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Changes in human bones boiled in seawater

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ABSTRACT

The differences between boiled or unboiled bones are not often studied. However, they are crucial to understand *postmortem* rituals and to establish defleshing procedures and mortuary practices. In this work, human bones boiled in sea or fresh water are characterized. The bone composition, as well as the compounds present in the resulting materials, shows that salt alters the boiling process mechanism. Hence, from structural and morphological criteria, it is possible to distinguish if a bone has been boiled in salt or fresh water. In both sets of samples, the smoothness of the bone surface depends on boiling time, but only in bones boiled in seawater, filaments are observed apparently pouring out of the pores.

Those differences which are mainly morphological (smoothness of the surface) are explained in terms of a collagen diffusional mechanism favored by sodium and chloride ions. For a boiling time of 6 h, the surface is covered by a thick layer or crusts of degraded collagen. Experiments with seawater may be used as model experiments to simulate taphonomical alterations in bones exposed to salt water.

1. Introduction

Bone alterations may be natural or cultural or both. Nature may transform bone through degradation in presence of a particular inorganic environment, through fungal contamination or by carnivore action, among other well known mechanisms. Furthermore, just weathering is known to modify significantly bone structure and morphology (Malgosa et al., 2008; White and Hannus, 1983). Bones altered by humans can present cut marks, intentional fractures, impacts due to pressure or percussion, perforations or thermal alterations. The extent of possible causes of modifications is such that it is a hard work to ponder which mechanism was preponderant on a given sample. For instance, bones treated thermally may, then, be altered by carnivores and, then, degraded by weathering.

The recognition of those phenomena is essential as, in archaeology and anthropology, the study of bones transformed by humans provides information on cultural habits. If bones are from animals, the alterations due to human practices are generally attributed to food preparation, whereas, if they are human, to ritual habits such as cannibalism or the incineration of bodies. However, the practice of boiling to easily deflesh bodies is often forgotten. In the Neolithic Andalucía, defleshing is thought to be a rather common ritual habit (Jiménez Brobeil, 1990). In this case, such evidence could be indicative of cannibalism as in the Sierra de Atapuerca or among the Anasazi (Cáceres et al., 2007; Roberts et al., 2002). To deflesh the corpse of the German Emperor Lothar I, who died in the 12th century, the body was, apparently, boiled for about 6 h to prevent *postmortem* decay during transit from the place he died which was 500 km from his castle where he was buried (Bada et al., 1989).

In this sense, model experiments should be very useful as the effects of temperature and boiling time on bone structure and morphology may be distinguished and studied experimentally using modern materials. Those effects are isolated from the degradation due to weathering or any other natural factor which

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are always present in archaeological materials. The characterization of boiled bones is crucial to anthropology not only to understand human habits but because boiling has been proposed as an analog for bone diagenesis (Hurlbut, 2000). In a previous work, we established the structural and morphological differences between unboiled and boiled bone, in fresh water (Bosch et al., 2011). In the present study the same experiments are presented but in seawater.

Indeed, in islands or coasts where fresh water may be precious, seawater is expected to be used in the boiling process if the purpose is just to obtain defleshed bones. The features of bones boiled in seawater must be different from those boiled in fresh water and those characteristics could be a criterion to discriminate between real cooking (boiling in fresh water) and utilitarian boiling (in seawater), *i.e*. boiling to eat or boiling to deflesh bodies and dispose of the bones either for ritual purposes or to produce ornamental objects or tools. Publications on boiled bones are scarce but, as far as we know, there are no characterization studies on seawater boiled animal or human bones. However, the effects of sodium chloride on hydroxyapatite and on collagen, which are the main components of bone, have been studied separately.

Salt alters collagen solubility and salt precipitation is a general protocol to recover isolated collagen (Hellauer and Winkler, 1975; Kangsanant et al., 2008; Kwon et al., 2009). In collagen-silica hybrid materials, sodium chloride decreases the rate of the collagen fibril assembly. The effect of sodium chloride is explained in terms of osmotic exclusion and influence on electrostatic interactions between collagen fibrils (Eglin et al., 2005). Hellauer and Winkler (1975), using soluble fractions of collagen have studied the influence exerted by salt concentration, kind of ions, and medium pH on hydrothermal stability. They report that Cl^- anion and even more I^-, being structure breakers corresponding to their position in the Hofmeister series, would disturb the regular order of the structural water narrowly bound to the polypeptide chains.

Hydroxyapatite is an ion exchanger which may retain calcium or sodium in the appropriate conditions. Hydroxyapatite in fish scales has been widely used recently for remediation of heavy metal pollution in water, soil, and sediment (Kalvoda et al., 2009). It has been already shown that when calcium is replaced by sodium, sodium is located preferentially in the calcium site II of the apatite structure; an extra shift of the OH^- ion outside of the metal II triangle is observed which may be attributed to the replacement of calcium by sodium and a vacancy (El Feki et al., 1999). NaCl strongly suppress hydroxyapatite dissolution kinetics, including at physiological ion concentrations (Kwon et al., 2009).

However, none of the previously mentioned studies is focused on the features of a bone boiled in seawater. Conventionally, thermal behavior of bones has been studied by the so called thermal methods, for instance calorimetry or thermogravimetric analysis (Lozano et al., 2003; Rogers and Daniels, 2002). These techniques study the modifications of bone in air environment and have not been able to determine any differences at low temperature treatments (Pijoan et al., 2007).

