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Cooked Bones? Method and Practice for Identifying Bones Treated at Low Temperature

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ABSTRACT Is it possible to determine low-temperature cooking in archaeological bones? The indirect exposure of bones to fire at low temperature (≤ 100 °C), linked to cooking, produces macroscopic modifications on these bones. These modifications have not been clearly or systematically described previously. Instead, physicochemical changes at nanometric level are only now beginning to be understood. In this paper, our principle aim is to explore new methods and techniques that correlate macroscopic features such as smoothness or light transparency with physicochemical characterization results that could aid towards detecting cooked bones in the archaeological record.

This study then selected 11 archaeological samples, both human and non-human. Bones were considered to be thermally treated or not, on the basis of macroscopic criteria. Complementary characterization techniques were used to study morphology (scanning electron microscopy and small angle X-ray scattering), structure (X-ray diffraction and transmission electron microscopy), local composition (energy-dispersive X-ray spectroscopy) and texture (gas adsorption). Indeed, fractal dimension, particle size, crystalline percentage or specific surface area may well explain some of the macroscopically observed modifications on these samples. The possibility that such apparent modifications may also be due to diagenesis is also considered.

From an archaeological point of view, the results are promising. Our characterization of human and non-human bones demonstrates that physicochemical techniques are complementary and provide good criteria against which to distinguish boiled from un-boiled archaeological samples. Copyright © 2013 John Wiley & Sons, Ltd.

Key words: archaeological bones; BET; cooked bones; EDS; indirect low-temperature exposure; SAXS; SEM; TEM

Introduction

Bone tissue is a complex material composed of organic proteins, *ca*. 30%, (mainly collagen type I) and a mineral compound (hydroxyapatite). Bone structure may be modified by natural or cultural agents, e.g. natural factors such as taphonomy and diagenesis (Lyman, 1994, 2002) and cultural agents including mortuary practices, worked bone, trophy taking, etc., (Duday *et al*., 1996; Cid & Romano, 1997; Turner & Turner, 1999; Hurlbut, 2000; Le Mort, 2003; Chacon & Dye, 2007; Emery, 2009). Among the multiple types of intentional anthropogenic use of bone, we can include cut marks, fractures, thermal alterations or human tooth marks (Shipman *et al*., 1984; Guillon, 1987; Bada *et al*., 1989; White, 1992; Etxeberria, 1994; Pearce & Luff, 1994; Botella *et al*., 2000; Degusta, 2000; Hurlbut, 2000; Caceres *et al*., 2007; Landt, 2007; Malgosa *et al*., 2008). In archaeology, the recognition of these is crucial as they provide data on the varied cultural practices of human groups.

Thus, identification of cooking techniques provides us with a better understanding of food processing and feeding practices, including boiling for marrow

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extraction and other intensive techniques of animal exploitation (Leechman, 1951; Montón Subías, 2002; Church & Lyman, 2003). This focus turns out to be crucial when we take into consideration the use of the associated artifacts and spaces required for these cooking activities in relation to ceramic, or indeed, pre-ceramic human groups. Identification of cooking techniques is also possibly a useful proxy in better understanding the manufacture of certain bone tools, and ornamental or symbolic objects (Pijoan *et al.*, 1989; Cid & Romano, 1997; Botella *et al.*, 2000; Solari, 2008; Emery, 2009; Rosell *et al.*, 2011).

Furthermore, the identification of cooked human bones opens interesting new lines of inquiry into new behavioral interpretations pertaining to death, including intentional de-fleshing in funerary rituals or cannibalism (Bada *et al.*, 1989; White, 1992; Turner & Turner, 1999; Botella *et al.*, 2000; Degusta, 2000; Hurlbut, 2000; Oestigaard, 2000; Pijoan *et al.*, 2007). Additionally, the correct identification of boiled bones is important in establishing the taphonomic agents, cultural or natural, that are responsible for site formation processes. The possibility of diagenesis cannot be discarded as it could mimic cooked alterations in bone tissue (Hurlbut, 2000; Roberts *et al.*, 2002; Koon *et al.*, 2003, 2010; Pijoan *et al.*, 2007; Bosch *et al.*, 2011; Trujillo-Mederos *et al.*, 2012).

Cooking for consumption is associated with two different types of processes of indirect fire exposure at low temperatures: (i) grilling, i.e. the bone is protected from direct exposure to fire by soft tissues, and (ii) boiling, i.e. bone with or without soft tissues is heated in water (temperature $\leq 100\,^{\circ}C$) (Botella *et al.*, 2000; Roberts *et al.*, 2002).

We should state that burning, where the bone is in direct contact with fire or an intense heat source (Botella *et al.*, 2000; Roberts *et al.*, 2002), is not the focus of this study. This is because burnt bones are usually related to cremation/incineration as part of funerary practices or are ascribed to another type of cultural practice not necessarily linked directly with food processing and cooking, but rather to food garbage disposal and to the use of bone as auxiliary fuel, given that its high grease content can aid in maintaining hearths (Théry-Parisot, 2002; Costamagno *et al.*, 2005; Yravedra *et al.*, 2005). Different experiments have analyzed the effects that direct fire exposure at high temperatures has on bones (Herrmann, 1977; Shipman *et al.*, 1984; Guillon, 1987; Buikstra & Swegle, 1989; Etxeberria, 1994; Stiner *et al.*, 1995; Pijoan *et al.*, 2007; Malgosa *et al.*, 2008); however, most of those changes are not important in identifying cooking, since they only occur at temperatures much higher than bones reach during normal cooking temperatures. The majority of these papers usually only study changes in cremated or incinerated bones, rather than in bones exposed to low temperatures probably destined for consumption or other cultural practices.

In previous studies, Pedro Bosch, a member of our team, established some structural and morphological differences between un-boiled and boiled bones (Pijoan *et al.*, 2007; Bosch *et al.*, 2011; Trujillo-Mederos *et al.*, 2012), from contemporary and archaeological contexts. Archaeological samples (with no thermal modification, direct and indirect thermal exposure) were compared against contemporary human bone samples boiled at different time intervals (2, 4 or 6 h) in fresh water and seawater and characterized through recourse to physicochemical techniques such as scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDS), X-ray diffraction (XRD), gas adsorption (Brunauer–Emmett–Teller (BET)), small-angle X-ray scattering (SAXS) and atomic absorption. These papers serve to inform the study at hand.

