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ORIGINAL PAPER

The tale of Henry VII: a multidisciplinary approach to determining the post-mortem practice

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Abstract During the Middle Ages in Europe, a different post-mortem funerary custom came to be used in order to transport and solemnly dispose of the bodies of high-status individuals. Because of their high degree of mobility, most medieval kings and queens rarely died where they had planned to be buried; thus, they had to be moved to the place of burial. Ancient writings describe some post-mortem funerary practices carried out to facilitate transport, such as boiling or burning of bodies after death.

The remains of Henry VII of Luxembourg were analysed in order to determine which post-mortem practices were utilized. A detailed chemical-physical analysis was conducted to highlight the changes in the bone matrix due to post-mortem alteration. Boiling and burning leave different marks in the bone that could be differentiated through the analysis of the inorganic and organic components of the bone. Accordingly, anthropological, X-ray diffraction (XRD), infrared spectroscopy (FT-IR), collagen ratio, and scanning electron microscopy (FE-SEM/EDAX) analysis were performed on two different bone fragments: cranial and tibial shaft. This multidisciplinary approach has enriched scientific understanding of the post-mortem practices to which the skull and tibial shaft of Henry VII were subjected. The results highlight that the tibial shaft was treated under higher temperature respect to the skull. Furthermore, this analysis also shed light on the state of preservation of the bone fragments analysed and has allowed us to initiate more complex molecular analysis, as well as ancient DNA analysis.

Keywords Taphonomic practice · FT-IR · SEM · Collagen · Boiling · Ancient bone

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Introduction

On August 24, 2013, after the seventh centenary of Emperor Henry VII's death, his coffin was opened (Fig. 1). Henry VII of Luxembourg was a very important historical figure. Born in 1275 in Valenciennes—a town in the Nord-Pas-de-Calais region of the country—he was the son of Count Henry VI of Luxembourg and Beatrice d'Avesnes. As such, he was a member of an important family of counts whose lineage dated back to the noble Siegfried house of Luxembourg between 936 and 998 AD. Henry VII was the count of Luxembourg, and on the sixth of January, 1309 AD, he was elected king of Germany in Aachen, despite the opposition of the King of France. While Henry was in Milan, he probably met and may have befriended Dante Alighieri. He also received the Iron Crown, the crown of the king of the Romans, while he was living in the city, in 1311. On June 29, 1312, Henry VII was crowned the Emperor of the Holy Roman Empire—a title he

Fig. 1 Henry VII's remains inside the coffin (Cathedral of Pisa, Italy)

held until his death in 1313 AD (Ferrai, 1889). During the struggle between Guelphs and Ghibellines, he ultimately sided with the latter, putting himself in conflict with the King of Naples, Robert of Anjou, the head of the Guelphs. He inspired poems of praise from Dino Compagnoni and Dante Alighieri (Rossetti 2011). He allied with Frederick II of Aragon, King of Sicily, to organize an expedition against Robert of Anjou, but Henry VII died in Buonconvento in 1313 (about 28 km from Siena) before he could restore the imperial authority (Ferrai, 1889).

Before his death, he requested that his corpse be buried in Pisa according to the local funereal rites (Mingozzi et al. 1893). Tradition dictated that the body was to be boiled or burnt to strip it of its flesh. As a result, only the bones were interred (Aries 1985).

Boncompagno from Signa (Signa 1170—Florence after 1240) in "*De cosuetudinibus sepelientium*" described boiling post-mortem important characters in the Germanic "*Mos Teutonicus*" (Latin: *the German custom*). This was a post-mortem funerary custom used in Europe during the Middle Ages as a means of transporting and solemnly disposing of the bodies of high-status individuals. Most medieval kings and queens had burial places assigned or constructed years before their deaths. But high mobility in the Middle Ages resulted in people rarely dying where they had planned to be buried. Records from the tenth and eleventh centuries prove that the wish for a royal burial place was taken seriously by the survivors, since several corpses of Holy Roman emperors of the Ottone and Salian dynasties were transported from their place of death to their intended burial place (Gerbektm et al. 1772).

