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SAVOURY RECIPES AND THE COLOUR OF THE TLATELCOMILA HUMAN BONES*

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Bones from Tlatelcomila (Tetelpan, México D.F.) were characterized by several complementary physical and chemical techniques, such as X-ray diffraction, scanning electron microscopy, transmission electron microscopy, atomic force microscopy, energy-dispersive spectroscopy and ultraviolet spectroscopy, to determine whether they were boiled or grilled. The usual correlation between thermal treatment and colour is revisited in terms of microscopic structure, morphology and texture. At temperatures less than 100°C, it is shown that colour depends not only on temperature or diagenesis but also on the cooking procedure; that is, on the presence of spice dyestuffs such as axiote (Bixa orellana) *or chilli* (Capsicum).

KEYWORDS: CANNIBALISM, TEMPERATURE, DRX, AFM, EDS, UV–VIS, SEM, PRE-HISPANIC, *AXIOTE*

INTRODUCTION

The study of altered bones has been shown to be a fundamental tool to determine cultural habits in human societies. For instance, at the Anasazi sites of Chaco Canyon (New Mexico, USA), the proper characterization of bone fragments has demonstrated intentional fragmentation, scattering of disarticulated elements, loss of vertebrae and pot polish, all of which have been attributed to anthropophagical practices (Hurlbut 2000). The same pattern has been reported at some Neolithic Spanish sites; for example, in the Cueva de Malalmuerzo in Moclín (Granada, Spain) or Las Majolicas in Alfácar (Granada, Spain) (Jiménez-Brobeil 1990; Solari *et al*. 2012).

However, such characterization is not obvious. Indeed, bone can suffer taphonomic and diagenetic alterations, similar to those ascribed to low-temperature thermal treatments (Shipman *et al*. 1984; Etxeberría 1994; Novak and Kollmann 2000; Roberts *et al*. 2002). Two levels of characterization may be distinguished, either macroscopic or microscopic. Macroscopically, a boiled bone turns out to be translucent and smooth, denser and often vitreous (Pijoan 1997; Botella *et al*. 2000). Another important parameter in macroscopic characterization is colour, which has been correlated with diagenetic alterations (White and Hannus 1983; Lyman 1994; Hedges and

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Millard 1995), or with ritual practices, which include intentional colouring with cinnabar or ochre (Wreschner 1980; Cervini-Silva *et al*. 2013). Furthermore, bones removed from the fire after 25 min display a colour diversity that includes black, brown, grey and white/blue; all those colours may be present in the same skeleton (Naysmith *et al*. 2007; Arora *et al*. 2010).

However, such correlations, although very useful, do not consider parameters as important as the thermal treatment time or the composition of the boiling liquid. In previous works, we have reported on the importance of boiling time, and on fresh versus salted water (Bosch *et al*. 2011; Trujillo-Mederos *et al*. 2012). Only micro-characterization, with physical and chemical techniques, seems to be sensitive enough to detect boiling or grilling times. Through focusing in this manner, differences in morphology and structure between boiled bones, in fresh water or seawater and boiled for 2, 4 or 6 h, have been clearly established.

Conventionally, local elemental composition may be obtained using energy-dispersive spectroscopy (EDS), compounds with X-ray diffraction (XRD) and morphology with scanning electron microscopy (SEM). Recently, the use of other techniques has been reported, such as NMR to measure pore-size distribution or specific surface area (Wang 2003), transmission electron microscopy (TEM), after demineralization of the samples, to observe the degradation of collagen fibres (Koon *et al*. 2003) or gravimetric analyses (DTA and DTG) to understand the progressive formation of crystalline phases with temperature (Lozano *et al*. 2002, 2003).

In the study of human skeletal samples from pre-Hispanic Mexico, it has been reported that some bones show modifications in colour, texture and morphology that could be interpreted as alterations due to heat exposure (Pijoan *et al*. 2004b, 2007). The aim of the present work is to understand the yellowish and reddish colour of Tlatelcomila human bones using various characterization methods. Colour will be correlated with materials science parameters in order to determine whether the Tlatelcomila bones were treated at low temperature, if they were intentionally coloured, or if the colour results from a particular cooking procedure. With this purpose, the Tlatelcomila bones, already reported in a previous work (Pijoan *et al*. 2007), will be revisited.

MATERIALS AND METHODS

The archaeological context

All the analysed materials were collected at the archaeological site of Tlatelcomila (Tetelpan, D. F., Mexico), where some of the first evidence of anthropophagy in Mesoamerica was reported (Pijoan and Pastrana 1987, 1989; see also Fig. 1). The evidence corresponds to the Late Preclassic period (700–500 BC). The excavation consists of four stratigraphic pits and the only associated cultural element worth mentioning is a *metate*. The remains constitute several broken and incomplete skulls and fragments of long bones. From the skull fragments, the minimum number of individuals (MNI) was estimated to be at least 18: seven infants, one sub-adult (probably a female; 16–18 years old) and 10 adults—six males, one female and three undetermined (Pijoan and Pastrana 1987, 1989).