We believe that given the results obtained in these previous studies (Pijoan *et al.*, 2007; Bosch *et al.*, 2011; Trujillo-Mederos *et al.*, 2012), the focus should be on applying these methods to archaeological samples including non-human bones. Therefore, this paper will correlate macroscopic changes to nanometric parameters in low-temperature thermally treated bones through indirect exposure to fire. As such, bone modification can only be understood through multi-technique characterization or via model experiments (Martin, 1984; Roberts *et al.*, 2002; Koon *et al.*, 2003, 2010; Pijoan *et al.*, 2007; Bosch *et al.*, 2011; Trujillo-Mederos *et al.*, 2012); in this paper, our objective is to test new methods and techniques comparing and contrasting the results obtained using SEM, SAXS, XRD, transmission electron microscopy (TEM), EDS and gas adsorption (BET) in the characterization of boiled and un-boiled human and non-human archaeological bones.

Materials and methods

Bone samples

All bone samples, human or non-human, came from archaeological sites in Northwestern Argentina. We chose six non-human samples (bone fragments of *Camelidae*, M1 to M6) from the Casa Chávez Montículos archaeological site and five human samples (skulls fragments, M7 to M11) from the La Rinconada archaeological site (Figures 1 and 2, Tables 1 and 2).

Figure 1. Macroscopic characteristics as observed in non-human samples 1 to 6, according to observational criteria by Botella *et al*. (2000) and Pijoan *et al*. (2004).

Casa Chavez Montículos site is located roughly 1.7 to 2 km south of the modern-day town of Antofagasta de la Sierra (Puna of Atacama, Catamarca Province, Argentina), on the left bank of the Antofagasta Punilla River (26° 5′ Lat S - 67° 25′ Long W).

The site consisted of a group of some ten mound-structures of varying heights, which were distributed

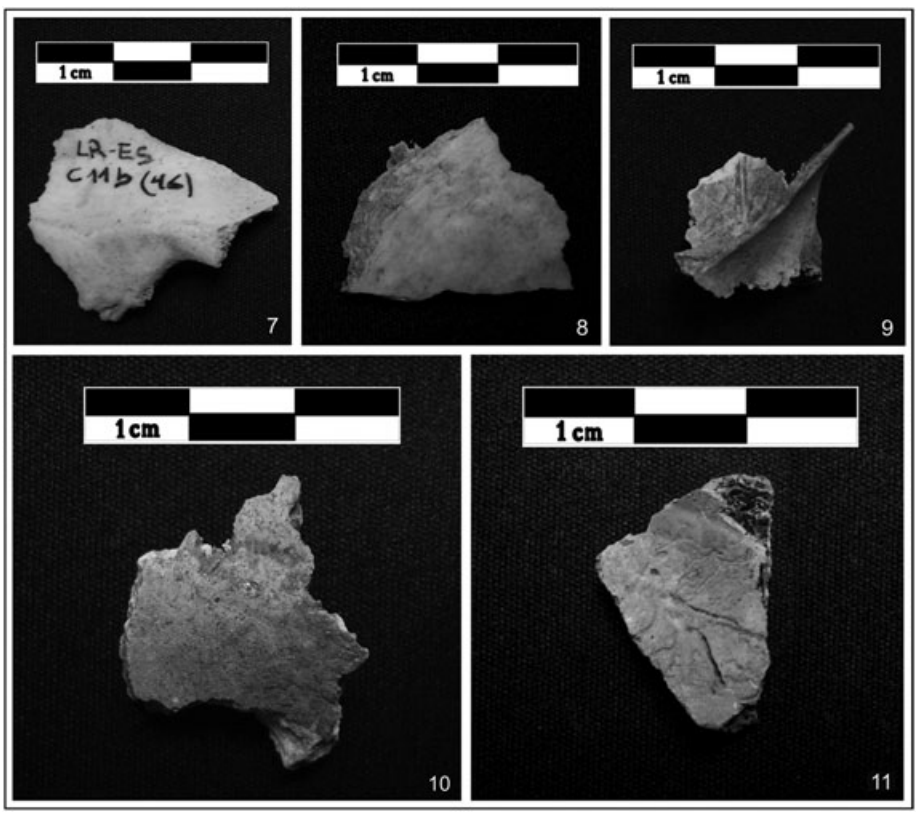

Figure 2. Macroscopic characteristics as observed in human samples 7 to 11, according to observational criteria by Botella *et al*. (2000) and Pijoan *et al*. (2004).

Table 1. Non-human samples from 'Casa Chávez' (Northwestern Argentina, 2400–1500 B.C.)

Sample label	Stratigraphical location	Species	Bone	Thermal treatment (based on macroscopic observation)
M1	X28Y30A-Level 3	*Camelidae*	Jawbone	Boiled
M2	X26Y31-Level 3	*Camelidae*	Metapodium	Boiled
M3	X28Y30-Level 4	*Camelidae*	Humerus	Boiled
M4	X28Y31B-Level 7	*Camelidae*	Metapodium	Probably un-boiled
M5	X28Y31A-Level 8	*Camelidae*	Femur	Probably un-boiled
M6	X27Y31B-Level 9	*Camelidae*	Scapula	Boiled

into two groups located on higher terrain surrounding a depression. The archaeological material uncovered was stratified within the mounds, but not in the spaces between the mounds or in the central depression.

Detailed study revealed that the mounds were mostly artificial augmenting pre-existing low natural elevations. The excavation profiles revealed certain homogeneity within the sedimentary matrix. In this, sand predominated together with smaller quantities of silt, clay and pebble of varied granulometry (small and medium in general). We observed a variable level of organic matter, the product of anthropogenic activities.

The site has been identified as a 'multiple activity residential base' (Olivera & Podesta, 1995), and the remains of different types of structures and features were recorded such as living space, waste areas, buried structures, hearths, lithic production zones, evidence for pottery manufacture, camelid processing and consumption areas, and artifacts related to agricultural tasks. Radiocarbon dates indicate occupation between *ca*. 2140 and 1320 years BP. The economic model underpinning the site was defined as pastoralism with ancillary agriculture. Camelids, both wild (*Vicugna vicugna*) and domestic (*Lama glama*), were the most important protein resource in the people's diet (Olivera, 1997; Olivera & Grant, 2009).