The defleshing occurred during the transport of his body to Pisa, near Paganico. After a boiling and a stripping, his corpse was placed on a pyre, as reported by Trenta (1893). Del Guerra (1931) suggested that his body was not cremated but quickly burnt in order to destroy only his soft tissue (Albertino Mussato: *De gestis Henrici VII Caesaris historia augusta*, lib. XVI, in *Rerum Italicarum Scriptores* di L.A. Muratori tomo X and *De gestis Italicorum* post-mortem *Henrici VII n Rerum Italicarum Scriptores di L.A. Muratori col. 583–586*). His bones therefore remained and were laid to rest with his imperial insignia (i.e. crown, scepter and gilded silver ball) inside his tomb in the church of Suvereto, where he was deposed behind the main altar of the cathedral until September 2, 1313, when his bones were moved to the tribune of the High Altar in the chapel of St. Bartholomew in Pisa. In 1493, the tomb of Henry VII was moved again to the Cathedral of Pisa, where it is still kept today. Henry VII's body has been located in the apse of the Cathedral of Pisa in a wooden coffin, during the second survey in the 1921.[1] The first was in 1725 (Dal Borgo F. Raccolta di Scelti Diplomi Pisani. Pisa. 1765); his coffin was opened and placed inside a lead tomb. Inside his coffin, disjointed and accumulated bones rest on a kind of silk bedsheet together with a scepter, a crown and a globe, all in silver gilt. These all rest on a layer of ash and burnt wood about 15 cm thick.

The bone is composed of two different components: inorganic (approximately 70–80 %) and organic (around 20–30 %) (Posner et al. 1984; Sillen and Parkington 1996). During the burial period, various chemical and biological processes could attack both aspects, resulting in the poor preservation state of the bones found (Hedges 2002). The dissolution of the inorganic component depends on the environmental conditions, particularly on the physical and chemical proprieties of the surrounding soil, such as groundwater, soil hydrology and pH (Nielsen-Marsh et al. 2000; Smith et al. 2007). The diagenesis of the organic component depends on the extent of the microbial attack, fungi (Marchiafava et al. 1974) and on the hydrolysis of the peptide bond (Nielsen-Marsh et al. 2000; Hedges 2002; Smith et al. 2007). The organic dissolution could be also promoted by bacteria through the attack of the gut's internal bacteria; after death, in the very early stages of degradation, these bacteria start to damage and destroy the biomolecules in the body, changing the preservation of the bones (White and Booth 2014; Jans et al. 2004).

There is no evidence from manuscript sources that the body of Henry VII was interred in the ground. Nord et al. (2005) suggested that a wooden coffin could preserve the skeleton if the grave was placed inside a protective structure which prevented contact between the soil and the skeletal remains. If the skeleton is not in contact with the soil, this prevents damage in the form of attack by soil bacteria and fungi, acid water percolation, groundwater ionic transport or moist environments. The natural process of bioerosion, due to the attack of the gut's internal bacteria, might also be affected by the removal of the soft tissues with the primary treatment, because

[1] Notaio Palamidessi Fausto: a) verbale di apertura del sarcofago dell'Imperatore Arrigo VII di Lussemburgo repertorio n. 2011 fascicolo 463; b) atto pubblico di traslazione e tumulazione dei resti mortali dell'imperatore Arrigo VII di Lussemburgo addì 26 settembre 1921, registrato a Pisa il 30 settembre 1921 al vol. 154 n. 325 fascicolo 639 repertorio 2678.

microbes are also eliminated in this process. Moreover, the diagenetic conditions which attacked the skeleton would probably also degrade the silk bedsheet, but this is well preserved. Thus, the action of the soil environment and microbial attack could be excluded from the diagenesis of Henry VII's body, suggesting that the diagenetic marks present on his skeletal remains should be attributed mostly to the post-mortem practices to which he was submitted.