The materials display all the distinctive features of anthropophagical practices (Turner 1983; White 1992), which include *perimortem* intentional breakage, anvil abrasion and percussion impacts, cut marks, facial mutilation, missing elements and thermal exposure. A full description of the archaeological data, as well as the features of the skeletal materials, has been previously published (Pijoan and Pastrana 1987, 1989).

Figure 1 *The location of the Tlatelcomila archaeological site.*

Samples

Six samples were cut from adult long-bone fragments. The samples were classified as boiled or grilled, following the macroscopic criteria proposed by Pijoan *et al*. (2004a) and Botella *et al*. (2000, 144). In Figure 2, the differences in colour and texture are evident, the yellowish samples (M1–M3) being assumed to have been boiled, whereas the reddish samples (M4–M6) may have been grilled.

For comparison purposes, a partially defleshed cow bone was boiled for 4 h in a water solution (1 litre of water) of *axiote*, also known as *achiote* (*Bixa orellana*). It was then washed in water and further defleshed to compare the bone colour under the meat and the colour of the bone that was in contact with the solution. *Axiote* is a spice containing bixine, which is a powerful carotenoid dyestuff (Bernal and Correa 1989; Jaramillo and Muñoz 1992); in Mexico, *axiote* has been used since pre-Hispanic times to flavour and colour food; for instance, it is an essential component in the preparation of '*cochinita pibil*' (Anderson 1963; Lentz 1991; Devia and Saldarriaga 2002; VanDerwarker 2010; Trabanino 2013).

Demineralized samples

The Tlatelcomila archaeological samples were demineralized following the modified procedure proposed by Labastida-Pólito *et al*. (2009). Human cortical bone samples were put in contact with a 0.6 M HCl solution in 250 ml of Milli-Q water, with constant agitation until softness.

Figure 2 *The adult long-bone fragments from which the samples were taken.*

The demineralized samples were then washed with Milli-Q water and were left in an extraction bell for 48 h at room temperature.

Characterization techniques

Powder X-ray diffraction (XRD) X-ray diffraction patterns were recorded using a Brucker AXS Advance D-8 diffractometer coupled to a cobalt anode tube. Cobalt radiation has the advantage that the peak resolution for high interplanar distances is better. The K_α radiation was selected, with a diffracted beam monochromator. In order to preserve preferred orientations, the samples were not powdered.

Scanning electron microscopy/energy-dispersive spectroscopy (SEM/EDS) For the SEM/EDS studies, a JEOL JSM-7600F field scanning electron microscope, coupled to an EDX Oxford INCAX-Act, was used. The samples were studied at low (×1000 and ×2500) and high (×5000 and ×10 000) magnifications. Note that the samples were not sputtered with any conductor material as, in these conditions, the images were of sufficient quality. The advantage was that any further contribution to the EDS spectra was avoided.

Transmission electron microscopy (TEM) Demineralized samples were dispersed on a holey carbon film. Note that the sample preparation was not the same as that reported by Koon *et al.* (2003, 2010) and, thus, the resolution was not the same. Only the outer coloured surface of the cortical bones was studied. Using transmission electron microscopy, most subtle heat-induced

changes in collagen, at fibril level, could be detected. For TEM analyses, a JEOL model JEM-1200eV microscope was used, at 120 kV.

Atomic force microscopy (AFM) The microscope was a JEOL JSPM, in tapping mode. The vibrating frequency of the cantilever was typically in the range of 350 kHz. All studies were carried out at room temperature in air. Images were recorded in both height and amplitude modes. The demineralized samples were studied, to check if the collagen fibres were degraded on the surface.

Ultraviolet visible spectroscopy (UV–Vis) The spectra were measured at room temperature in the wavelength range 380–800 nm (1 nm resolution), which includes vibrations from violet (380–436 nm), blue (437–495 nm), green (496–566 nm), yellow (567–589 nm), orange (590–627 nm) and red (628–770 nm). The area under the curve for each wavelength colour interval was calculated and referred to the total area under the spectrum. In this way, the contribution of each colour to the spectrum may be evaluated. The bone fragments were not treated and the spectra were taken from three different zones for each single bone. The spectrophotometer was an Ocean Optics model USB4000 instrument.

RESULTS

Powder X-ray diffraction (XRD)