The stratigraphy showed that site occupation was discontinuous rather than constant. An important episode of non-occupation of the site occurred between levels V and VI observed in Mound 1 stratigraphy; this period was estimated to have been, on the basis of taphonomic controls, about 15 years (Olivera & Nasti, 1993). Camelid bone samples were selected taking in account these lapses in site occupation so as to evaluate possible differences between the early and later samples. Samples 1, 2 and 3 (Levels III and IV) were associated to primary or secondary waste areas, while samples 4, 5 and 6 (Levels VII, VIII and IX) were from near to or from within habitation areas. In both cases, bones remains were clearly associated with other archaeological materials such as pottery and lithic.

The human bones samples were from La Rinconada in the Ambato Valley (Catamarca Province, Argentina). This archaeological site is part of the settlement system of the area for the period between ca. 600 and 1200 AD belonging to the socio-cultural temporal classification known as the Middle Period or Regional Integration Period (Gordillo, 2007, 2009).

The Northeastern sector of this site was composed of several structures and a large courtyard (E5) covering 550 m2. Excavations revealed that the buried archaeological surface lay 0.80 m beneath the modern level. At this earlier level, we uncovered a variety of different materials (ceramics, bones, stones, etc.), which in turn was covered by an irregular layer of burned wooden logs and branches (this last perhaps from a burned roof). This burnt layer was subsequently overlain by natural post-occupational refuse. In all these deposits, the sediment was a slightly consolidated silty-clay loam interlaced by a few small roots.

Three samples (7, 10 and 11) came from the Northern sector of the courtyard (E5), where a set of human bones were found on the buried surface and associated to ceramic containers, bones of *Camelidae* and other materials. These included a complete jawbone, a skull (without jaw) and a certain number of fragments from other skulls.

Table 2. Human samples from 'La Rinconada' (Northwestern Argentina, 700–1100 A.D.)

Sample label	Stratigraphical location	Species	Bone	Thermal treatment (based on macroscopic observation)
M7	LR-E5-C11b	*Homo sapiens*	Skull	Boiled
M8	LR-E7-C3c	*Homo sapiens*	Skull	Boiled
M9	LR-E7-C3c	*Homo sapiens*	Skull	Un-boiled
M10	LR-E5-C13d	*Homo sapiens*	Skull	Un-boiled
M11	LR-E5-C13b	*Homo sapiens*	Skull	Burnt

These remains had an MNI = 6 (adults and sub-adults, both sex) and showed evidence of having been in contact with fire, including possible cooking as well as intentional cut marks (Gordillo & Solari, 2009).

The remaining samples (8 and 9) come from a nearby structure (E7). On this surface, we uncovered two upper maxillary bones, a jawbone fragment, a broken occipital and small burned fragments of another skull. They correspond to an MNI = 2, male adults (Gordillo, 2007, 2009; Gordillo & Solari, 2009).

The criteria followed for the sample selection were initially predicated on the macroscopical appearance of bones and second on observed evidence of intentional manipulation, such as butchery marks and intentional fractures on non-human bones; or cut marks on these cranial human bones founded on a strikingly non-funerary context.

The samples selected for comparison were classified as un-boiled (normal bone, without any thermal treatment), boiled (indirect fire exposure at low temperature) or burnt (direct fire exposure at high temperature), following the macroscopical criteria established by Botella *et al*. (2000), i.e. bones exposed to fire indirectly and at low temperature, become smoother, denser and had a vitreous appearance. Also, the generalized bone color turned out to be yellowish, conservation is also better, and when knocked against something, the sound emitted is analogous to that of ceramic or dried wood. Furthermore, thin bones appear translucent when exposed to direct light (Figures 1 and 2). Last, spongy tissue becomes harder, and it is hard to destroy it using one's fingers as happens with untreated bones (Pijoan *et al.*, 2004).

The only sample of burnt bone showed all the typical marks of damage and surface color change associated to this type of treatment and which many researchers have alluded to (Herrmann, 1977; Shipman *et al.*, 1984; Guillon, 1987; Buikstra & Swegle, 1989; Etxeberria, 1994; Stiner *et al.*, 1995; Pijoan *et al.*, 2007; Malgosa *et al.*, 2008). As burning was not covered by this study, the burnt sample was only selected as a reference for contrasting purposes. Finally, un-boiled samples presented all the macroscopical characteristics common to untreated bones.

Characterization techniques

Thermally treated or untreated bones may be distinguished according to macroscopic criteria, with these differences having a nanometric origin. Thus, they should be observed utilizing physico-chemical methods of characterization. As the materials studied here are bones, we know that they are composed of hydroxyapatite and collagen. No compound identifications are therefore required; instead, the main problems arising are those related to morphology and texture.

We chose SEM to determine the shape of the particles at a micrometric level. At a nanometric level, TEM and small angle X-ray scattering were the most appropriate. Furthermore, with small angle X-ray scattering, the fractal dimension can be estimated; this parameter has scarcely been used when characterizing archaeological bones. Gas adsorption provides the specific surface area; this was crucial in understanding porosity in bone at the nanometric level.

Nitrogen adsorption (BET)

Specific surface area is one of the more easily measured variables that relate directly to bone texture (Robinson *et al.*, 2003; Kolmas *et al.*, 2007). The specific surface area as determined by gas adsorption in this study, i.e. after a thermal treatment *in vacuo* at 120 °C, is sensitive to thermal alterations and may be correlated to macroscopical observations. In previous studies, it has been noted that bone, treated from room temperature up to 200 °C, loses water; from 200 to 450 °C organic matter decomposes and, from 450 to 650 °C, organic residues are burnt; above 650 °C, only inorganic residues are found (Bigi *et al.*, 1997; Lozano, 2002). However, organic matter is altered when water is used as a medium even at 100 °C (Koon *et al.*, 2003, 2010), yet such modifications cannot be observed using gravimetric thermal analysis.

For pore structure analysis, nitrogen adsorption–desorption isotherms (Rouquerol *et al.*, 1999) were determined. All samples were characterized using N_2 adsorption at 77 K applying the BET equation to the N_2 adsorption isotherm on a sample treatment *in vacuo* at 120 °C. A Micromeritics ASAP 2020 was used to record the isotherms.

SAXS

We determined the fractal dimension and the Guinier radius or radius of gyration from the SAXS curves (Guinier & Fournet, 1955). The SAXS is due to the inhomogeneities present within the sample, whose size is between 1 and 50 nm. This technique is sensitive to the morphology (size and shape) of the inhomogeneities, in this case, the mineral crystals present in bone. Recently renewed interest in this method stems from the possibility of determining the fractal dimension of the scattering objects (Harrison, 1995). The fractal dimension has been used as a useful parameter to

describe connectivity in bone tissue, because it is closely linked to density and roughness.