The aim of the present work is to try to better understand what happened to the Henry VII remains after his death and the taphonomic practices to which he was subjected. Boiling and burning will have two different impacts on the bone; indeed, there is a distinct difference in crystal size between higher and lower temperatures heated bone (Shipman et al. 1984). Moreover, due to the direct contact with the fire, the intensity of the heat source resulted higher in the burning, compared to the boiling procedures. At about 100 °C, exchange between bioapatite phosphate and water is very slow relative to structural carbonate–water exchange (Lee-Thorp 2002). Shemesh (1990) and Person et al. (1996) proposed that changes in crystallinity are inherently linked to diagenetic alteration. The crystallinity index (CI) or splitting factor (SF) is a measure of the degree of order within the bioapatite or hydroxyapatite structure. It is a function of crystal size and strain, structural defects and composition (Person et al. 1995). Following death, crystallinity can increase as bioapatite crystals grow and reorganize (Stiner et al. 2001), processes that are facilitated by heating.

Moreover, both the inorganic component and organic component may undergo changes in their chemical composition and structure due to post-mortem alterations of bone (Reiche et al. 2003); indeed, the burning destroyed the organic component faster (Roberts et al. 2002).

For this reason, a combined approach was used in order to clarify what happened to the bone remains of Henry VII. Bone structure x-ray diffraction (XRD) and infrared spectroscopy (FT-IR) were applied, because these techniques inform the crystallinity of bone apatite and the precipitation of foreign minerals in the bone matrix. The state of preservation of the organic component was performed, measuring gravimetrically the amount of collagen in the bone sample. In addition, this information has accompanied with an anthropological analysis of the bone remains and surface morphology of the samples, using scanning electron microscopy (FE-SEM), to highlight changes and ruptures of the microstructure of the bone matrix.

Materials and methods

Sample

The skeletal elements analysed were cranial fragment and a portion of the tibial shaft. An anthropological analysis was performed, and the colour of different bone districts was attested in order to highlight the presence/absence of burning traces. For the inorganic component analysis, five different repetitions were performed.

The analysis of organic and inorganic component of modern human bones (taken from a deceased subject about 30 years ago and provided by the laboratories of Forensic Medicine of the University the University of Rome Tor Vergata) was used as a reference standard.

X-ray diffraction

XRD data were collected using a Rigaku Miniflex diffractometer, operating in step-scan mode and employing Cu Kα radiation at 30 kV and 100 mA. Flat fragment of the tibia and skull were gently front-packed into glass holders, without any other preparation. The measurements were collected from 10 to 80°, with a 0.010° step size and a scan speed of 0.25° min^{-1}.

Fourier transform infrared spectroscopy

The FTIR-ATR (attenuated total reflectance mode) analysis was performed by using both the FT-IR IS50 (Thermo Fisher Scientific, Whaltman, MA, USA) and FT-IR Spectrum One (Perkin Elmer, Whaltman, MA, USA), using the following instrumental parameters (Surovell and Stiner 2001): resolution = 2 cm^{-1}, gain = 4, range = 4000–400 cm^{-1}, mode = absorbance, scans = 64.

This is a non-destructive analysis because the bone fragment is placed on the ATR cell. Samples from the tibia and skull were subjected to this analysis. For the FT-IR analyses, the most important parameter analysed is the crystallinity index (CI_{FTIR}) or splitting factor (SF), which corresponds to the degree of diagenesis of hydroxyapatite. This is calculated summing the heights of the peaks around 565 cm^{-1} ($\nu4$ PO_4^{-3}) and 605 cm^{-1} ($\nu4$ PO_4^{-3}) and dividing this value by the height of the valley between them, approximately at 595 cm^{-1} (Featherstone et al. 1984; Lee-Thorp and van der Merwe 1991; Nielsen-Marsh and Hedges 2000). Moreover, the C/P ratio was calculated using the height of peaks around 1035 cm^{-1} ($\nu3$ PO_4^{-3}) and 1415 cm^{-1} ($\nu3$ CO_3^{-2}). The FT-IR spectra also showed an increase of calcite ($CaCO_3$) in the bone, with a peak at 710 cm^{-1} and the presence of francolite (F-apatite) with a peak at 1096 cm^{-1} (Shemesh 1990). In addition, the following parameter associated with heat-induced changes across the spectra was also calculated: carbonyl to carbonate ratio ($CO/CO_3 = A_{1415}/A_{1455}$) (Thompson et al. 2009). C/P and CO/CO_3 parameters are calculated by dividing the height at the different wavenumbers. The increase of calcite is determinate with the presence of peak at 710 cm^{-1}. All heights are measured from a common baseline drawn (Fig. 2).