The X-ray diffractograms of the yellowish and reddish Tlatelcomila samples are presented in Figure 3. Although, macroscopically, samples M1–M3 and M4–M6 are similar in colour and texture, their X-ray diffraction patterns are very different. In samples M1 and M2, the observed peaks are sharp and intense; these materials are then very crystalline. The locations of the main peaks correspond to armbrusterite, $K_5Na_6Mn^{3+}Mn^{2+}_{14}[Si_9O_{22}]_4(OH)_{10}{\cdot}4H_2O$, and a small amount of hydroxyapatite, $Ca_5(PO_4)_3(OH)$: no amorphous compounds are observed as the background is very low. Instead, in sample M3 only hydroxyapatite and a very small amount of calcium phosphate ($Ca_2P_2O_7$) are identified. The background line is high, showing a rather large amount of an amorphous compound. As the peaks ($35° < 2\theta < 40°$) are very broad and the small-angle X-ray scattering ($0° < 2\theta < 10°$) is large, the particles of hydroxyapatite and calcium phosphate must be in the nanometric range. The X-ray diffraction patterns of samples M3 and M4 are rather similar. Sample M4 consists of only nanometric hydroxyapatite. In sample M5, an intense and very broad peak ($15° < 2\theta < 45°$) appears, which corresponds to an amorphous material. The crystalline compounds are hydroxyapatite and calcium phosphate. Finally, in sample M6, the observed peaks are neither sharp nor intense; the material is not very crystalline and the locations of the main peaks correspond to armbrusterite, a small amount of hydroxyapatite and calcium phosphate. The background line is intense, showing a rather large amount of an amorphous compound. Again, as the peaks ($35° < 2\theta < 40°$) are very broad and the small-angle X-ray scattering ($0° < 2\theta < 10°$) is large, the particles of hydroxyapatite and calcium phosphate must be nanometric.

Scanning electron microscopy/energy-dispersive spectroscopy (SEM/EDS)

The micrographs obtained by scanning electron microscopy are shown in Figure 4. In the upper sequence (samples M1–M3), a layered morphology is observed. The open pores, whose size is

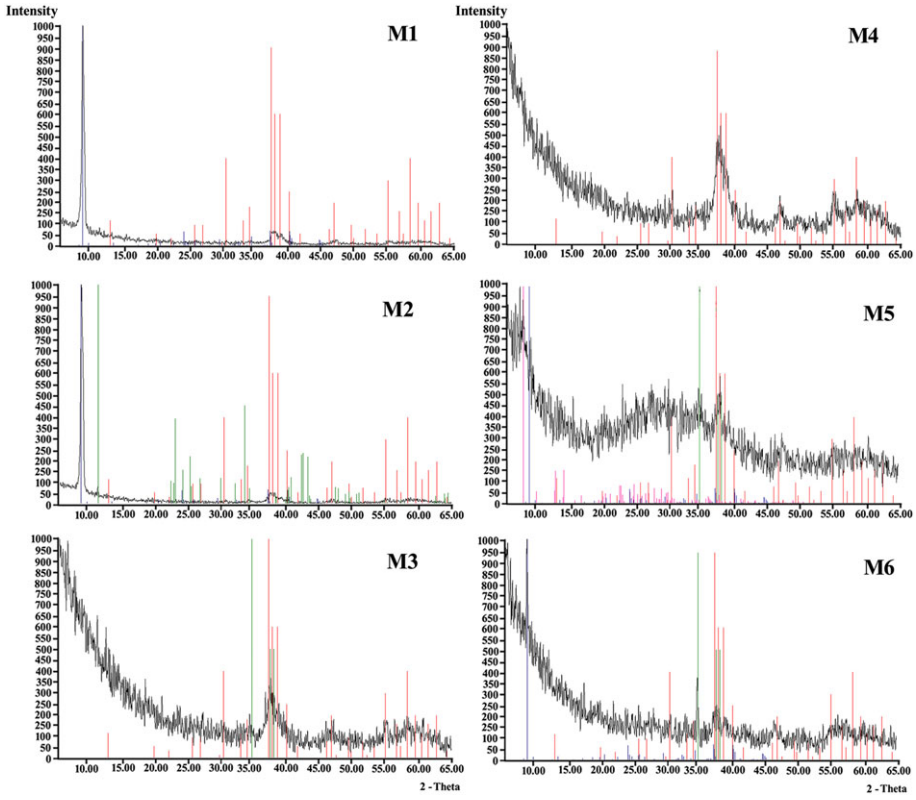

Figure 3 *A comparison of the sample diffractograms: red bars correspond to hydroxyapatite, blue bars to armbrusterite and green bars to $Mg_3(Si_4O_{10})(OH)_2$ in M2 and $Ca_2P_2O_7$ (calcium phosphate) in M5 and M6 (see online for a colour version of this figure).*

D = 0.2–0.5 μm, must correspond to the *canaliculi*. All samples present a packing of feather-like particles, the size of which turns out to be ~0.4 μm wide by ~2 μm long. The M3 image reveals (in the arrowed zone) how the bone surface is really flat, whereas the edge is slightly rough. All samples show a smooth surface, like the one reported in a previous work for boiled samples (Trujillo-Mederos *et al*. 2012).

The atomic molar ratio Ca:P determined by EDS in sample M1 is 1.54, which corresponds, within the error range (±0.1) to the expected value. Indeed, hydroxyapatite has a Ca:P ratio of 1.66 (Miculescu *et al*. 2011; see also Table 1). The Ca:P ratios of the M2 and M3 bones are slightly lower, 1.23 and 1.42, respectively. Only the value 1.23 is significantly different from the expected value for hydroxyapatite.