Fractal dimension should help in determining bone density and gyration radius, sometimes called the Second Moment of Inertia. This is useful as a size parameter independent of the shape of the scattering objects. If the shape is spherical, the radius of the sphere, R, is related to the Guinier radius, R_g, as follows:

$$R = (5/3)^{1/2} R_g$$

Identifying gyration radius provides a parameter related to the size of the scattering objects and is independent of their shape (Guinier & Fournet, 1955). As previously mentioned, gyration radii correspond to the heterogeneities or scattering objects at the origin of SAXS. These scattering objects may be pores, molecules or mineral clusters with a diameter between 0.8 and 40 nm. Cross-striated collagen fibrils, whose diameter is close to 200 nm (Parry & Craig, 1980), are constituted by collagen type I molecules, tropocollagen, which in turn consist of three α chains, about 300 nm in length and 1.4 nm in diameter (Ramachandran & Ramakrishnan, 1976). Each α chain has about 1050 amino-acid residues. Therefore, if the reported radii of gyration correspond to α chains, it is not surprising that with rises in temperature, α chains decrease in size as they fracture or break.

The untreated samples were placed in front of the X-ray beam. A Kratky camera coupled to a copper anode tube was used to measure the SAXS curves. The intensities were recorded with a position sensitive counter.

SEM/EDS

Using SEM, we identified the surface morphology at micrometric level. In SEM (Goldstein *et al.*, 2003), an electron beam is focused on the sample in a vacuum environment. The information obtained 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 the bone, but it is not possible to identify the compounds unless their morphology is known.

Local composition can be estimated using EDS. At the microscopic level, the elemental identification provided by the EDS is very useful. When the sample is homogeneous, one EDS analysis will be representative; whilst if the sample is inhomogeneous, an EDS analysis of each zone will have to be performed. EDS do not detect elements with an atomic weight lower than fluorine. EDS analysis is applicable to a depth of 2 to 3 μm over an area of 1 μm^2.

The samples were sprinkled with gold to ensure the necessary conductivity required to avoid charge problems. For SEM/EDS studies, a JEOL JSM-7600 F (Field Scanning Electron Microscope) coupled to an EDX Oxford INCAX-Act was used. The samples were studied at low (x 2000 and x 5000) and high magnifications (x 10,000).

TEM

In TEM, collagen molecules are packed into long rope-like structures called fibrils. With TEM (Egerton, 2005), most subtle heat-induced changes in collagen at fibril level can be detected, as demonstrated by Koon *et al.* (2003, 2010).

The samples were scattered on a holey carbon film. For TEM analyses, a JEOL model JEM-1200 eV microscope was used at 120 kV.

Results

Porosity and specific surface area

Human samples

Table 3 compares the specific surface area due to micropores, to mesopores and to both[1] . Table 3 also shows that low-temperature treatment decreased the specific surface area and generated some microporosity.

Macroscopically, samples M7 and M8 seem to have been boiled whereas samples M9 and M10 were labeled as un-boiled; sample M11 was burnt. The specific surface area (BET method) of un-boiled samples (62, 94.7 m^2/g) is higher than the specific surface area of boiled samples (41.6 and 43.8 m^2/g). Furthermore, the boiling process creates a small amount of micropores (Smicrop = 3 and 4 m^2/g). Sample M11 presents a high specific surface area (67.6 m^2/g). It would seem that the boiling process blocks nitrogen access to the samples pores. It is likely that collagen residues migrate, driven by a rising temperature gradient, towards pore mouths blocking the entrance. This mechanism is confirmed by the surface area value (67.6 m^2/g) obtained for the burnt sample which is very close to the values found in the un-boiled samples. In sample M11, collagen residues were burnt (Bigi *et al.*, 1997; Lozano, 2002), and therefore nitrogen access to pores was unhindered.

[1] Tables 3 and 4: 'S' = total specific surface area; 'Smicrop' = specific surface area due to micropores; 'Smesop' = specific surface area due to mesopores.

Table 3. Specific surface areas of human bone samples using nitrogen adsorption (BET)

Sample	Thermal treatment (based on macroscopic criteria)	S (m^2/g)	S_{microp} (m^2/g)	S_{mesop} (m^2/g)
M7	Boiled	41.6	3.0	38.6
M8	Boiled	43.8	4.1	39.7
M9	Un-boiled	62.0	0.0	62.0
M10	Un-boiled	94.7	0.0	94.7
M11	Burnt	67.6	2.2	65.4

Non-human samples

Table 4 compares the specific surface areas of the non-human samples. The values obtained correlated well with the range evidenced amongst the human samples in Table 3. Samples M1, M2, M3 and M6 were boiled as demonstrated by their macroscopical features, whereas samples M4 and M5 seem to be 'probably un-boiled'.

However, only samples M2 and M4 were from the same type of bone, metapodium. Sample M4, presented the highest value obtained for specific surface area (92.6 m2/g). Sample M2, turned out to have a specific surface area of 87.9 m2/g. What we therefore saw here was that specific surface area decreased with thermal treatment, and these results reproduced the human skull evidence discussed above.

The specific surface area of the other non-human boiled samples, as determined by their macroscopical features, samples M1, M3 and M6, are 49.2, 59.9 and 74.1 (m2/g), respectively. All of these values, although they correspond to other types of bones, were lower than 96.2 (m2/g), sample M4, which was the specific surface area obtained for the un-boiled sample. The value obtained for sample M5, which was stated as being 'probably un-boiled', 46.2 m2/g, fitted well within the range of the boiled samples. The microporous specific surface area of samples M3 to M6 fell between 2.7 and 5.0 and was consistent with the human bone results.

Fractal dimension and radius of gyration

Human samples

Table 5 compares the fractal dimension and the gyration radii of the human bones studied. The three samples M7, M8 and M9 had similar fractal dimension and similar gyration radius, whereas the values for the burnt sample M11 were similar to those of un-boiled sample M10. Note that, excepting sample M9, the trend was the same as in the specific surface area values presented above. A high fractal dimension could indicate that the bone is dense and well packed (with or without collagen), whereas a low fractal dimension could reveal that bone components are disaggregated. The values can be compared to those obtained in fresh dog bone (Lima *et al.*, 2006), whose fractal dimension varied between 1.3 and 2.9, depending on the degree of bone densification. Such variations could also be attributed to protein content that may regulate the remodeling of bone (Wess *et al.*, 2001). Sample M9 would then be un-boiled, but with a certain degree of disaggregation due perhaps to a diagenetic process. Such results are in agreement with those from nitrogen gas adsorption (BET).