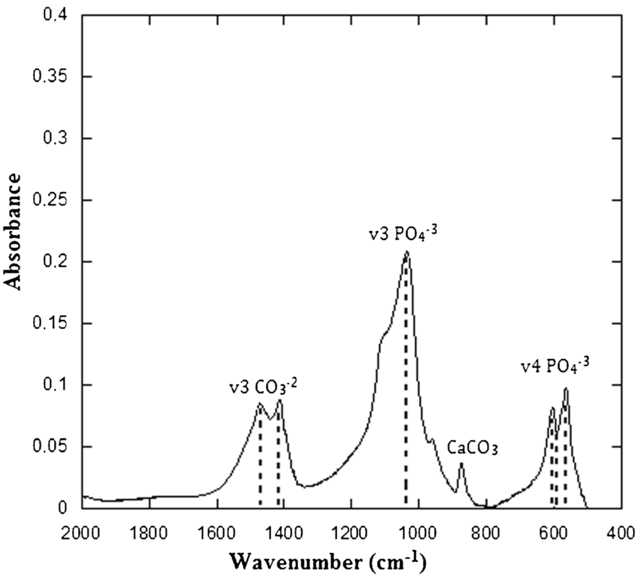

Fig. 2 Typical FT-IR spectrum of modern bone with the absorbances attributed to each peak

Collagen extraction

The extraction of collagen was performed using the modified protocol delineated by Longin and colleagues (Brown et al. 1988). In addition, the extraction was performed in parallel on a modern sample used as a control reference.

This method also provides cleaning of bone, demineralization, gelatinization and lyophilization. The cleaning of bone involves scraping the entire surface of the bone with a sterile surgical knife (bistoury). Subsequently, a bone fragment was taken and pulverized with a mortar. To obtain a satisfactory yield of collagen, the extraction has to be performed on at least 500 mg of bone powder. The bone powder thus obtained has to be demineralized so that 8 ml of 0.6 M HCl at 4 °C is added to the powder. The test tubes are agitated manually to allow a first action of the acid and then are placed on a horizontal shaker overnight in a cold room (4 °C). Acid was replaced until complete dissolution of the inorganic component of the bone. The samples are then centrifuged for 10 more minutes at 4000 rpm, and the acid is removed: the remaining pellet is subjected to 3 cycles of washing with 10 ml of ddH_2O in order to eliminate the acid completely. Once the 3 cycles of washing with ddH_2O was completed, 8 ml of HCl with pH 3 (0.001 M) at room temperature was added and, subsequently, the samples were placed in a stove at 75 °C for 24 h. The acid enables the insoluble collagen to gelatinize, and subsequently, the soluble collagen is separated from the rest of the insoluble matter by means of a centrifugation at 4000 rpm at 26 °C for 15 min then lyophilised.

Deproteinization of modern bone

The deproteinated modern bone was used as a control in both analyses of bone preservation. The modern bone was used as a non-burned and non-boiled control, while the deproteinated material represents a bone which has lost its organic fraction. It could be used to determine whether bioerosion or post-mortem practices were applied. The deproteination of bone was performed using the following protocol, described in the literature (Nielsen-Marsh and Hedges 1999). About 4 g of bone powder are placed in a closed container with hydrazine hydrate, 98 % ($N_2H_4 \cdot H_2O$), and about 15–20 ml of hydrazine are used for each gram of bone. The bone is left in a solution for 1 h at room temperature; subsequently, hydrazine is removed by decantation, and 15–20 ml of hydrazine (for each g of bone) are added and incubated in an oven at a temperature of about 70 °C for 1 h. The hydrazine is changed after 1, 10, 14 and 32 h at the same temperature (70 °C). The last hydrazine is removed by washing, and the sample is diluted with absolute ethanol; three washes are performed with this reagent at 30-min intervals with mixtures of 1:1, 3:1 and 7:1 (*v*/*v*) of absolute ethanol and hydrazine hydrate and finally three times with absolute ethanol. The sample is dried in an oven at a temperature of 110 °C.