In this work, we prepared reference materials boiled in seawater to be characterized at a nanometrical level through physical methods. Human bones treated in seawater are expected to be different from bones boiled in fresh water as each of the components of bone, hydroxyapatite and collagen, are prone to exchange ions or react with solved sodium chloride. We chose to work on human bones to compare the results with those reported previously (Bosch et al., 2011), but the study may be expanded to animal bones. The anthropological implications would be the differentiation between cooking or utilitarian boiling in the zones with scarce fresh water and the understanding of some taphonomical phenomena.

2. Materials and methods

2.1. Bone samples

Fresh samples were obtained from two different human bodies, deposited in the Departamento de Anatomía, Anatomía Patológica y Citología of the Universidad de La Laguna. Approval from the research ethics committee of the Universidad de La Laguna and family consent for the donation of organs for autopsy and procedures included in this research were obtained. The samples labeled 137 come from the distal third of the right tibia of an adult young male. This body was impregnated with a formaldehyde solution to be able to study it during next days. It has to be said that the impregnation was superficial and that, when the bones were cut, they were intact, even the marrow was fresh. Thus such pretreatment does not alter the present samples. Samples 131 were taken from the distal third of the right femur of an adult mature male which had not been impregnated in formaldehyde. In both cases, three cylinders of bone were cut, approximately 2 cm thick.

These contemporary bones were not boiled with flesh. Indeed, temperature in water would not be significantly different and the cleanliness of the experiment was improved. Furthermore, as bones were degreased the results would be the same. The time periods (2, 4, and 6 h) were chosen as it is known that stews are cooked in water for *ca*. 2 h, for instance “rabo de toro”, but tendons require 4 h. Tendons may be eaten or used to construct bows, as Trojans did. A sample treated for 6 h is an extreme that has no reference in the everyday life; still, as already mentioned, German Emperor Lothar I was boiled for 6 h to deflesh the corpse (Bada et al., 1989).

2.2. Boiling procedure

The bone samples were weighed, and, then, boiled in water for 2, 4, or 6 h. This means that each sample was at *ca*. 100 °C in contact with water. In this way the temperature and treatment time complete our previous work (Bosch et al., 2011). The samples were individually boiled in an open precipitation glass in order to avoid the use of metal autoclaves or closed metal pots to prevent contamination. To control as many parameters as possible, samples 131 were boiled in distilled water, Milli-Q, to eliminate ionic exchanges with impurities. They were, thus, the reference for samples 137 which were boiled in seawater (100.56 °C) from the Canary Island coast, Spain. The boiling temperature of water with salt increases 0.5 °C per mol of salt in 1 L of water. The samples were left at ambient temperature in an extraction bell for 72 h and then weighed again. The waste water was collected to analyze it by atomic absorption.

2.3. Sample degreasing

After boiling, the materials were all frozen for 2 days at −14 °C, they were then defrosted at ambient temperature for 2 h and immersed in trichoroethylene for 11 days to degrease them. The bones were heated at 37 °C for 1 day, in an oven, to dehydrate them before cutting a small piece (*ca*. 1 g) of the central part. Those pieces were milled to obtain a fine powder appropriate for the X-ray diffraction techniques.

Unfortunately the degreasing process has not been normalized and several procedures may be used. For instance bones may be kept in ammonia solution for less than one week followed by bleaching with hydrogen peroxide solution and cleaning with commercial detergent (Hussain et al., 2007). Our procedure is close to the method reported by Dayal et al. (2009) which has been shown to be very effective.

2.4. Characterization techniques

2.4.1. Atomic absorption (AA)

Atomic absorption was used to quantify the calcium and sodium content of waste water after boiling the bone samples. Of course, seawater was also analyzed for comparison purposes. Atomic absorption spectroscopy employs the absorption of optical radiation (light) by free atoms in the gaseous state. Atomic absorption spectroscopy determines over 70 different elements in solution or directly in solid samples. Perkin Elmer model Analyst 700 was used and the wavelengths for Na and Ca were 589 and 422.7 nm, respectively.

2.4.2. Powder X-ray diffraction (XRD)

X-ray diffraction is a conventional analytical method that identifies the crystalline compounds present in the sample, since each compound has a unique diffraction pattern. Compounds may be identified by comparing the experimental pattern with those reported in the cards of the JCPDS (Joint Committee on Powder Diffraction Standards). Only if compounds are crystalline, with a crystallite size larger than *ca*. 3 nm, and if the corresponding contents are higher than *ca*. 3%, peaks are observed; otherwise, a broad and undefined enhancement of the background line is registered. Therefore, in bone, the sharp peaks, present in the diffractograms, correspond to crystalline hydroxyapatite, and the broad background line is due to collagen as collagen is not crystalline. A Bruker-axs D8-advance diffractometer coupled to an X-ray diffraction anode tube was used to obtain the X-ray diffraction patterns which were recorded with a scintillation counter. A nickel filter selected the CuKα radiation.

2.4.3. Scanning electron microscopy/Energy dispersive X-ray spectroscopy (SEM/EDS)

In scanning electron microscopy, an electron beam is focused on the sample in a vacuum environment. The information is local and corresponds to a selected fraction of the bone. From the micrograph, it is possible to determine the morphology of the various compounds that constitute bone but it is not possible to identify the compounds unless their morphology is known. Note that in SEM micrographs, the observed objects are in the micron range, whereas in XRD the patterns are due to the atomic structure of the full sample, *i.e*. to the arrangement of atoms in space and the results correspond to the bulk of the sample. In the microscope, the elemental identification provided by the EDS probe is very useful. When the sample is homogeneous, one EDS analysis is representative, instead, if the sample is inhomogeneous an EDS analysis of each zone has to be performed. Elements with an atomic weight lower than fluorine are not detected by EDS. It has to be emphasized that carbon peak is due to the sample as well as to the layer sputtered on the sample to guarantee conductivity. For SEM/EDS studies, a Leica Cambridge microscope Stereoscan 440, with software Leica Optics Electron was used. Again, the bone splinters were sputtered with carbon to avoid charge problems. The samples were studied at 2000, 5000 and 10,000 magnifications.