Non-human samples

The comparison of the two metapodium samples, M2 and M4, indicated that the fractal dimension, 2.36, was not altered by thermal treatment (Table 6). In boiled sample (M2), the gyration radius value turned out to be 0.506 nm; whereas in the un-boiled metapodium (M4), it was 0.631 nm, once again, this replicates the pattern found in the human bones samples, i.e. boiling process diminishes gyration radius as collagen α-chains are partially destroyed. The other boiled samples, i.e. M1, M3 and M6 samples showed fractal dimension values from 2.31 to 1.90 (Table 6), the degree of variance in bone values most probably related to the amount of boiling time. As suggested

Table 4. Specific surface areas of non-human bone samples using nitrogen adsorption (BET)

Sample	Thermal treatment (based on macroscopic criteria)	S (m^2/g)	S_{microp} (m^2/g)	S_{mesop} (m^2/g)
M1	Boiled	49.2	13.4	35.8
M2	Boiled	87.9	14.6	73.3
M3	Boiled	59.9	3.0	56.8
M4	Probably un-boiled	96.2	5.0	90.2
M5	Probably un-boiled	46.2	2.7	43.5
M6	Boiled	74.1	4.5	69.6

Table 5. Fractal dimensions and gyration radii of human bones using SAXS

Sample	Fractal dimension	Radius of gyration (nm)
M7	1.90	0.518
M8	1.80	0.531
M9	1.90	0.533
M10	2.70	0.783
M11	2.60	0.773

Table 6. Fractal dimensions and gyration radii of non-human bones using SAXS

Sample	Fractal dimension	Radius of gyration (nm)
M1	2.36	0.631
M2	2.36	0.506
M3	2.31	0.532
M4	2.36	0.631
M5	2.70	0.667
M6	1.90	0.486

by the nitrogen adsorption results and by previous studies (Bosch *et al.*, 2011; Trujillo-Mederos *et al.*, 2012), collagen migrates from the internal structure of the bone to the surface depending on boiling time. In a similar fashion, the gyration radius values varied between 0.631 and 0.486 nm. The un-boiled sample M5 presented a fractal dimension of 2.70, which provided the highest value in this group of samples; it was indeed a denser sample being a femur. The gyration radius, 0.667 nm, was as expected much higher than the gyration radii measured for the boiled samples. Once again this result was in agreement with the values obtained from the non-human metapodium bones and the human bones, previously described

Surface morphology, local composition and collagen thermal alterations

In checking the results, some samples were studied using SEM used in determining the surface morphology at the micrometric level. EDS was used to estimate the local composition. Out of the all the existing samples, M1, M5, M9 and M11 were chosen. M1 had a high microporous surface area suggesting it had been boiled. Similarly, samples M5 and M9 seem to be un-boiled even though the specific surface area obtained for M5 sample would imply a boiled sample. Sample M11 was burnt and therefore contained no collagen.

Human samples

The surface of sample M9 was layered, and some fibrils could be observed. Although its morphology was not typical of fresh bone, neither was it typical of boiled bone. This different morphology might have been due to a diagenesis process. On the right side of Figure 3, the layers may be clearly observed. The surface morphology of burnt sample M11 was made up of layers of particles and agglomerates (Figure 4). Some cracks and fissures were evident to the naked eye on this sample.

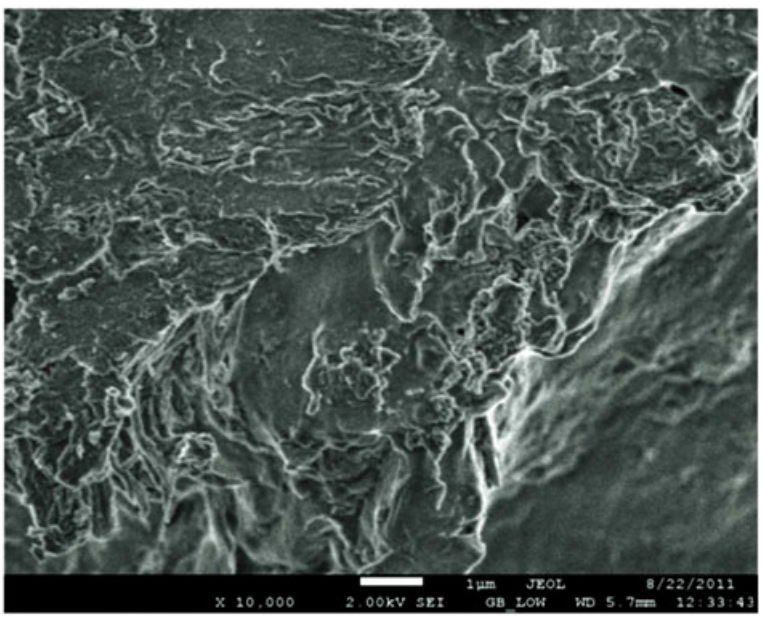

Figure 3. Scanning electron microscopy image of sample M9. Particles of 0.5 µm may be observed on/and between the layers (magnification of × 10,000). This figure is available in colour online at wileyonlinelibrary.com/journal/oa.

