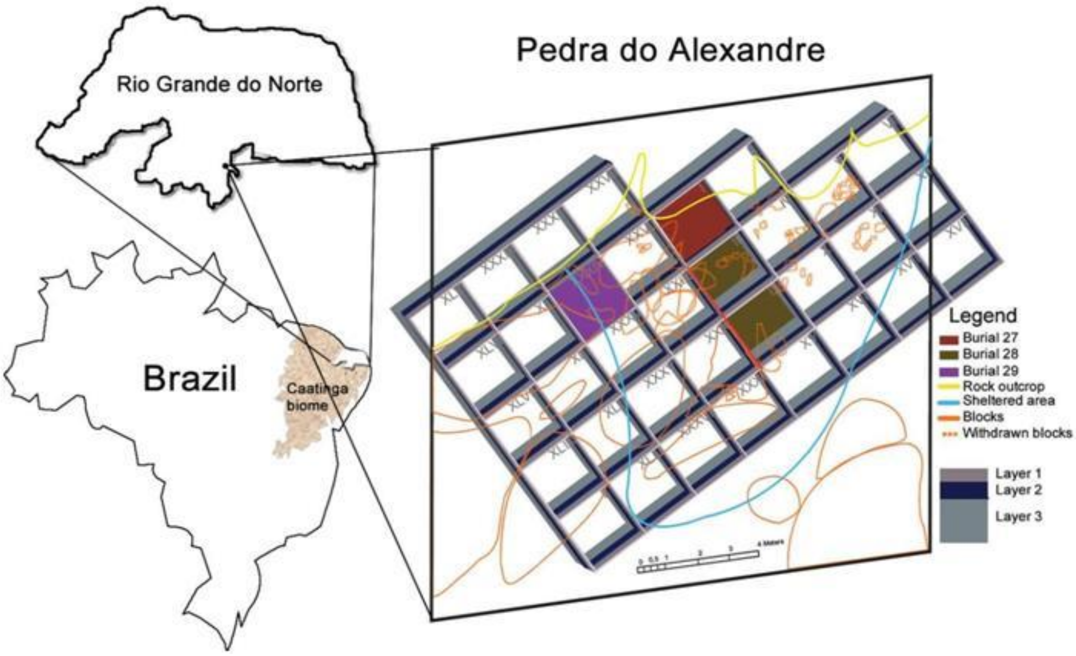

Figure 3: Localization of CaatingaBioma and the state of Rio Grande do Norte including Pedra do Alexandre site. Georeferenced map adapted from Mutzenberg (2007) giving the height of the burials in three distinct layers and its sectors distributed in space.

According to past research (Martin 2008) about this site, there were 31 burials until the 17th campaign that occurred in November 2011. During the campaign 16 - conducted in October 2010 - three burials, 27, 28 and 29, were found and these numbers were used as a prefix to identify each burial in the samples (e.g.: 284045).

The 16th campaign was divided into two parts, field and laboratory. In the field, the procedure adopted was an excavation by artificial levels with 10 centimeters each one, gathering bones and sediments after the georeferencing procedure. Along the excavation, neither roots nor other plant remains were found near the bones. In laboratory, the samples were treated with screening, dry and wet cleaning and fragment count. The minimum number of individuals (MNI) was one for burials 27 and 29 and five in burial 28 (Silva et al. 2011).

Gravels and micro fragments of quartz were mainly found between the sediments, the presence of organic matter is higher near the surface layer of the soil, and a major part of the soil in the deepest layer is sandy. Another characteristic is linked to the bedrock mainly composed of mica schist (Martin, 2008). Dripping and infiltrating water can appear in the site during the rare wet season – i.e. about one or two months (March-April) in each year. The temperature around the site is 28,7°C in a place with shadow and litter the soil near the surface, and in places with intense sunlight is 36,3°C, both temperatures collected in November 2011.

Results

The determined CI, C/C and C/P based on values from spectra showed a slightly variation between archaeological to modern ones (Table 2).

The CI averages of archaeological bones ranges from 2.64 to 3.49 in overall; for stratigraphic approach, there was a roughly difference between the burials, near the soil layer (burial 27) we had the values from 2.74 to 3.21, in the intermediate layer (burial 28) we had averages from 2.64 to 3.39, in the deepest layer (burial 29) we had the biggest value 3.46. The corresponding values for C/C and C/P do not vary as much as the CI for the studied bones, an extraordinary low carbonyl/carbonate ratio content was found in one long bone fragment (294380), also another long bone presented a high level of carbonate instead of phosphate (284343). The two more crystallinity indexes 1030/1020 cm-1 and 1060/1075 cm-1 ratios showed no abrupt variation between the samples. As expected, the amide 1 peak (~1637 cm-1) showed an extant of low presence of collagen in Paleoamericanbone fragments compared to modern bone (Figure 4).