3. Results

3.1. Atomic absorption

The calcium and sodium content of the waste water collected after boiling was determined. Table 1 shows the differences in waste water composition between bones boiled in distilled water and seawater. For comparison purposes the original seawater was also studied, it contains 12,250 $\pm$ 0.47 and 420.71 $\pm$ 1.04 mg/L of sodium and calcium respectively (molar ratio Na:Ca = 29.11). Water in Granada (Spain) contains 180–250 mg of $CaCO_3$/L, *i.e*. 64–92 mg of Ca/L.

Table 1
Sodium and calcium amounts found in waste waters. Seawater initially contained 12,250.0 $\pm$ 0.47 and 420.71 $\pm$ 1.04 mg/L of sodium and calcium respectively (molar ratio Na:Ca = 50.65). Water in Granada contains 64–92 mg/L.

Boiling time (hours)	Waste water from boiling in seawater		Waste water from boiling in distilled water	
	Sodium (mg/L)	Calcium (mg/L)	Sodium (mg/L)	Calcium (mg/L)
2	22,680 $\pm$ 1070	887.13 $\pm$ 5.21	149.83 $\pm$ 7.45	37.180 $\pm$ 0.58
4	34,150 $\pm$ 1770	1262.4 $\pm$ 6.67	148.66 $\pm$ 11.45	29.610 $\pm$ 0.13
6	41,430 $\pm$ 1090	1168.0 $\pm$ 9.82	121.18 $\pm$ 14.38	21.740 $\pm$ 0.27

Samples boiled in distilled water leach calcium and sodium depending on boiling time; after 6 h (sample 131) the waste water contains up to 121.18 mg of sodium/L and 21.740 mg of calcium/L. The corresponding Na:Ca molar ratios are 7.04, 8.73 and 9.69 after 2, 4 and 6 h. Sodium contained in bone is more easily released than calcium.

Instead, if bone samples are boiled in seawater whose molar ratio Na:Ca is already 50.65, the Na:Ca molar ratios turn out to be 44.47, 47.04 and 61.71 for 2, 4 and 6 h. Furthermore, after a boiling time of 6 h, the amount of calcium dissolved per liter of seawater (1168.0 mg/L) is significantly higher than the amount of calcium dissolved by distilled water (21.74 mg/L) even if seawater already contained 420 mg/L. The comparison of the molar ratios Na:Ca in both, distilled and salt waste waters, shows that sodium leaching per calcium atom increases with boiling time. However, if the water is seawater after 2 h the Na:Ca in waste water is lower than in the original seawater showing that sodium ions are incorporated into the bone structure which releases calcium.

3.2. X-ray diffraction

Fig. 1 compares the X-ray diffractograms of bones boiled in distilled water (patterns a, b, and c). The peaks correspond to hydroxyapatite, $Ca_5(PO_4)_3OH$ (JCPDS card 9-432), and the large bump from 5 to 50° (2θ) has to be attributed to non-crystalline compounds, most probably collagen. The amount of amorphous compound (collagen) diminishes as boiling time increases.

In the samples boiled in seawater (patterns d, e, and f), no other crystalline compounds than hydroxyapatite and halite (NaCl) appear. Halite (NaCl) peaks are observed in the 4 and 6 h boiled samples at $2\theta = 45.5°$ and 56.5°. The amorphous percentage, which initially is high, again, diminishes with boiling time as the peaks due to crystalline material sharpen. If the corresponding diffractograms of bones boiled in distilled and salt water are compared, in samples boiled in seawater the crystallinity seems to increase as peaks become sharper and the percentage of amorphous material decreases.

3.3. Scanning electron microscopy

The images obtained by scanning electron microscopy are shown in Fig. 2. The sequence a, b, and c compares the bones treated in distilled water; the bone surface becomes smoother with increasing boiling time as it has been already reported (Bosch et al., 2011). The series d, e, and f, corresponds to bones treated in seawater. Fig. 2d shows the typical morphology of bone and two faceted crystalline particles which must be halite, they are *ca*. 4 μm. As boiling time is increased the surface becomes smoother, Fig. 2e, porosity is definitely obstructed.