The second row (samples M4–M6) shows some differences from the previous one. The M4 sample morphology is constituted by ordered fibres, resulting in a dense and smooth surface that is very flat. The general features of samples M5 and M6 are that the surfaces are regular and soft. Some zones are darker (marked ‘a’ in the micrographs), while some others are whiter (marked ‘b’ in the micrographs). The channels and galleries reveal an inner feather-like packing of the particles in the dark patches; at lower magnification (×1000 and ×2500), the pattern of clear and dark zones is evident. Pores like those found in the first sequence are observed. Samples M5 and M6 are similar: they both present the two types of texture already mentioned, ‘a’ and ‘b’ zones.

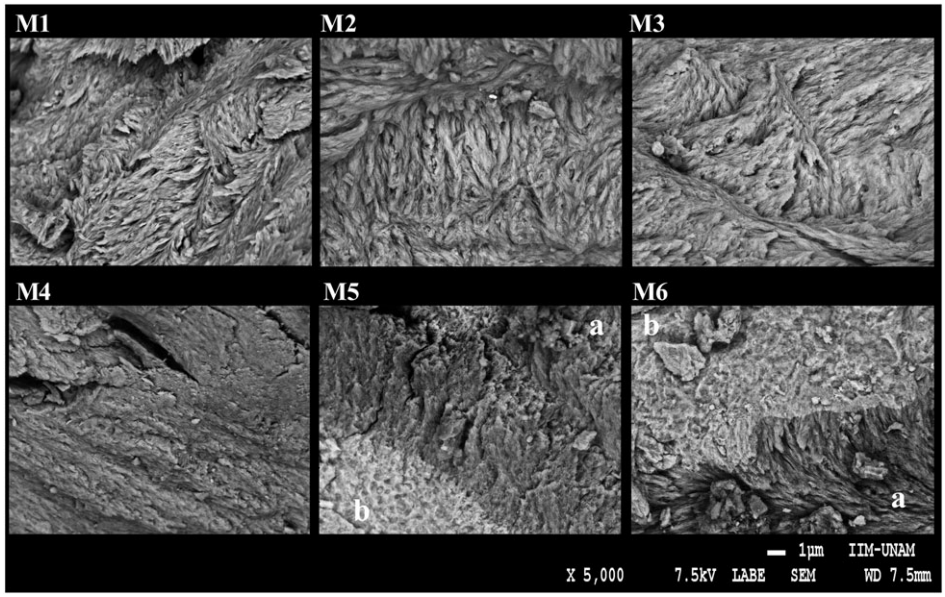

Figure 4 *The surface morphology of samples M1–M3 is similar and homogeneous. On the other hand, in samples M5 and M6 it is heterogeneous: the zone labelled 'a' is similar to the surface of boiled bones, while the zone labelled 'b' is similar to the surface of cremated bones.*

Table 1 *The atomic molar ratio Ca:P as determined by EDS (accepted value for hydroxyapatite: Ca:P = 1.66)*

					M5		*M6*	
Sample Elements	*M1*	*M2*	*M3*	*M4*	*Zone a*	*Zone b*	*Zone a*	*Zone b*
Ca (at%)	19.49	9.43	7.97	14.46	12.65	13.43	14.40	16.70
P (at%)	12.06	7.62	5.59	8.89	18.88	24.92	8.88	11.18
Atomic molar ratio Ca:P	1.54	1.23	1.42	1.62	1.49	1.85	1.60	1.49

Sample M4 has a Ca:P ratio of 1.62, in agreement with the expected value. In samples M5 and M6, the Ca:P atomic ratios are 1.49 and 1.60 in zone 'a', whereas in zone 'b' they are 1.85 and 1.49 (Table 1).

Transmission electron microscopy (TEM)

Samples M2 and M5 were chosen to be studied by TEM as they seem to be the most representative: from their macroscopic features, the first one (M2) is assumed to have been boiled and the second (M5) grilled. Figure 5 (a) shows a collagen fibril, present in demineralized sample M2, the length and diameter of which are ~1600 nm and ~300 nm, respectively; these values are in agreement with those observed by Koon *et al*. (2003) for denatured collagen (diameter 200–250 nm). The band pattern is rather faint, but it can be estimated to be ~80 nm. It has to be emphasized that this denatured fibril has a diameter six times larger than that reported for fresh

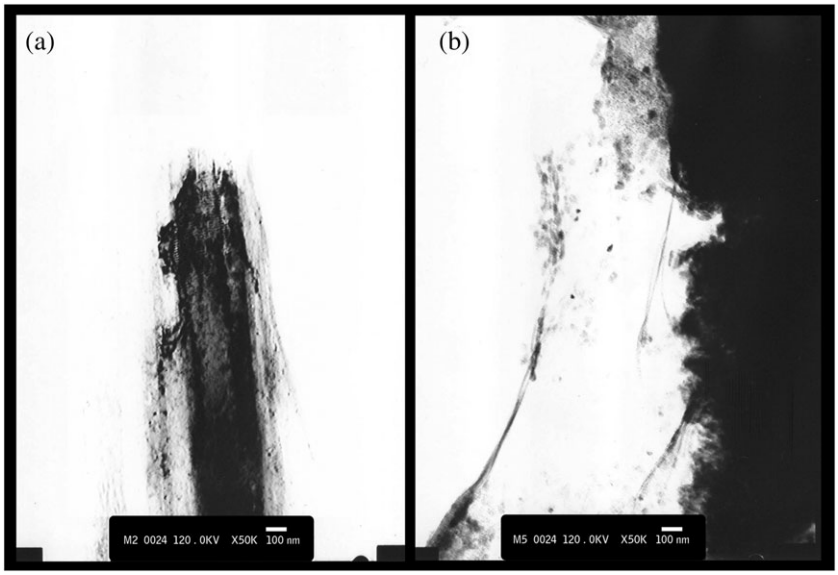

Figure 5 *TEM images of (a) collagen fibril present in demineralized sample M2 and (b) fibrils packed and stuck in demineralized sample M5.*