Table 2: Averages and standard deviations from five diagenetic parameters from three distinct archaeological burials and one modern bone. Respectively: Crystallinity Index; Carbonyl/Carbonate; Carbonate/Phosphate; 1030/1020 and 1060/1075 ratios.

Samples	C/P	C/C	CI	1060/1075	1030/1020
274385	0.20±0.03	0.93±0.02	2.84±0.36	1.23±0.09	0.95±0.01
274385ol	0.22±0.04	0.92±0.01	3.21±0.11	1.29±0.03	0.95±0.01
274405	0.26±0.01	0.91±0.04	2.74±0.61	1.21±0.12	0.95±0.01
284045	0.38±0.16	0.84±0.03	3.39±0.31	1.29±0.03	0.96±0.04
284329	0.33±0.10	0.91±0.08	2.64±0.19	1.22±0.01	0.94±0.04
284343	0.74±0.51	0.81±0.04	3.08±0.50	1.24±0.09	0.94±0.01
294221	0.30±0.07	0.87±0.01	3.39±0.31	1.24±0.01	0.92±0.01
294380	0.36±0.19	0.36±0.24	3.46±0.49	1.30±0.07	0.96±0.04
Modern	0.37±0.01	0.90±0.01	2.64±0.01	1.22±0.01	0.92±0.01

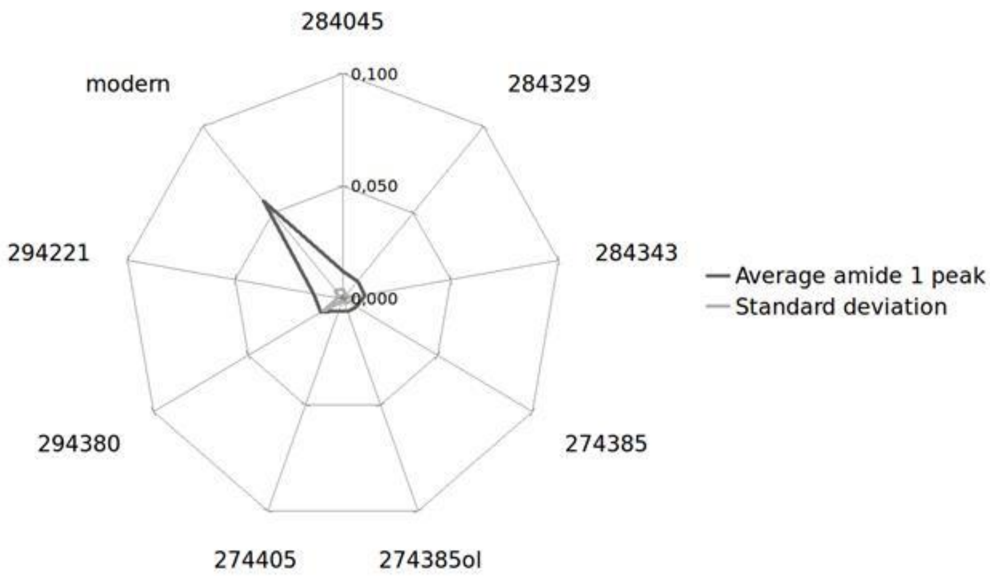

Figure 4: Amide 1 (~1637 cm-1) peak as functional band indicator of type-1 collagen.

Discussion

The macroscopic analysis by naked-eye showed that bone fragments were not well-preserved (Behrensmeyer1978). The scale considers the preservation going from bones without cracks in the same position of collagen fibers (0) to splitting and cracks scattered throughout the entire bone structure (5). All the samples had poor preserved external surface structure (4).

Burial microenvironment at rock shelter had similar preservation from three distinct stratigraphic layers. The similar preservation among the samples must be akin to the similar characteristics of burial microenvironment, and it was determined by macroscopic and spectroscopic physicochemical characteristics. Conversely, the CI values were nothigher than expected among stratigraphic layers, nor between the layers and the modern bone value, indicating an initial step of bioapatite recrystallization of the studied bone fragments. Moreover, there was no clear distinction between the modern and archaeological samples using Crystallinity Index, only a slightly variation for burials 27 and 28, and a strong deviation for burial 29 (Table 2). Values of CI and C/P from burned bones are still known with results higher than 4.0 for crystallinity index and under 0.2 for carbonate/phosphate ratio; none of these patterns was observed in our study (Squires et al. 2011, DalSasso et al. 2016,Lebon et al. 2016). The bone fragments were not calcined or charred conform 1060/1075 crystallinity ratio, nor macroscopically color signals of heating were observed (Lebon et al. 2010).