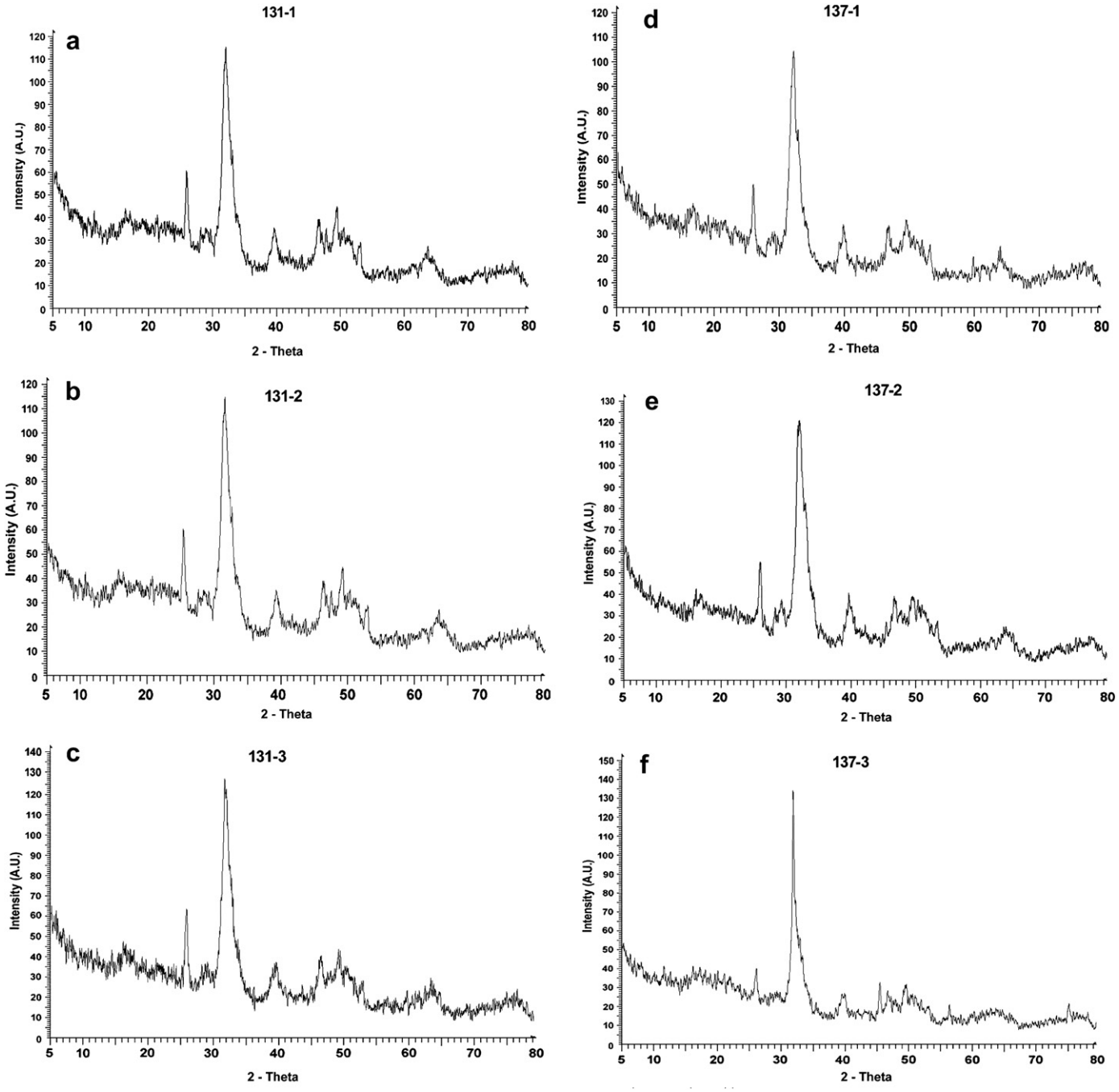

Fig. 1. X-ray diffraction patterns of the bone samples boiled in distilled water for 2, 4 and 6 h (a, b and c, respectively) and in seawater for 2, 4 and 6 h (c, d and f, respectively). The sharp peaks correspond to hydroxyapatite which remains.

However, when samples treated in seawater for 2 or 4 h were studied at a higher magnification, it was discovered that some zones close to pore mouths present a filamentous morphology, Fig. 3; fibrils seem to pour out of the pores. Such features were not found in samples treated with distilled water neither in the sample treated in seawater for 6 h. The diameter of the fibrils is *ca.* 0.1 μm and their length may reach 5 μm, they are non-crystalline, cross-linked, and very long.

3.4. Energy dispersive spectroscopy

Although EDS associated to the scanning electron microscopy is a surface technique, the results correspond to a depth of around 3 μm; the analysis is local and only detects correctly elements with an atomic mass higher than 16 g/mol. The only elements found in the most representative zone of bone samples boiled in distilled water were calcium and phosphorus; instead, in samples boiled in seawater chlorine and sodium were present, as expected. In Table 2, molar compositions are presented. In distilled water more calcium was lost as boiling time was increased. This result is in agreement with the atomic absorption analysis of the waste waters. The boiling process extracts calcium from the sample. Such conclusion is confirmed by the results obtained for samples treated in seawater.

When the electron beam was focused on the fibril rich zones the obtained spectrum, Fig. 4, shows that the corresponding