samples. Remember that thermal treatment modifies the fibril structure, enlarging it until it becomes a gelatin with a disordered structure. In Figure 5 (b), the fibrils seem to be packed and stuck. Furthermore, they are not structured and the full image corresponds to an amorphous compound, most probably the non-crystalline fraction already detected in the corresponding X-ray diffraction pattern.

Atomic force microscopy (AFM)

The two previous demineralized samples (M2 and M5) were also studied by atomic force microscopy. The purpose of this characterization is to determine whether the collagen fibres are denatured and to check the conclusions of the TEM. Figure 6 (a) displays the array of the collagen fibrils in sample M2. If this image is compared with those reported in the literature (Hassenkam *et al*. 2004; Orgel *et al*. 2006), the collagen fibrils appear to be disordered and broken, in agreement with the TEM results: they do not follow the typical cross-striation pattern. The diameter of the demineralized fibrils turns out to be 257 ± 5 nm. The topographic analysis along the longitudinal axis of several denatured collagen fibrils indicates a faint mean banding periodicity of 130 nm (±20 nm). This value is twice that reported by Grover *et al*. (2012), of 64.4 (±2.91 nm) for all films containing collagen (both non- and cross-linked). Furthermore, Grover *et al*. (2012) observe that the gelatin films did not show this band structure. Nevertheless, the band periodicity is subject to alterations due to hydration: Bozec and Odlyha (2011) found that the band periodicity varies between 67 and 69 nm, depending on the fibril hydration, or on thermal treatments. The tropocollagen units constituting the fibril are expected to increase their size as the fibril is denatured by heat.

Sample M5 (apparently grilled) consists of denatured collagen fibrils stuck together; the length is much shorter, less than ~650 nm (Fig. 6 (b)). The diameter of the fibril is 120 nm (±20 nm),

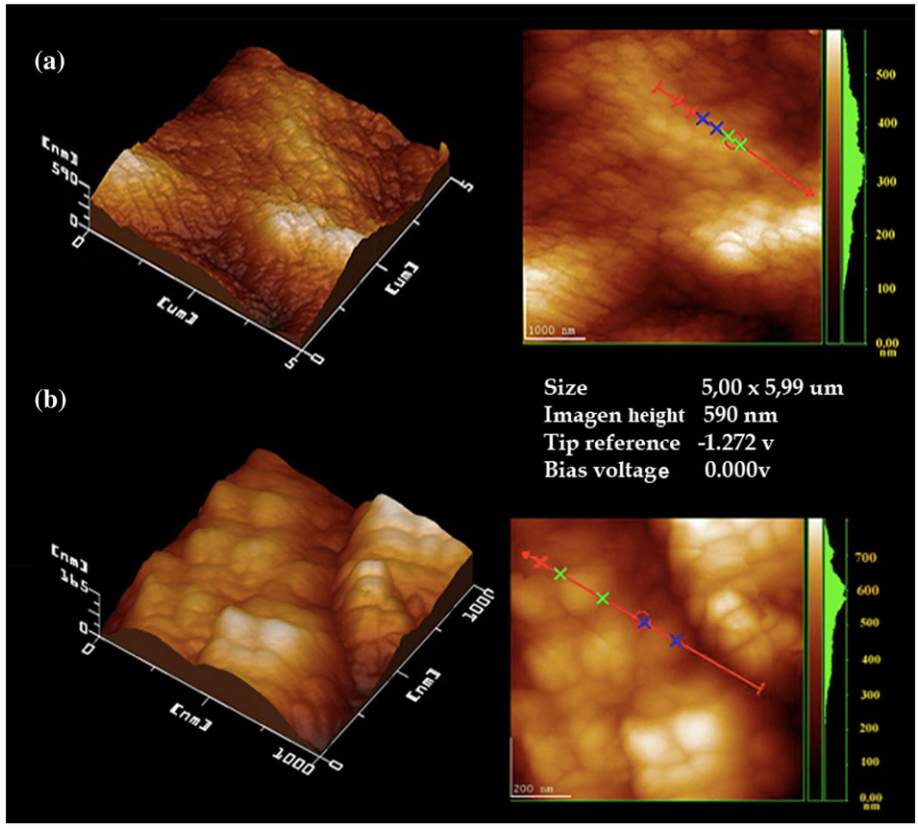

Figure 6 *Three-dimensional and two-dimensional atomic force microscopy images of demineralized samples (a) M2 and (b) M5. The topographic profile was measured following the red line on the second image (see online for a colour version of this figure).*

much less than the diameter found for the collagen fibrils of sample M2, assumed to be boiled bone. The banding pattern is, in this sample, 78 nm (±3 nm). These differences could also be attributed to experimental artefacts. The width of the fibrils may be affected by the dilatation of the cylindrical shape of the fibril and the geometry of the AFM tip (Kato *et al*. 2001). However, both samples were prepared in the same way. Therefore, both samples should present the same error, if any.