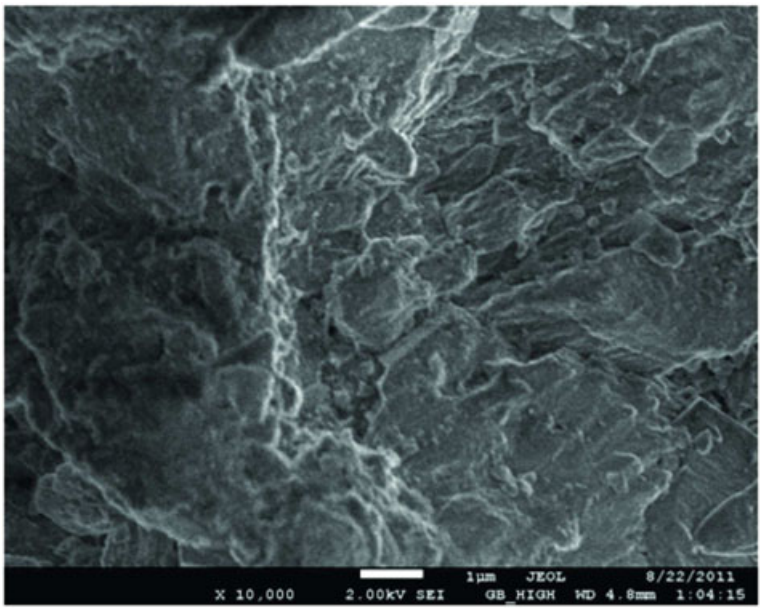

Figure 4. Scanning electron microscopy image of sample M11. Sample surface is constituted by particles and agglomerates of ca. 2 μm. A large number of cracks are apparent (magnification of × 10,000). This figure is available in colour online at wileyonlinelibrary.com/journal/oa.

Non-human samples

Bone M1 had a smooth surface, as was to be expected from a boiled sample. Furthermore, it did not show the presence of crusts implying that it was probably boiled for less than 6 h; this compares well previous results (Bosch *et al.*, 2011). The Ca/P atomic ratio was 1.85; this value together with the surface morphology indicates that this sample was boiled for *ca*. 2 h, also comparable with other studies (Trujillo-Mederos *et al.*, 2012). The elemental composition showed important quantities of Si and Mg because of the presence of quartz or clays (Table 7). The sample was demineralized so that the collagen could be observed, i.e. a 0.6 M solution of HCl was put in contact with the bone (Labastida-Pólito *et al.*, 2009). After 1 h, the bone became beige and almost transparent. The dissolution was interrupted when the sample became soft and tender, approximately after 2 h. We should note that human burnt sample M11 dissolved totally after 90 min, because of the absence of collagen.

In the demineralized M1 sample, only fibers of a diameter of ca. 200 nm were identified. This size corresponds to the cross-striated collagen fibrils (Parry & Craig, 1980). Labastida-Pólito *et al*. (2009) have stated that fibrils are packed and do not form a lattice; in this condition, they diffuse in disorder as suggested by the specific surface area values creating the interstitial microporosity visible in Figure 5.

Figure 6 contrasts the surface morphology of sample M5, before and after demineralization. Before demineralization, the surface was smooth and very similar to that of M1 thus corresponding to a boiled sample. The elemental composition of this sample is shown in Table 7. The atomic ratio Ca/P turned out to be 1.49; according to previous studies, this value was closer to 1.59, the ratio reported for bones boiled for *ca*. 4 h (Trujillo-Mederos *et al.*, 2012). These observations are in agreement with the BET and SAXS results that seemed to indicate a boiled sample and in disagreement with the macroscopic observation that suggested a 'probably un-boiled' material. These contradictory results most likely serve to show that the sample was inhomogeneous. Again, the demineralization process eliminated hydroxyapatite, and cross-striated collagen fibers were observed. The HCl solution seemed to act preferentially on P, which was totally eliminated as shown by EDS in Table 7. The fibers were packed in the mouths of the pores in accordance with the previously proposed mechanism.

To check the SEM results, i.e. to see whether the samples had been thermally treated or not, demineralized samples M1 and M5 were studied in the transmission electron microscope (TEM) as demonstrated in Koon *et al*. (2003, 2010). Figure 7 compares the TEM images of mineralized and unmineralized sample M5. In both images, collagen was degraded, and the gelatinous clumps reported by Koon *et al*. (2003, 2010) in damaged bones were observed. Again, most probably, M5 sample was partially cooked or suffered some kind of diagenesis that altered the collagen fibrils.

Table 7. Comparison of the atomic elemental composition of samples M1 and M5 using EDS. Results corresponding to demineralized sample M5 are included to show the efficiency of the process. Only selected elements are shown, for instance the content of O or C has no particular meaning; the total content is not 100%

Element	Sample M1 (Before demineralization)	Sample M5 (Before demineralization)	Sample M5 (After demineralization)
Mg	1.74		
Si	5.82		0.86
P	2.27	7.60	
S	1.33		
K	1.26		
Ca	4.20	11.38	0.31
Ca/P ratio	1.85	1.49	

Discussion

The results can be summarized as follows boiled bones presented a lower specific surface area than un-boiled bones probably because diffusing blocked the pores degraded collagen. The low fractal dimension obtained from boiled bones, which in itself correlated to partially disaggregated bone, further supported this concept. Gyration radius correlated to collagen α chains diameter; these diminished with thermal treatment indicating that these chains were degraded. The surface of boiled bones when observed using SEM turned out to be smooth at a μm level. Nevertheless, EDS analysis served to show that a variable degree of diagenesis could affect some of the results obtained from archaeological bones. Last, those observations were confirmed when some of the samples were subjected to TEM.

Our characterization techniques were shown to be complementary, with each method emphasizing a different focus on the samples. Parameters that were employed less frequently such as fractal dimension or gyration radius, could be used to describe roughness and size without having to hypothesis as to shape. Gas adsorption was a very convincing technique, even though the bones were pretreated at 120 °C *in vacuo* and, at this temperature, water was eliminated, the bone structure remains still showed the expected values.

Also, as we were testing new methods and techniques, we came across some problematic results. Sample M5 was the most problematic because the macroscopic criteria indicated that it was 'probably un-boiled', while the nanometrical characterization seems to show that the opposite was true. This contradiction could be explained by assuming that this sample suffered a diagenesis process that affected the bone tissue surface; thus, macroscopically, it was rougher than it should have been. However, the bulk of the sample's texture and structure at a nanometrical level correspond to boiled material. Therefore, we propose that this sample was boiled but suffered from some diagenesis process. Another possibility might be that it was thermally treated for a very short period so the macroscopical appearance was maintained but the collagen was locally altered.