The low signal of amide I peak (Figure 4) is due to the semi-arid burial microenvironment which is not proper to conservation of collagen extant. Other studies for Sudan, Morocco and France contexts found low content of collagen using the Amide 1/PO4 ratio, but emphasized the assessment of collagen molecule by ATR-FTIR as standard pre-scanning technique for dating procedure and DNA extraction procedure (DalSasso et al. 2016, Lebon et al. 2016).

Enamel (274405) has less intense peaks than archaeological fragments and internal part of modern bone tissues, also could be better analyzed from different archaeological sites with more samples to create a reliable database. This structure has an important role in the preservation of ancient DNA extracted from dentine (Beasley et al. 2014).

The results from diagenetic parameters resembles one diagenetic trajectory proposed by (Smith et al. 2007), mainly from well-preserved (Type 1, C/P 0.4+ and CI 3 typical values) to accelerated collagen hydrolysis (Type 2, C/P 0.1 and CI>3.9 typical values) (see Table 2). Regarding only the ATR-FTIR data, the amide I was used as signal of collagen content in substitution of OHI and porosimetry (Smith et al. 2007).The diagenetic trajectory identified using crystallinity indicates a case of ACH (accelerated collagen hydrolysis), this type of degradation is rare and it was found in bones from Apigliano (Smith et al. 2002). As an initial step, the number of samples and measurements in this note should be improved to ensure that prehistoric human bones from Pedra do Alexandre rock shelter are proper to extraction of ancient DNA and collagen in the future.

Conclusion

The bone degradation in the semiarid soil from Pedra do Alexandre site has similar local conservation processes in a case of three stratigraphic layers with a diagenetic trajectory resembling accelerated collagen hydrolysis (ACH). The fragments have cracking and flaking, however the samples do not have diagenetic parameters that indicate heating or boiling. The bones in semiarid context and Caatinga biome are overall more microenvironmental dependent than stratigraphically. ATR-FTIR technique is essential to deal with archaeological remains specially the Paleoamerican bone fragments and it should be standardized for field expeditions holding on handheld and portable mass spectroscopy devices.

Acknowledgments

We thank Fernanda Pimentel by the useful commentaries about ATR-FTIR. Claudia Oliveira, Gabriela Martin and Ana Catarina Torres by the coordination of field work. This article is the first part of master's dissertation of AAF financed by CAPES and Seridó Foundation.

References

BEASLEY, M. M, BARTELINK, E. J.; TAYLOR, L. and MILLER R. M. 2014.Comparison of transmission FTIR, ATR, And DRIFT spectra: Implications for assessment of bone bioapatite diagenesis. J ArchaeolSci 46:16-22.

BEHRENSMEYER, A. 1978. Taphonomic and ecologic information from bone weathering.Paleobiology 4:150-162.

BUENO L.; DIAS, A. and STEELE, J. 2013.The Late Pleistocene/Early Holocene archaeological record in Brazil: A geo-referenced database.QuatInt 301:74-93.

CASTRO, W., HOOGEWERFF, J., LATKOCZY, C., ALMIRALL, J. 2010. Application of laser ablation (LA-ICP-SF-MS) for the elemental analysis of bone and teeth samples for discrimination purposes. Forensic SciInt 195:17-27.

COLONESE, A.;COLLINS, M.; LUCQUIN, A.; EUSTACE, M.; HANCOCK, Y. 2014.Long-term resilience of Late Holocene coastal subsistence system in southeastern South America.PLoS One 9:e93854.

DAL SASSO, G.; LEBON, M.; ANGELINI, I.; MARITAN, L.; USAI, D., et al. 2016. Bone diagenesis variability among multiple burial phases at Al Khiday (Sudan) investigated by ATR-FTIR spectroscopy. PalaeogeogrPalaeoclimatolPalaeoecol 463:168-179.

QUEIROZ, A. and CARVALHO, O. 2008.Problems in the interpretation of Brazilian archaeofaunas: Different contexts and the important role of taphonomy.QuatInt 180:75-89.

HEDGES, R. 2002. Bone diagenesis: an overview of processes. Archaeometry 44:319-328.