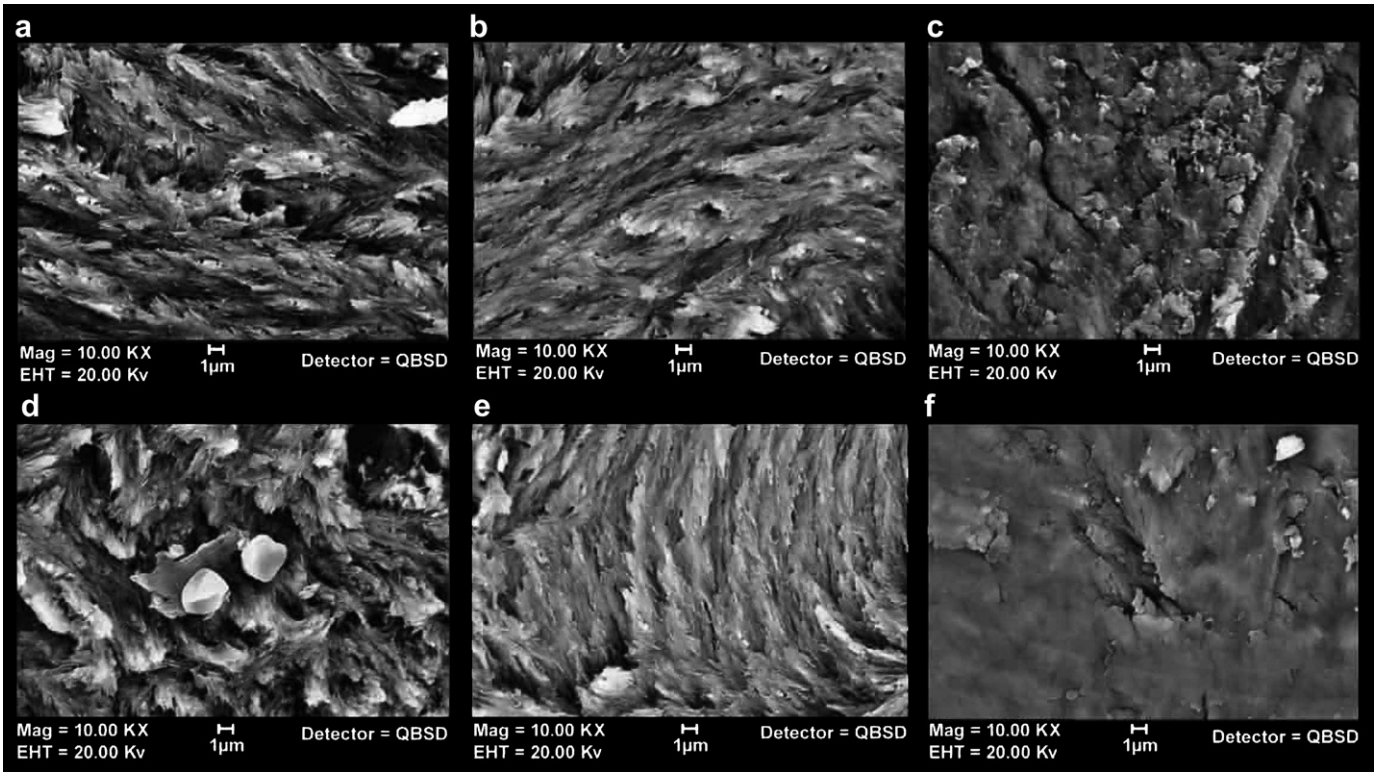

Fig. 2. SEM images obtained at a magnification of ×10,000. They correspond to the bone samples boiled in distilled water for 2, 4 and 6 h (a, b and c, respectively) and in seawater for 2, 4 and 6 h (c, d and f, respectively). As boiling time increases the surface becomes smoother. However, the morphology of bones boiled in distilled or salt water is different; in the samples boiled in salt water filaments appear.

composition is similar to bone composition. Only phosphorus (36.01 at.%), calcium (55.96 at.%), sodium (3.30 at.%), and chlorine (4.74 at.%) are identified. The difference is that they contain more phosphorus and less calcium.

4. Discussion

4.1. Main results

Our results show that no other crystalline compounds than hydroxyapatite are formed in bones boiled in distilled water or in seawater, only some crystals of halite are found in the later case. Given the drying procedure, the formation of such crystals is not surprising. Indeed, after boiling the wet samples were left at ambient temperature in an extraction bell for 72 h. In both sets of samples, the smoothness of the bone surface depends on boiling time, but filaments are only observed in bones boiled in seawater. Therefore, it is possible to distinguish bone that was boiled in salt water versus bone boiled in fresh water within 4 h of boiling. However, after 6 h the differences fade out. In the present work the Ca:P values do not correspond to those expected. Hence, the three main points to be discussed are: a) bone surface morphology, b) Ca:P ratio and c) formation of fibrils. A last paragraph on archaeological context is presented.

4.2. Bone surface

The bone surface is progressively smoothed with boiling time until the crusts already reported (Bosch et al., 2011) appear on the surface after 6 h. In the present samples the layer is continuous, but in the previously cited work it appears as islands, such difference depends on the collagen content of the bone which may vary with age or disease (Surovell and Stiner, 2001). Furthermore, it depends on the composition of the broth as in the salted material this effect seems to be more pronounced; thick layers cover the rather inhomogeneous morphology of bone surface. Then, these results are in agreement with our previous experiments, *i.e.* with boiling time the bone surface is smoothed as degraded collagen deposits on the top. However, with seawater, a fibrillar morphology was observed in samples boiled for 2 or 4 h. These features will be discussed in a paragraph centered on that point.

4.3. Ca:P ratio

The molar ratio Ca:P, determined by EDS, diminishes with boiling time in both groups of bones either boiled in distilled water (1.83 for 2 h, 1.59 for 4 h, and 1.54 for 6 h) or in seawater (1.80 for 2 h, 1.77 for 4 h, and 1.66 for 6 h). The diminution of Ca:P ratio is higher in distilled water, *i.e.* that more calcium per atom of phosphorus is lost. In our previous study (Bosch et al., 2011) it was found that Ca:P ratio increased with boiling time, from 1.11 to *ca.* 1.61. This contradiction can be solved considering the X-ray diffraction results. X-ray diffractograms correspond, in the present work, to hydroxyapatite, $Ca_5(PO_4)_3OH$, whose molar ratio Ca:P is 5:3 = 1.66; instead, the crystalline compound found in the study by Bosch et al. (2011) was highly hydrolyzed, $3Ca_3(PO_4)_3.Ca(OH)_2$, molar ratio Ca:P = 9:10 = 0.90. The difference may be understood if the solubility products of hydroxyapatite and calcium hydroxyde are compared. The solubility product of $Ca(OH)_2$ is 9×10^{-6} which is much higher than the solubility product of hydroxyapatite (2.34×10^{-59}). Low values of the solubility product correspond to poorly soluble compounds. Therefore, the $Ca(OH)_2$ is initially solved producing a high concentration of Ca in the solution and, only then, some hydroxyapatite is ionized. The total concentration of calcium