Nevertheless, this last explanation can be disregarded when we consider boiling time. As has already been

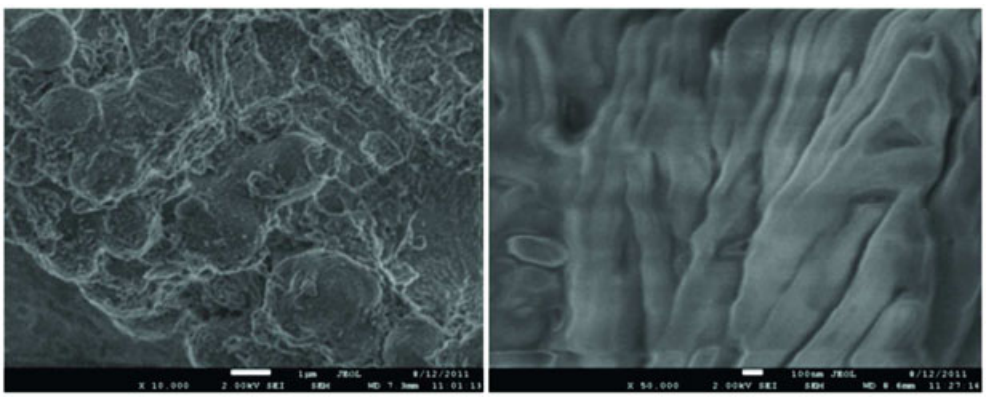

Figure 5. Surface morphology of sample M1 as observed in the SEM. A) Before demineralization, large smooth and globular particles of ca. 3 μm are present (magnification of × 10,000). B) After demineralization (magnification of × 50,000). This figure is available in colour online at wileyonlinelibrary.com/journal/oa.

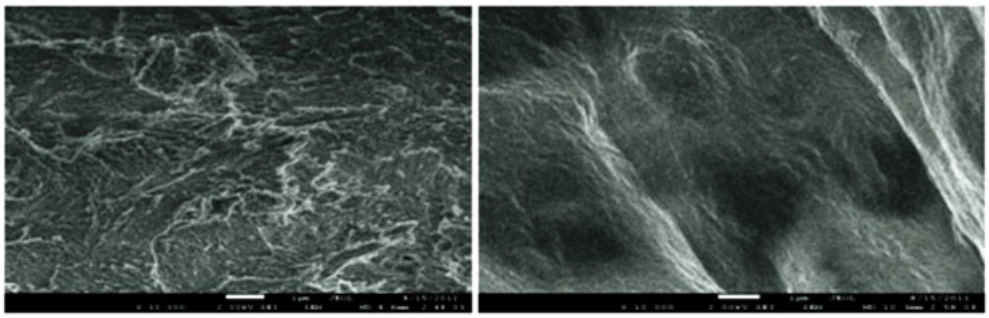

Figure 6. Surface morphology of sample M5 as observed in the SEM. A) Before demineralization (magnification of × 10,000). B) After demineralization (magnification of × 50,000). This figure is available in colour online at wileyonlinelibrary.com/journal/oa.

stated by Bosch *et al.*, 2011 and Trujillo-Mederos *et al.*, 2012, as boiling time increases, bone surface becomes smoother until non crystalline crusts appear on the surface after 6 h of boiling. That is why boiling time would explain variations in some of the data. The specific surface area cannot be the same in a sample boiled for 6 or 1 h, if we assume a collagen diffusion mechanism. The longest boiling times will correlate to smaller specific surface area values.

If this assumption is accepted, the non-human samples could be ordered relative to boiling time thus:

$$M4\left(\text{un-boiled}, 96.2\,m^2/g\right) > M2 > M6 > M3 > M1 > M5\left(46.2\,m^2/g\right)$$

And the human samples thus:

$$M10\left(\text{un} - \text{boiled}, 94.7\,m^2/g\right) > M9\left(\text{un-boiled}, 62.0\,m^2/g\right) > M8 > M7\left(41.6\,m^2/g\right)$$

Note the correlation between un-boiled samples M4 and M10, and between boiled samples M5, M8 and M7 at the other extreme. This correlation was independent of bone type or species. Differences between non-human and human samples are not noticeable by any methods employed here.

These values may be compared with those reported in previous studies using fresh human bones boiled for 2, 4 or 6 h (Bosch *et al.*, 2011). The corresponding surface areas were all comprised of between 2 and

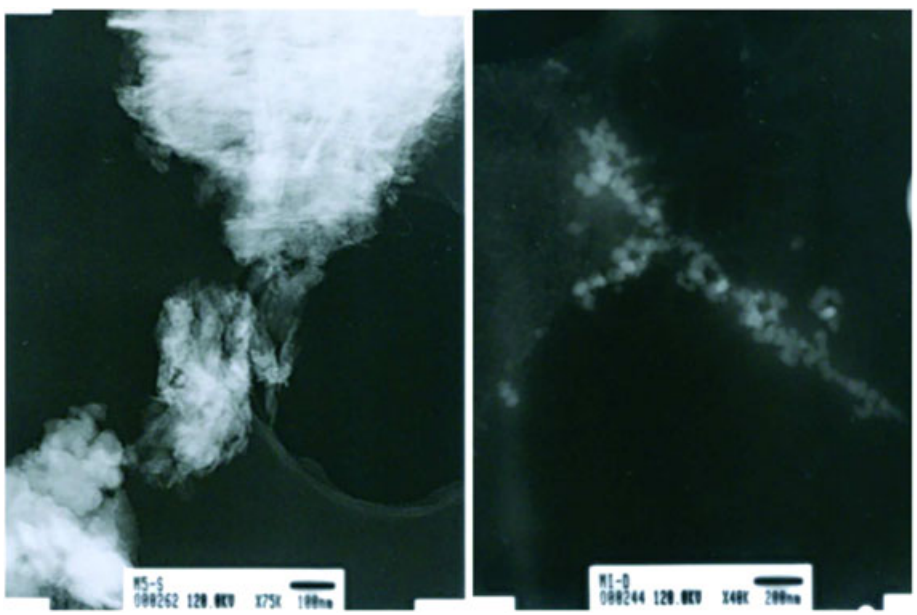

Figure 7. Sample M5 studied by TEM. In the non-demineralized material (A), no collagen fibrils are found. In the demineralized bone (B), only gelatinous clumps are present showing that collagen is damaged and, hence, cooked. This figure is available in colour online at wileyonlinelibrary.com/journal/oa.

7 m^2/g, and the differences between samples was not significant. In Neolithic bones, the determined areas were *ca*. 20 m^2/g. The differences may be attributed to pore accessibility, which is determined by bone pretreatments. In these earlier studies, the samples were treated *in vacuo* at 80 °C so as to be certain that the structure was not altered. In the present study, the samples were pretreated at standard conditions, *in vacuo* at 120 °C. Thus, the gas adsorption results are always relative. This explains why when reviewing the literature, the values stated cannot be assumed to be absolute values. Smith *et al*. (2008) in comparing modern and old bovine bones obtained specific surface areas comprising between 0.19 m^2/g and 101 m^2/g depending on pretreatment methods (degasification method, liophilization or treatment with solvents).