Field emission-scanning electron microscopy/energy dispersive X-ray analyser

The morphological study of the bone samples were carried out by using a field emission-scanning electron microscope/energy dispersive X-ray analyser (FE-SEM/EDX, LEO 1550) equipped with a sputter coater (Edwards Scan Coat K550X). Afterwards, the samples were fixed in 2.5 % glutaraldehyde in a 0.2 M phosphate buffer, pH 7.4 for 3 h. Subsequently, the fragments were washed three times in 0.1 M phosphate buffer, pH 7.4 and dehydrated in alcohol (Lander et al. 2014). Therefore, the samples were placed in a dessicator for 40 h and fixed on an aluminum stub with carbon tape and then coated by a thin Au layer (with a thickness of 20 nm), deposited by sputtering. The experimental conditions of the sputtering are 2 min (as deposition time) at $I = 25$ mA.

Results and discussion

In this paper, we present the results obtained by applying the different methods of investigation carried out in different laboratories involved in research.

Anthropological analysis

Henry VII was beheaded. Signs of cracks can be seen on the right mastoid and on part of the inner surface of the angle of the left branch of the jaw (Fig. 1 in Supplementary). Moreover, Rossetti (2011) highlighted that burn marks only appear on the bone fragments and not on the skull, indicating that Henry VII's body was likely burned after death (Fig. 2 in

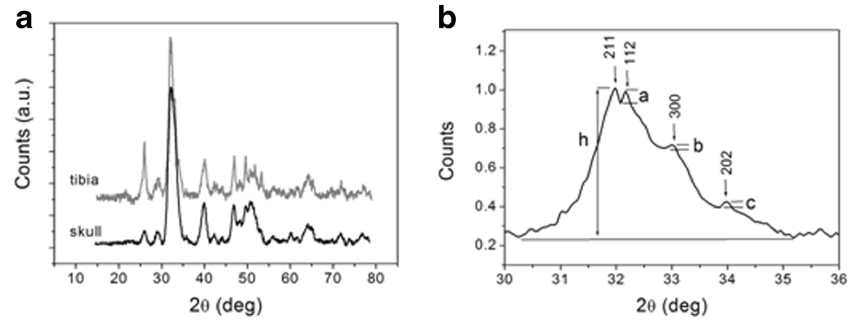

Fig. 3 X-ray diffraction spectra: **a** obtained from the bone fragments analysed; **b** zoom of the particular range between 30 and 36° of the skull remain analysed

Supplementary). It appears that the body of Henry VII was first stripped and then burned. The post-cranial bones of Henry VII do not show cut and scraping traces, meaning that it was probably manually stripped of tissue. Subsequently, the post-cranial bones were quickly burnt, and—in the areas where small fragments of meat, tendons and fat were still present—the high temperature changed the colour of the bones (Fig. 2 in Supplementary). In fact, if the corpse was burned with all its tissues, the heat—because of the presence of the soft parts and especially of fats—would have reached very high temperatures, resulting in the burning of the flesh and the deformation of the bone, at which point it would take on different colours (from yellow to black) and would cracked in a conchoidal manner (Petiti, 2009).

X-ray diffraction

Figure 3 shows the XRD patterns obtained from the skull and tibia bone. The bone presents a typical poorly crystallized XRD pattern of HA in a hexagonal symmetry (ref.: JCPDS no.9–432). The peaks at 2θ = 25.8° (002), 28.1° (102), 29° (210), 31.8° (211), 32.9° (300), 34.1° (202), 39.6° (310), 46.7° (222), 49.5° (213) and 53.2° (004) exist in all samples. No calcite seems to be present. Again, within the error range of 0.01°, peaks are not shifted and, therefore, no significant recrystallization is present.