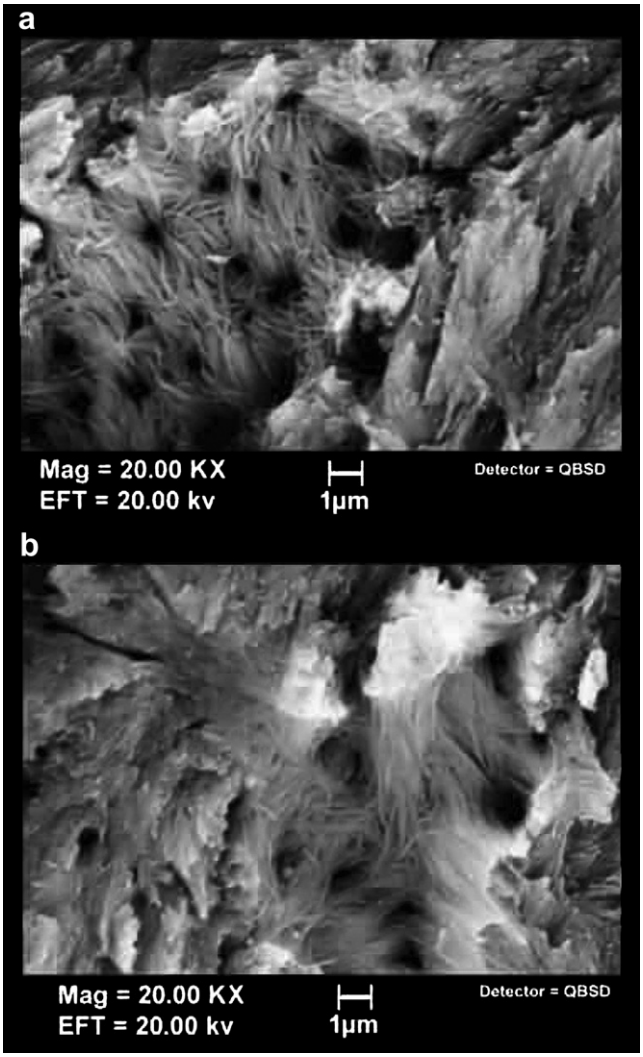

Fig. 3. SEM images obtained at a magnification of ×20,000. They correspond to the bone samples boiled in seawater for 2 and 4 (a and b, respectively). The filaments pour out from pores and cracks.

in the solution would be the concentration from the dissolution of calcium hydroxyde added to the concentration of calcium coming from hydroxyapatite. The only phosphorus source is, of course, hydroxyapatite. Therefore, the Ca:P should be very high. Instead, in the samples of the present study, there is no contribution of calcium coming from calcium hydroxyde. Calcium and phosphorus both come from non hydroxylated hydroxyapatite, the Ca:P value is therefore low.

After a boiling time of 6 h, all experiments reach a Ca:P value close to 1.66 which is the value of pure hydroxyapatite. It is clear, then, that, hydroxylated or not, the boiled bone tends to equilibrium where the composition of hydroxyapatite is "ideal", Table 3. Remember that biological hydroxyapatite is not compositionally pure (non-stoichiometric), often being calcium deficient. It may be enriched in CO_3^{2-} which replaces PO_4^{3-} ions in various lattice sites. NaCl is known to strongly suppress hydroxyapatite dissolution kinetics, including at physiological ion concentrations (Kwon et al., 2009). The mechanism of this process involves selective dissolution of the smallest and less crystalline apatite crystals, which precipitate again as more crystalline and thermodynamically stable forms (Surovell and Stiner, 2001; Wess et al., 2002). Therefore, biological hydroxyapatite may present different degrees of hydroxylation but with boiling time or diagenesis phenomena it tends to lose collagen and reach a Ca:P value close to 1.66.

Table 2
Elemental analyses by EDS, atomic percentage.

Boiling time (hours)	Bone boiled in distilled water (samples 131)		Bone boiled in salted water (samples 137)			
	Phosphorus	Calcium	Phosphorus	Calcium	Sodium	Chlorine
2	35.31	64.69	33.09	59.57	3.03	4.32
4	38.48	61.52	33.74	59.76	3.05	3.45
6	39.23	60.77	34.35	57.37	4.46	3.82

4.4. Fibrils

X-ray diffraction shows that no other crystalline compounds than hydroxyapatite are formed. EDS measurements conclude that the elemental composition of the fibrils corresponds to the elemental composition of bone. Hence, fibrils are constituted by collagen and hydroxyapatite; bone is a hierarchically composite material whose basic building block is the mineralized collagen fibril (Fratzl et al., 2004). The mineralized collagen fibril is about 100 nm in diameter which corresponds to the size of the observed fibers.

Collagen fractions may be obtained by extraction with water–salt solutions containing 1–10% sodium chloride (Neklyudov et al., 2003). The values of the effective constant of extraction rate vary with pH. However, no mechanism of extraction is proposed by Neklyudov et al. (2003). From the reports of Eglin et al. (2005), and Hellauer and Winkler (1975), it seems that Cl^- modifies the order of water bound to the polypeptide chains and alters the electrostatic interactions between collagen fibrils.

The presence of the collagen fibrils on the bone surface suggests that the fibrils solved in seawater were deposited on bone surface with time as salt crystals do. A second mechanism could be that the collagen fibrils diffuse through the large channels or cracks present in the bone, driven by a temperature gradient. The distribution in which the collagen fibers appear in the SEM micrographs discards the first proposition. Therefore, sodium chloride favors the collagen extraction through a diffusion process. The resulting materials should not present the same mechanical properties as unboiled bone, boiled bone in fresh water or raw bone.