The following pattern emerges. With heat, the collagen fibre is denatured and the beam of disordered collagen fibrils becomes apparent. The fibrils increase their diameter depending on boiling or grilling or, most probably, with cooking time.

Ultraviolet visible spectroscopy (UV–Vis)

To the naked eye, the colour surface of the thermally treated bones of Tlatelcomila is either yellowish or reddish, in contrast with the inside of the samples, which turns out to be ivory white (Fig. 7). In the first sample series, the shape of the UV–Vis spectra is very different (Fig. 8). In sample M1, the spectrum increases homogeneously towards higher wavelengths, showing that red and orange colours are dominant. In sample M2, the spectrum presents a very broad and intense peak from 400 to 700 nm, centred at $\lambda \approx 650$ nm (red) and with a maximum value at 669

Figure 7 *Colour differences between the surface and the core of sample M6 (bar = 2 cm).*

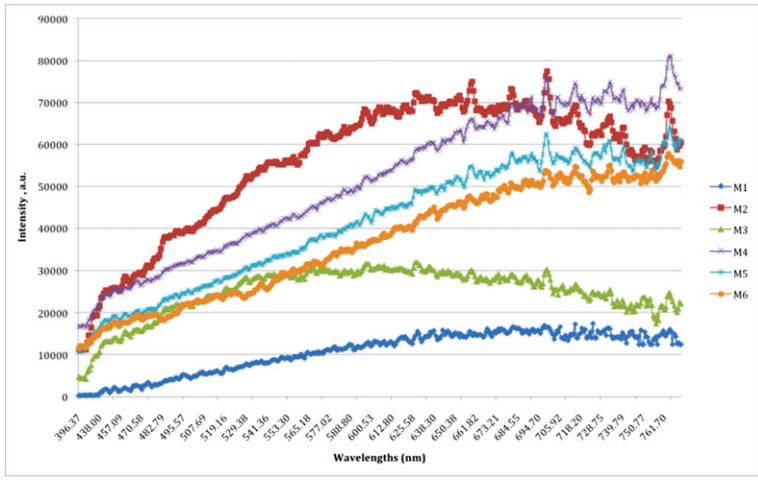

Figure 8 *The UV–Vis spectra used to determine the colour percentage in each sample (see online for a colour version of this figure).*

nm (red). Sample M3 shows a broad peak from 400 to 700 nm, centred at 609 nm (orange) and a maximum at 625 nm (orange). The three spectra corresponding to samples M4–M6 are similar and may be decomposed in a uniformly increasing section from $\lambda \approx 200$ nm to $\lambda \approx 680$ nm and a horizontal line from 680 to 770 nm (red).

To quantify the amount of each colour, a histogram was constructed from the percentages of each colour in each sample. In this way, the colour proportion may be evaluated (violet, 380–436 nm; blue, 437–495 nm; green, 496–566 nm; yellow, 567–589 nm; orange, 590–627 nm; red, 628–770 nm). The colour of sample M1 is mainly composed of red (61%); the following dominant colours are green (14%) and orange (12%). In the first series (samples M1–M3), which includes the more reddish samples, sample M2 is 52% red, 19% green and 13% orange, whereas sample M3 is 46% red, 23% green and 13% orange. In all these samples, yellow is ~7%. Note that as red decreases, green and blue increase. All samples of the second series, samples M4–M6, are similar: red (58%), green (15%), orange (11%) and yellow (6%). It has to be emphasized that the stain does not infuse into bone; it is found only on the surface.

Figure 9 compares the appearance of a fresh cow bone boiled in the *axiote* solution described in the experimental section. Initially, the bone was covered by flesh, which retracted with temperature and over time, uncovering some zones of the bone. After 4 h the bone was cleaned, and the meat removed and washed. The zones where the bone was in direct contact with the cooking liquid are definitely reddish. This finding is not new, as bone may be dyed in the presence of many species (Devia and Saldarriaga 2002).