Powder X-ray diffraction (XRD) was used to measure the crystallinity (CI) of archaeological bone phosphate. The crystallinity index was determined according to Person et al. (1995, 1996): the goal is to discriminate the separation of peaks corresponding to reflections [211], [112], [300] and [202]. As in Fig. 2b, which shows the region between 30° and 36° of the XRD spectrum relative to the tibia bone, height is measured between the average value at the top of a peak and the value of the 'valley' separating it from the following peak. By this measure, the tibia and the skull appear to have CI_{XRD} of 0.22 and 0.10, respectively (Table 1).

Fourier transform infrared spectroscopy

The FT-IR spectra obtained from our samples are shown in Fig. 4. All the samples show the following apatite characteristic bands: 1028–1100 cm^{-1} (ν3, P-O stretch), 960 cm^{-1} (ν1 sym, P-O stretch), 603 cm^{-1} (ν_4, P-O stretch) and 564 cm^{-1} (ν_4, P-O stretch and P-O bending).

Through the study of the IR absorption spectra (Fig. 4), it was possible to calculate the diagenetic parameters of both samples of Henry VII and of the reference standards, modern bone and modern bone deproteinated. These are reported in Table 1. These parameters are a useful tool to evaluate the post-depositional history of bone remains.

Generally, the C/P ratio ranges from 0.5 in modern, undegraded bone to around 0.1 as diagenesis progresses. In addition, this relationship may also increase if the sample absorbs calcite from the environment (Smith et al. 2007). The decrease of the C/P ratio value may indicate a loss of carbonate during rearrangements or dissolution of the inorganic phase for bones that have suffered alteration (Nielsen-Marsh and Hedges 2000). This is evident in both samples of Henry VII and in the modern deproteinated bone. Moreover, this ratio allows one to comment upon changes to the carbonate content of the bone following burning, which decreases as consequence of the carbonate decomposing at higher temperatures (Thompson et al. 2009, 2013).

Table 1 Diagenetic parameter values, obtained by analysing the modern control and Henry VII bone samples

Samples	CI_{FTIR}	CI_{XRD}	C/P	CO/CO_3	% $CaCO_3$	% Collagen
Modern	2.77 ± 0.01	0.07 ± 0.03	0.62 ± 0.08	0.93 ± 0.01	< 3	19.80
Henry VII cranial fragments	3.30 ± 0.08	0.10 ± 0.03	0.31 ± 0.09	0.89 ± 0.02	< 3	–
Henry VII tibial shaft	3.62 ± 0.05	0.22 ± 0.03	0.31 ± 0.07	0.92 ± 0.02	< 3	10.60
Modern deproteinated	3.20 ± 0.14	0.19 ± 0.02	0.33 ± 0.05	0.95 ± 0.05	< 3	0.00

– no data for the corresponding analysis were acquired

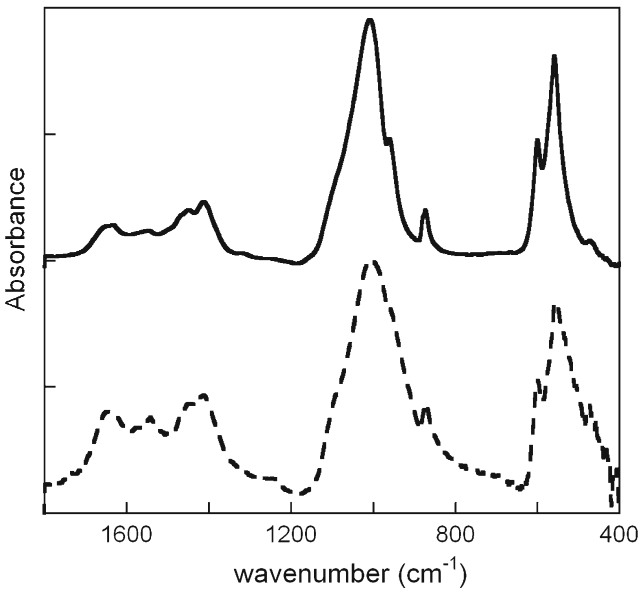

Fig. 4 FTIR-ATR spectra of (*solid line*) tibia and of skull (*dashed line*) of Henry VII. Spectra are stacked for clarity

Also the carbonyl/carbonate ratio (CO/CO_3) was evaluated to glean information about the organic and inorganic material present in the bone (Thompson et al. 2009, 2013). Generally, the peaks for carbonyl and carbonate are visible when bones are submitted to 500 °C reducing in intensity with increasing temperature due to decomposition of collagen (Thompson et al. 2009). CO/CO_3 ratio values higher than 1.2 suggest that the bones are burning at a high temperature, though the specific temperature cannot be determined. Table 1 shows that the CO/CO_3 values are similar to the modern samples and slightly higher in the tibial shaft sample excluding high temperature burnings.