4.5. Archaeological context

In the past, bone was generally available to the domestic producer as discarded subsistence remains. Such point is crucial as most probably these remains were cooked, *i.e.* treated at a temperature close to 100 °C for 2 or 3 h in presence of water when meat with bone were boiled; when they were grilled, the temperature reached by bone, determined by the great amount of water present in fresh meat, is as well 100 °C. The domestic production of bone artifacts, tools or decorative objects had, then, as starting material, a boiled bone with a low salt content. Unfortunately, most studies on bone technology describe the work made to manufacture artifacts, but they do not describe the starting materials (D'Errico and Henshilwood, 2007; Emery, 2009; Rosell et al., 2011). Furthermore, the bones are studied with the scanning electron microscope at low magnifications searching cut marks, but not at high magnifications as required to determine the bone micrometrical morphology. As an example (D'Errico and Henshilwood, 2007), an analysis of 28 bone tools from Southern Africa (Middle Stone Age) showed that 25 were awls made on long-bone shaft fragments and further manufactured by scraping, which

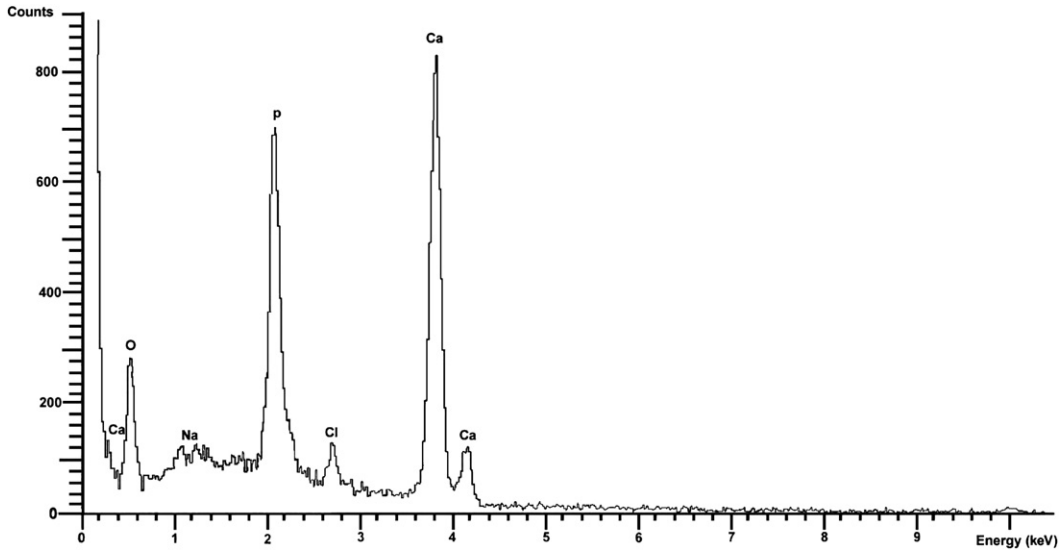

Fig. 4. EDS analysis of the filaments present in Fig. 3a. Their composition does not reveal any elements not present in bone.

Table 3
Ca:P ratio of boiled bones in distilled and seawater, remember that in pure hydroxyapatite Ca:P = 1.66.

Boiling time (hours)	Molar Ca:P previous work	Molar Ca:P bone boiled in distilled water	Molar Ca:P bone boiled in seawater
Unboiled	1.097	–	–
2	1.118	1.83	1.80
	1.068		
4	1.166	1.59	1.77
6	2.61	1.54	1.66
	1.61		

were then used to pierce soft material such as leather or pierce shells to make beads. Another example, from another period and culture, is the one discussed by Emery (2009) who studied Maya bones. Emery (2009) distinguishes between the domestic and the non domestic producers of bone artifacts as the domestic producer uses a large variety of species and skeletal elements, and the diversity of bone raw materials is as great as the diversity of discarded subsistence remains. Instead, it is explained that the non domestic producer used raw materials highly uniform.

The present results show that it is possible to determine if the starting materials were boiled or not, in salt or fresh water, *i.e.* if they were the result of the "recycling of some cooked garbage" or if they were prepared to be manufactured boiling them in seawater. When bone is boiled in salt water it is more resistant to aging. It is not surprising then that tools recovered from sea or from drowned worlds are always said to be immaculately preserved (Arnaud et al., 1978).

5. Conclusion

Boiling human bones in sea or fresh water may be a model experiment to simulate diagenesis phenomena. The comparison of materials boiled in sea or fresh water shows that the presence of ions as chlorine promotes the diffusion of collagen. When bone is boiled for 2 or 4 h the collagen fibrils appear at the surface of the bone emerging from cracks and channels. But, when bones are boiled for 6 h in sea or fresh water, hydroxyapatite composition and surface morphology are similar: Ca:P = 1.66 and a smooth surface.

The characterization techniques show that boiled human bones can be distinguished from unboiled bones. The effect of seawater is clear if the bone is boiled for 2 or 4 h as fibers of collagen appear on bone surface, but, if bone is boiled for 6 h, the differences with fresh water fade out.