Collagen gyration radius also seems to depend on the length of time it is subjected to thermal treatment. In this case, the length of time diminishes the radius of gyration playing out on the samples thus:

$$M2(\text{metapodium}) < M3(\text{humerus}) < M1(\text{jawbone}) < M5(\text{femur})$$

This sequence reproduces exactly the sequence obtained for surface areas. Hence, thermal treatment reduces specific surface area and radii of gyration. Only the sample M6 (scapula) did not fit well in this correlation, probably because it was a less dense bone as revealed by its fractal dimension value (1.90); fractal dimension tends to become low if the sample is rough but not dense. There was no clear tendency between fractal dimension and a boiled or un-boiled condition, although it would seem that the fractal dimension is lower in boiled than in the un-boiled samples. SEM-EDS observations alerted us to the fact that in archaeological samples, diagenesis and taphonomic processes may mimic or mask the effects of boiling (Roberts *et al*., 2002; Koon *et al*., 2003, 2010; Pijoan *et al*., 2007; Bosch *et al*., 2011; Trujillo-Mederos *et al*., 2012), as could be seen with problematic results obtained from samples M5 and M9.

Finally, from an archaeological point of view we conclude that these results are promising. There was a significant overlap between our initial macroscopic observations to determine the presence of boiled or un-boiled bones and the results of physicochemical analysis performed on these same samples. This study therefore highlights the importance of integrating various methods to attain more reliable conclusions.

The application of BET evidenced an interesting concurrence with the results from macroscopic observation. When we integrate this data with SAXS, the results become even more consistent with those from macroscopic observation.

Determining the presence of boiled bone is highly relevant in a series of different ways. First, boiling implies the need for particular culinary practices revolving around the necessity of having suitable containers, in particular ceramics. As such, in early archaeological sites, when doubts concerning the presence of pottery exist, the identification of boiled bones may contribute indirectly to postulate its presence. Second, there is also a significant economic impact related to subsistence and human behavior, such that the presence of boiled bones allows for the testing of optimization models (see, e.g. Eldredge, 1989; Smith & Winterhalder, 1992; Grayson & Cannon, 1999; Gremillion, 2002). For example, the presence of boiling may suggest optimization in the use of non-humans animals consumed by the extraction and use of marrow, fat and tissues that remain attached to the bone after removing the meat. Also, the boiled bones of smaller size tend to indicate the same trend. These factors are vital in the testing of optimization models hypotheses of stress and/or differential management intensification and exploitation of non-humans animals.

In the case of boiled human bones, there is also a promising array of data and how it reflects on mortuary rituals. The presence of this boiling allows us to hypothesis the possibility of cannibalism or other complex funerary treatment of the bones.

Our research also has implications for the field of taphonomy and the study of post-depositional natural processes such as diagenesis. Specifically, the SEM-EDS observations are of great interest since they contribute not only to contrast positively or negatively previous results but also serve to indicate probable diagenetic or other thermal situations that are not related to boiling activities.

The macroscopic observation of sample M5 as well as BET, XRD and SAXS analyses including observation prior to bone demineralization indicates that M5 was a 'probably un-boiled' bone. Yet observation after demineralization suggests that surface alteration may be due to heat treatment – perhaps even boiling – or as the result of diagenetic processes. In the case of sample M9, the SEM revealed some alterations that may correspond better to diagenetic processes and thus becomes a useful proxy in determining post-depositional processes of the samples. It is significant that in the M1 sample, the SEM-EDS analysis corresponded to the macroscopical observations of a boiled sample, while the M11 burnt sample results confirmed our observation of a direct heat treatment of certain intensity on the sample. Importantly, in these cases, the results were shown to be independent of species or bone type sampled.

Conclusion

In exploring these new methods and techniques and testing their potential for Andean South American archeology, we consider the results obtained to date to be promising opening new investigative avenues in the detection of cooked bones at low temperatures. Nevertheless, at the same time, some of the more problematic results serve to reinforce the necessity of continued research aiming to expand both the variety of samples and the physicochemical analyses used. From an archaeological point of view, it is very important to discern how cooking processes changed bone structure, at both macroscopical and microscopical levels of observation. New experiments, alternative applied methods, as well as on discreet and larger bone samples, should overcome some of the problems caused by natural taphonomic processes and diagenesis, whilst also helping us to further understand them.

The characterization of human and non-human bones demonstrated that physicochemical techniques are complementary and that they provide some new criteria for distinguishing boiled from un-boiled archaeological samples. With boiling, the specific surface area is reduced; thus, the pore openings are obstructed. Collagen gyration radius also diminishes accompanied by the degradation of collagen. On the other hand, fractal dimension does not seem to be an adequate parameter to determine if bone is boiled or not, although it seems to be lower in boiled samples, indicating that boiled bones are lighter and that the structure is more complex. As we can appreciate from previous studies (Bosch *et al.*, 2011; Trujillo-Mederos *et al.*, 2012), boiling time could explain some of the differences observed between samples.

In general, the results obtained are in accordance with macroscopic observations such as the smoothness or transparency that characterize boiled bones (Botella *et al.*, 2000; Pijoan *et al.*, 2004). Specific surface area, gyration radii and SEM micrographs help to indicate the diffusion and degradation of collagen fibrils. These broken fibrils could be seen when the sample was subjected to TEM. Note that these results are independent of the bone type and species. However, we also realized that diagenesis or taphonomic processes could partially mask the boiling effects described above.

Finally, it is important to note that determination of bone condition, boiled or un-boiled, on archaeological material goes beyond the merely analytical. The discussion of the presence or absence of boiling, post-depositional and taphonomic processes leads us to other important issues such as culinary practices, availability of technology (presence of ceramic, even in the absence of ceramic vessels from the archaeological site record), ritual practices (e.g. cannibalism) and optimization strategies (e.g. use of bone marrow and fat, differences in the treatment of different taxa, etc.). All these themes serve to highlight the importance of juxtaposing these results with other studies such as process formation, human manipulation (cut marks, bone fracture, etc.), ceramic or other type of cooking technology, spatial distribution, etc., which all link to the further study of past human societies.

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