DISCUSSION

Evidence of thermal treatment

The scanning electron micrographs (SEM) showing the texture and morphology of the six samples are similar to those already described for boiled or grilled bones (Stiner and Kuhn 1995; Weiner and Wagner 1998; Lozano *et al*. 2002; Forancelli-Pacheco *et al*. 2012; Grover *et al*. 2012). Such a clear conclusion matches the macroscopic observations and is confirmed by the other characterization techniques used in this work. Indeed, collagen begins to denature around 40°C (Bonmatí-Limorte and León-Albert 1983), although the total denaturation temperature of collagen in bone may be very high (about 155°C) due to the reduced water content induced by mineralization (Avery and Bailey 2008). In our study, collagen, observed in TEM, turns out to be a rather

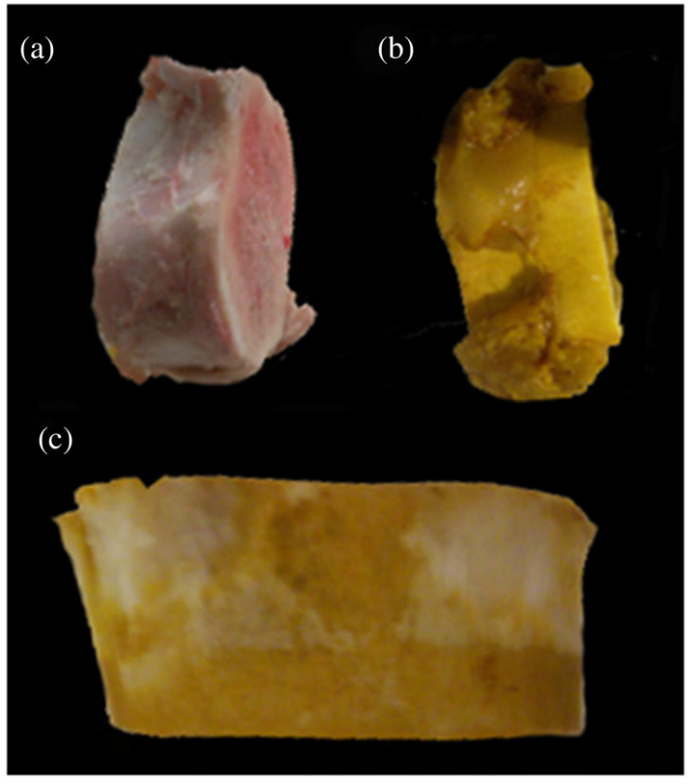

Figure 9 *The colour of a boiled cow bone in* axiote: *(a) fresh bone; (b) bone cooked in* axiote *for 4 h; (c) bone cleaned and washed.*

amorphous mass, without any clear structure. The fibrils are degraded and disordered. The molecular packing is deformed, and the fibrils lose their oval morphology as well as their periodic patterns. In this study, the rather faded banding pattern was 130 nm (±20 nm), this value is larger than the one usually reported (67 nm). The TEM and AFM observations are supplementary evidence of thermal treatment (Bonmatí-Limorte and León-Albert 1983; Kirsch *et al*. 1998; Holmes *et al*. 2001; Wells *et al*. 2005; Orgel *et al*. 2006). Therefore, all samples were either boiled or grilled.

Grilling versus boiling

To distinguish boiling from grilling is a hard task. Although, in boiling water, the temperature is maintained at 100°C, while in grilling the temperature is expected to reach much higher values, as the bone is covered by meat and meat is mostly constituted by water, the temperature reached by bone in grilling should also be 100°C or less. Only the areas of bone not covered by meat are exposed to very high temperatures. These areas may be exposed directly to heat from the start of the cooking process or progressively as the meat contracts with temperature.

There are other complementary criteria. Indeed, there are differences between boiled bones due to the treatment time. The morphology of a bone boiled for 6 h turns out to be different from that of a bone boiled for 2 h. In boiling, the bone salts tend to dissolve, promoting collagen surface migration, as shown by bones boiled in seawater. In boiled bones, the texture is homogeneous and dark patches or inhomogeneous degradations are not observed. This is the way, then, to distinguish boiling from grilling. During the grilling process, the meat juices concentrate around the bone and diffuse into it slightly, and dark patches are observed. The resistance of grilled bones is heterogeneous, as the temperature it attains is different in the meat-covered zones compared to the directly exposed areas.

The three samples of the first group fulfil the already described features for boiled bones for 2 or 4 h; that is, mainly smooth homogeneous surfaces (Botella *et al*. 2000; Bosch *et al*. 2011; Trujillo-Mederos *et al*. 2012; Solari *et al*. 2013). On the other hand, the second group shows dark areas, and a morphology similar to that described in previous works for burnt bones (Holden *et al*. 1995; Quatrehomme *et al*. 1998; Pijoan *et al*. 2004a, 2007). As in grilled bones, there are burnt fragile areas and small flakes of the surface can be taken out, through taphonomic processes, revealing the inner material, which then presents the features of a low-temperature treated bone, as we observed in samples M5 and M6.

The collagen fibres were found to be degraded differently in the samples of the first group compared to those of the second group. They constitute an amorphous crust in the first group, whereas in the second their morphology is less altered. Hence the samples from the first group were thermally treated for a longer time than those from the second group. Again, this difference is consistent with boiling for 2–4 h. On the other hand, as grilling is fast—because otherwise the meat burns—the collagen fibres maintain some of their initial morphology (Koon *et al*. 2010; Solari *et al*. 2013). The Tlatelcomila bones, grilled and boiled, were found in the same location; therefore, the cooking of the meat was through a grilling or a boiling process. It is not surprising to find two procedures to cook human meat at the same site. Both boiling and grilling were used in Mesoamerican ritual anthropophagy (Pijoan and Pastrana, 1987, 1989; Pijoan 1997; Pijoan *et al*. 2007).