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References

Arnaud, G., Arnaud, S., Ascenzi, A., Bonucci, E., Graziani, G., 1978. On the problem of the preservation of human bone in sea-water. Journal of Human Evolution 7, 409–414.
Bada, J.L., Herrmann, B., Payan, I.L., Man, E.H., 1989. Amino acid racemization in bone and the bowling of the German Emperor Lothar I. Applied Geochemistry 4, 325–327.
Bosch, P., Alemán, I., Moreno-Castilla, C., Botella, M., 2011. Boiled versus unboiled, a study on neolithic and contemporary human bones. Journal of Archaeological Science 38, 2561–2570.
Cáceres, I., Lozano, M., Saladié, P., 2007. Evidence for bronze age cannibalism in El Mirador Cave (Sierra de Atapuerca, Burgos, Spain). American Journal of Physical Anthropology 133, 899–917.
Dayal, M.R., Kegley, A.D.T., Strkalj, G., Bidmos, M.A., Kaykendall, K.L., 2009. The history and composition of the Raymond A. Dart collection. American Journal of Physical Anthropology 140, 324–335.
D'Errico, F., Henshilwood, C.S., 2007. Additional evidence for bone technology in the southern African Middle Stone Age. Journal of Human Evolution 52, 142–163.
Eglin, D., Coradin, T., Giraud Guille, M.M., Helary, C., Livage, J., 2005. Collagen-silica hybrid materials: sodium silicate and sodium chloride effects on type I collagen fibrillogenesis. Bio-Medical Materials and Engineering 15, 43–50.
El Feki, H., Savariault, J.M., Ben Salah, A., 1999. Structure refinements by the Rietveld method of partially substituted hydroxyapatite: $Ca_9Na_{0.5}(PO_4)_{4.5}(CO_3)_{1.5}(OH)_2$. Journal of Alloys and Compounds 287, 114–120.
Emery, K.F., 2009. Perspectives on ancient Maya bone crafting from a Classic period bone-artifact manufacturing assemblage. Journal of Anthropological Archaeology 28, 458–470.
Fratzl, P., Gupta, H.S., Paschalis, E.P., Roschger, P., 2004. Structure and mechanical quality of the collagen-mineral nano-composite in bone. Journal of Materials Chemistry 14, 2115–2123.
Hellauer, H., Winkler, R., 1975. Denaturation of collagen fibers in NaI, NaCl and water of different pH values as studied by differential scanning calorimetric measurements. Connective Tissue Research 3, 221–230.

Hurlbut, S.A., 2000. The taphonomy of cannibalism: a review of anthropogenic bone modification in the American southwest. International Journal of Osteoarchaeology 10, 4–26.
Hussain, M., Hussain, N., Zainab, H., Qaiser, S., 2007. Skeletal preservation techniques to enhance veterinary anatomy teaching. International Journal of Agro Veterinary and Medical Sciences 1, 21–23.
Jiménez Brobeil, S.A., 1990. Rituales funerarios neolíticos en la alta Andalucía. Estado actual de la cuestión. Zephyrus XLIII, 125–130.
Kalvoda, J., Novák, M., Bábek, O., Brzobohatý, R., Holá, M., Holoubek, I., Kanický, V., Skoda, R., 2009. Compositional changes in fish scale hydroxylapatite during early diagenesis; an example from an abandoned meander. Biogeochemistry 94, 197–215.
Kangsanant, S., Vittayanont, M., Tongraung, C., 2008. Effect of NaCl on texture modification of cuttlefish mantle (*Sepia brevimana*). Songklanakarin Journal of Science and Technology 30, 11–17.
Kwon, K.-Y., Wang, E., Chung, A., Chang, N., Lee, S.-W., 2009. Effect of salinity on hydroxyapatite dissolution studied by atomic force microscopy. Journal of Physical Chemistry C 113, 3360–3372.
Lozano, L.F., Peña-Rico, M.A., Heredia, A., Ocotlán-Flores, J., Gómez-Cortés, A., Velázquez, R., Belío, I.A., Bucio, L., 2003. Thermal analysis study of human bone. Journal of Materials Science 38, 4777–4782.
Malgosa, A., Piga, G., Mazzarello, V., Bandiera, P., Enzo, S., 2008. Estudio de cremaciones españolas a través del análisis de difracción de rayos X (XRD). In: Nieto Amada, J.L., Obón Nogués, J.A., Baena Pinilla, S. (Eds.), Genes, ambiente y enfermedades en poblaciones humanas. Prensas Universitarias de Zaragoza, Zaragoza, Spain, pp. 331–340.
Neklyudov, A.D., Berdutina, A.V., Ivankin, A.N., Mitaleva, S.I., Evstaf'eva, E.A., 2003. Collagen fractions obtained by water–salt extraction from raw materials of animal origin. Applied Biochemistry and Microbiology 39, 426–430.
Pijoan, C.Ma., Mansilla, J., Leboreiro, I., Lara, V.H., Bosch, P., 2007. Thermal alterations in archaeological bones. Archaeometry 49, 713–717.
Roberts, S.J., Smith, C.I., Millard, A., Collins, M.J., 2002. The taphonomy of cooked bone: characterizing boiling and its physico-chemical effects. Archaeometry 44, 485–494.
Rogers, K.D., Daniels, P., 2002. An X-ray diffraction study of the effects of heat treatment on bone mineral microstructure. Biomaterials 23, 2577–2585.
Rosell, J., Blasco, R., Campeny, G., Díez, J.C., Alcalde, R.A., Menéndez, L., Arsuaga, J.L., Bermúdez de Castro, J.M., Carbonell, E., 2011. Bone as a technological raw material at the Gran Dolina site (Sierra de Atapuerca, Burgos). Journal of Human Evolution 61, 125–131.
Surovell, T.A., Stiner, M.C., 2001. Standardizing infra-red measures of bone mineral crystallinity: an experimental approach. Journal of Archaeological Science 28, 633–642.
Wess, T., Alberts, I., Hiller, J., Drakopoulos, M., Chamberlain, A.T., Collins, M., 2002. Microfocus small angle X-ray scattering reveals structural features in archaeological bone samples; detection of changes in bone mineral habit. Calcified Tissue International 70, 103–110.
White, E.M., Hannus, L.A., 1983. Chemical weathering of bone in archaeological soils. American Antiquity 48, 316–322.