To enhance our interpretation of the diagenesis of Henry VII's bones, the crystallinity index (CI) was also calculated using the FT-IR technique. Table 1 indicates that both the bone fragments analysed show diagenesis, and the tibial shaft presents the highest value. Usually, the CI value in samples with a good preservation state falls within a range from 2.7 to 3.3, as during the diagenesis of bone, the CI value increases around 4.0. This could suggest a post-burial growth in crystal size (Smith et al. 2007); a value around 7.0 is caused by fossilization. Moreover, reflecting the structure order of the crystal within the bone, CI values change if the crystals are heated and change in size, becoming more ordered (Stiner et al. 2001). Thus, the combination of CI_{FTIR}, C/P and CO/CO_3 ratio are able to discriminate between bones heated at high and low temperatures. An increase of calcite in bone is caused by the formation of post-mortem authigenic calcite, which probably derives from dissolved carbonate in the burial environment (Nielsen-Marsh and Hedges 2000).

The diagenetic parameters (CI_{XRD}, CI_{FTIR}, C/P and CO/CO_3) obtained for the inorganic component seem to suggest a diagenesis of Henry VII's bone remains, and in order to determine whether the sample had been burned or boiled after death, we compared our results with earlier studies. Several authors (Roberts et al. 2002; Munro et al. 2007; Thompson et al. 2009, 2013) have suggested that an increase of CI_{XRD} associated with an increase of CI_{FTIR} and with a decrease of C/P and CO/CO_3 are due to exposure of the bone remains at high temperatures. Therefore, the highest values of CI_{XRD} and CI_{FTIR} calculated for the tibial shaft, with respect to the skull, seem to suggest that different practices were used for Henry VII' skull and post-cranial bones. The higher temperature was probably used for the post-cranial bones, rather than the skull. The colour of the bones and available textural evidence suggests that the skull fragment was boiled and the post-cranial bone was burnt.

Although our measurements cannot provide absolute estimates of the boiling and burning temperature, the CI_{FTIR} suggests that the skull was boiled for at least 9 h (3.5 ± 0.1 as Roberts et al. 2002 suggested). The C/P and CO/CO_3 ratios exclude the use of high temperatures in the post-mortem practice (Thompson et al. 2009, 2013).

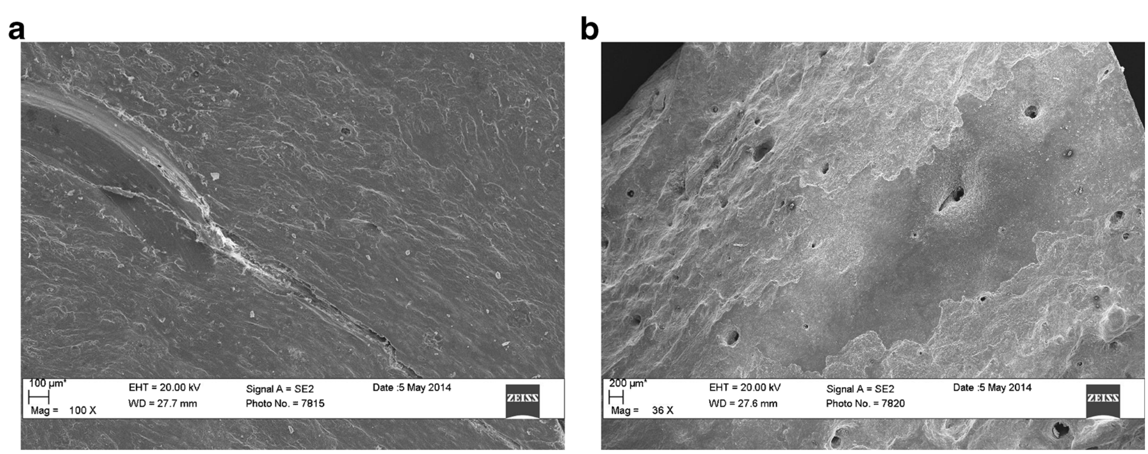

Fig. 5 SEM analysis obtained on bone fragments analysed: **a** shows microcracks and cracks on bone surfaces; **b** highlights the pit formation