Colour and cooking

As already discussed, all samples were thermally treated, through either boiling or grilling, but this does not explain their colour. In this work, we observe that grilled and boiled bones are

yellowish up to intense red, as shown by the UV–Vis spectra and the naked eye. However, despite the fact that all the bones were embedded in one sedimentary area, they show two well-defined palettes that match the type of heat treatment.

Conventionally, colour is attributed to several factors, the first and most usual being taphonomy (White and Hannus 1983; Lyman 1994; Hedges and Millard 1995): it can also be due to intentional pigmentation resulting from mortuary rituals (Wreschner 1980; Cervini-Silva *et al*. 2013; Ávila *et al*. 2014) or, lastly, to thermal treatments.

In the Tlatelcomila bones, the colour could be attributed to armbrusterite, which is dark reddish brown. However, armbrusterite is found only in some bones and not in others; thus it cannot lie at the origin of the colour—nor can soil minerals, such as hematite or ochre (Lyman 1994), because the corresponding elements are not detected in EDS. As, again, no Hg or Fe are detected, the possible use of *post mortem* ritual cinnabar or any other inorganic red pigment has to be discarded. Hence, the colour must have been acquired before burial, either through thermal treatment or through some organic dye that has gone undetected by EDS or X-ray diffraction.

In the literature, we find that thermal treatments have been often associated with colour modifications. In cremated bones, colour has been correlated with heating time and temperature (Shipman *et al*. 1984; Buikstra and Swegle 1989; Correia and Beattie 2001), while in boiled or grilled bones it has been associated with exposure time (Botella *et al*. 2000). Botella *et al*. (2000) report that it necessarily takes 3.5–4.5 h of boiling time to observe changes in bone cortex colour. However, in a previous work we found that colour does not vary with boiling time (Trujillo-Mederos *et al*. 2012). The degradation of collagen, at temperatures less than 100°C, is not linked to colour variations. Furthermore, as already stated, the inside of all the Tlatelcomila bones is ivory white, which reveals that the colour is only present on the external surface and, thus, the colour is not due to time exposure at low temperature, but it must be due to surface reactions with exogenous agents during cooking (Huculak and Rogers 2009).

The Tlatelcomila grilled bones are all similar; they all present the same colour, showing that the temperature and diffusional processes are the same in the three samples. This is not surprising, since they were all in contact with the meat for a short time (less than 1 h; otherwise the meat would be burnt) at a similar temperature. In the grilling process, the meat juices concentrate around the bone and diffuse into it slightly, and dark patches are observed. The reddish patches, then, are due to blood coming from the meat as the temperature increases (Huculak and Rogers 2009). The resistance of grilled bones is heterogeneous, as the temperature reached is different in the meat-covered zones compared to the directly exposed areas.

On the other hand, bones corresponding to boiled samples are all heated at the same temperature, less than 100°C, but their colour is homogeneous and it varies from sample to sample. As already stated, there are no patches. The exogenous agent responsible for the colour then has to be taken from the solution used for boiling. In this sense, the boiled bones must have been in contact with solutions the compositions of which could have been different (salted or unsalted, and with spices or grease, amongst others).

In Pre-Hispanic Mexico, meat was cooked at low heat with ingredients such as *axiote* (*Bixa orellana*), which was also used as a dye, *pipián* (*Cucurbita argyrosperma*) and a vast variety of chillis (*Capsicum*), amongst others, which are usually red or green (Guirola 2010; Powis *et al*. 2013). These condiments contain high amounts of carotenoids, which are well known as dyeing agents (Devia and Saldarriaga 2002).

To illustrate our proposition, we boiled a cow bone in an *axiote* solution. *Axiote* was chosen as it is a strong dyeing agent, still used in Mexican cooking; it is, of course, an extreme case to exemplify how bone may be coloured through a boiling process in the presence of some

condiments. The resulting bone presented the same colour as the archaeological bones (Fig. 9), showing that the bone surface colour can be attributed to the cooking of savoury dishes.

In this sense, all correlations linking temperature, bone colour and cultural habits have to be reconsidered. At temperatures less than 100°C, bone colour is an indicator of the cooking procedure, but not of the thermal treatment time. The colour of the Tlatelcomila bones is due to the cooking procedures.

CONCLUSION

The comparison between boiled and grilled archaeological human bones from Tlatelcomila shows that the bone surface morphology in boiled or grilled bones is different. The macro and nano observations converge and provide the same results. As far as the collagen is concerned, it is denatured in both series of samples, and the fibrils expand until they progressively lose their features. Boiling for 2–4 h denatures collagen. Last but not least, the colour is not indicative of the temperature or exposure time to the boiling or grilling processes. The differences in colour have to be attributed to the cooking recipes used in Mesoamerica. The surface colour of the Tlatelcomila boiled bones may be explained by a combined effect of temperature, cooking time and the composition of the cooking liquid soup.

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