HOLLUND, H.I.; ARIESE, F.; FERNANDES, R.; JANS, M.M.E. and KARS, H. 2012. Testing an alternative high-throughput tool for investigating bone diagenesis: FTIR in attenuated total reflection (ATR) mode.Archaeometry 55:507-532.

HOLLUND, H.; TEASDALE, M.; MATTIANGELI, V.; SVERRISDÓTTIR, O.; BRADLEY, D. et al. 2016. Pick the right pocket. Sub-sampling of bone sections to investigate diagenesis and DNA preservation.Int J Osteoarchaeol 27:365-374.

LEBON, M.; REICHE, I.; BAHAIN, J.; CHADEFAUX, C.; MOIGNE, A. et al. 2010. New parameters for the characterization of diagenetic alterations and heat-induced changes of fossil bone mineral using Fourier transform infrared spectrometry. J ArchaeolSci 37:2265-2276.

LEBON, M.; REICHE, I.; GALLET, X.; BELLOT-GURLET, L.; ZAZZO, A. 2016. Rapid quantification of bone collagen content by ATR-FTIR spectroscopy.Radiocarbon58:131-145.

MARTIN, G. 2008. Pre-história do Nordeste do Brasil. 5th ed. Recife, PE, Brazil: Ed. Univ./UFPE.

MUTZENBERG, D. 2007. Gênese e ocupação pré-histórica do Sítio Arqueológico Pedra do Alexandre: uma abordagem a partir da caracterização paleoambiental do Vale do Rio Carnaúba – RN. Master dissertation, Department of Archaeology, Federal University of Pernambuco, Brazil.

PATONAI, Z.; MAASZ, G.; AVAR, P.; SCHMIDT, J.; LORAND, T. et al. 2012. Novel dating method to distinguish between forensic and archeological human skeletal remains by bone mineralization indexes.Int J Legal Med 127:529-533.

PUCÉAT, E.; REYNARD, B. and LÉCUYER, C. 2004. Can crystallinity be used to determine the degree of chemical alteration of biogenic apatites? Chemgeol 205:83-97.

REICHE, I.; FAVRE-QUATTROPANI, L.; VIGNAUD, C.; BOCHERENS, H.; CHARLET, L. et al. 2003. A multi-analytical study of bone diagenesis: the Neolithic site of Bercy (Paris, France).MeasSciTechnol 14: 1608-1619.

SILVA, S.F.S.M.; SULASSI, H.S;L.; ALCANTARA, T.M.; BORGES, M.A.S. and OLIVEIRA, A.P. 2011. Report of 16ª field campaignPedra do Alexandre, Carnaúba dos Dantas, RN.Recife: Federal University of Pernambuco, Brazil.

SMITH, C.; NIELSEN-MARSH, C.; JANS, M.; ARTHUR, P.; NORD, A. et al. 2002.The strange case of Apigliano: early 'fossilization' of medieval bone in southern Italy. Archaeometry 44: 405-415.

SMITH, C.; NIELSEN-MARSH, C.; JANS, M. and COLLINS, M. 2007. Bone diagenesis in the European Holocene I: patterns and mechanisms. J ArchaeolSci 34:1485-1493.

SNOECK, C.; LEE-THORP, J. and SCHULTING, R. 2014. From bone to ash: Compositional and structural changes in burned modern and archaeological bone. PalaeogeogrPalaeoclimatolPalaeoecol 416:55-68.

SQUIRES, K.; THOMPSON, T.; ISLAM, M. and CHAMBERLAIN, A. 2011. The application of histomorphometry and Fourier Transform Infrared Spectroscopy to the analysis of early Anglo-Saxon burned bone. J ArchaeolSci 38:2399-2409.

SUROVELL, T. and STINER, M. 2001. Standardizing infra-red measures of bone mineral crystallinity: an experimental approach. J ArchaeolSci 28:633-642.

TERMINE, J. and POSNER, A. 1966.Infrared Analysis of Rat Bone: Age dependency of amorphous and crystalline mineral fractions. Science 153:1523-1525.

THOMPSON, T.; ISLAM, M.; PIDURU, K. and MARCEL, A. 2011. An investigation into the internal and external variables acting on crystallinity index using Fourier transform infrared spectroscopy on unaltered and burned bone.PalaeogeogrPalaeoclimatolPalaeoecol 299:168-174.

WEINER, S. and BAR-YOSEF, O. 1990.States of preservation of bones from prehistoric sites in the Near East: A survey. J ArchaeolSci 17:187-196.