Organic component

Data on collagen yields (Table 1) suggests only a limited loss of organic matrix in the sample of Henry VII. Fantner et al. (2004) proposed that the organic component is lost between 250 and 500 °C. Henry VII's organic component results showed a low loss of organic material excluding bacterial attack and suggesting that the tibial shaft was probably not exposed to high temperatures (Bigi et al. 1997).

The analysis of the organic and inorganic components seems to indicate a different post-mortem treatment for the skull and the tibial shaft, but both skeletal fragments were not subjected to high temperatures of burning.

SEM

Since the same results were obtained using both protocols, only SEM morphological figures obtained with fixation in glutaraldehyde protocols have been reported, as they provide data at higher resolution (Fig. 5).

SEM observation shows that the examined bones have a generally smooth surface, but a more detailed SEM investigation reveals microcracks and cracks on the surface of all the samples of bone (Fig. 5a). Microcracks are commonly observed, due to factors such as indicators of an increase in mechanical loading (Martin 2007), implications of skeletal fragility, stress fractures (O'Brien et al. 2005) and tension and compression forces due to muscle movement (Currey 2003). Moreover, as already discussed in the literature, chemical modifications of the bone matrix inevitably lead to morphological changes. SEM analysis revealed the presence of the larger cracks on the surface of the bone, probably due to thermal treatment (Fantner et al. 2004) as a result of prolonged exposure to high temperatures which can result in shrinkage and cracking. Furthermore, as suggested by Fantner et al. 2004, the boiled bone fracture is not a single crack but a set of multiple cracks that form the fracture (Fig. 5a). The sample's surface is also wrinkled probably due to the indirect thermal exposure associated with the action of boiling water (Pijoan et al. 2004, Bosch et al. 2011) (Fig. 5b). This surface erosion could create weak spots, and the heat of the water caused the formation of pits, which penetrated into the cortex of the bone (Fig. 5b).

Conclusion

In this work, a multidisciplinary approach was applied in order to better study the skeletal remains of Henry VII.

A series of physicochemical characterizations have been applied to the bone remains to determine the morphological, structural and compositional traits of the bone fragments. Given the importance and uniqueness of the skeletal material and, therefore, the impossibility of using large amounts of bone material, innovative techniques and minimally invasive analyses were performed.

XRD spectra showed the typical structure of bioapatite but did not reveal the presence of crystal structures compatible with thermal processes at high temperatures. Similar results were obtained by FTIR spectroscopy, which demonstrated that the mineral analysed mainly consists of apatite, with a high degree of substitution of carbonate groups. Morphological analysis revealed heterogeneous picture. Generally, the surface of the fragments examined is smooth and homogeneous. Moreover, the anthropological analysis shows evidence of burning only in the post-cranial districts. Therefore, through the different analyses, it was possible to assume that after death, the skull of Henry VII was probably boiled. This result confirms that the boiling was used during this period and that the tibial shaft and the skull were treated in different ways. The diagenetic parameters, evaluated by the FT-IR, seem to especially suggest that the tibial shaft was subjected to a higher temperature than the skull. Probably, as suggested by the absence of signs of cutting or scraping on the surface of the bones, the corpse of the Henry VII was boiled after death in order to skeletonize the body, and subsequently, only the corpse and not the skull was quickly burnt.

In conclusion, taking into account all the parameters calculated, the finding indicates a good state of preservation, enabling us to consider further molecular analysis (Scorrano et al., 2015), for example, the analysis of ancient DNA might provide additional useful information to reconstruct the story of Henry VII.

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