THE TAPHONOMY OF COOKED BONE: CHARACTERISING BOILING AND ITS PHYSICO-CHEMICAL EFFECTS

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ABSTRACT

Cooking is perhaps the most common pre-burial taphonomic transformation that occurs to bone, yet it is still one of the least understood. Little progress has been made in determining a method of identifying cooked bone in the archaeological record, despite its import for various branches of archaeology. This paper attempts to describe boiling in terms of its physico-chemical effects on bone, and uses a suite of diagenetic indicators to do this.
It is shown that cooking for brief periods of time has little distinguishable effect on bone in the short term, but that increased boiling times can mirror diagenetic effects observed in archaeological bone. The relationship between the loss of collagen and alterations to the bone mineral is explored through heating experiments, and the results compared with archaeological data. The possibility of boiling being used as an analogue for bone diagenesis in future studies is raised, and the key relationship between protein and mineral is once again highlighted as vital to our understanding of bone diagenesis.

KEY WORDS

BONE, DIAGENESIS, BOILING, CRYSTALLINITY, COLLAGEN, POROSITY

INTRODUCTION

Taphonomic studies in archaeology have become increasingly important since site formation processes were highlighted as fundamental in determining the patterning that we observe in archaeological sites. Valid interpretation of archaeological remains depends upon an understanding of how those remains came to their final resting place. Taphonomic processes include human and non-human effects, and usually archaeological remains will have been exposed to a mixture of both during their history.

In dealing with human settlements and animal bone assemblages, one of the main taphonomic transformations involving bone will be concerned with food preparation and consumption. However, it can be very difficult to discern whether a bone has been cooked. Previous investigations have attempted to identify differing taphonomic features exhibited by cooked and uncooked bones in order to recognise cooking in the archaeological record. These features include fracture patterns (Gifford Gonzalez 1989, Oliver 1993), location of cutmarks (Gifford Gonzalez 1989, Luff 1994), patterns of burning damage (Binford 1981, Gifford Gonzalez 1989, Albarella & Serjeantson 2000) and relative frequencies of skeletal elements within bone assemblages (Speth 2000). However, studies into fracture patterns have obtained contradictory results (Alhaique 1997), and cutmarks are often not present or difficult to recognise due to other post-depositional taphonomic alterations. This issue can also complicate assemblage analysis (Todd & Rapson 1988, for a review of all these topics see Lyman 1994). Some burial experiments have included cooked bone in their burial assemblages, however none of these have established unequivocally whether, or how, cooked bone behaves differently when buried (Bell *et al*. 1996; Nicholson 1996, 1998).

Despite these difficulties, the ability to detect cooked bone remains a desirable objective within archaeology (Millard 1998). The unidentifiable bias which cooked bone might impart to archaeological assemblages raises doubts as to the efficacy of assemblage analyses as well as analyses of single bones

by any of the scientific techniques currently proliferating in archaeological science. Without understanding the limitations which cooking might place on the data, it will be impossible to know how much faith can be placed in results. This is notwithstanding the other lines of investigation that might profit from such a method, such as examining gender relations manifested within food processing and consumption (Hastorf 1991), and the development and introduction of new technologies (Movius 1966, Lupo & Schmitt 1997).

When considering cooked bone, we are mainly dealing with three different processes:

1. Burning / Incineration (where the bone is normally in direct contact with fire, or an intense heat source)
2. Roasting / Baking (where the bone is protected from the heat source via insulating flesh)
3. Boiling (where the bone and flesh are heated at a constant temperature, moderated by liquid)

Of these, the first category (burning and incineration) is the only one that has had its physical effects studied in any depth (Shipman *et al*. 1984, Buikstra & Swegle 1989, David 1990, Nicholson 1993, Stiner *et al*. 1995), and as it is more often associated with rubbish disposal than with cooking, it is excluded from this study. Experimental work has also shown that when roasted or baked the bone is insulated from the direct heat source such that temperatures experienced by the bone are much reduced. A study by Alhaique (1997) showed that temperatures did not exceed 85°C around the bone at an oven temperature of 200°C, and in most cases was much lower; furthermore cooking times for roasting and baking are generally short. We therefore concentrate on boiled bone, which experiences more extreme heating, and may be prolonged (e.g. soup making and the rendering of bones for grease and fat; Brink 1997, Lupo & Schmitt 1997, Saint-Germain 1997).

Using a suite of techniques designed to determine the diagenetic state of the bone, boiling has been characterised in terms of the diagenetic effects it has on bone, allowing comparison with altered bone in the burial environment. Bone is a composite of protein (mainly collagen) and mineral (a form of hydroxyapatite, often referred to as *bioapatite*; Millard 1998, Collins *et al*. this volume,). Our approach has been designed to examine the various components separately as well as their interaction, and how their alteration affects the bone during diagenesis.

MATERIALS AND METHODS

Heating experiments have been carried out on whole bones and bone powders, and their effects characterised using the techniques detailed below. Whole bovine rib bones were boiled in pans on a gas stove, with samples taken after 3, 9, 27 and 81 hrs. Bone powder (bovine tibia) was sealed with excess water (in the presence of air) in airtight tubes, and then heated at 95°C, 85°C, and 75°C for durations of 1 week, 1 month and 4 months respectively. Those experiments carried out on whole bones were intended to replicate cooking conditions, with cooking times reflecting those which might be obtained through normal food preparation practises (3 and 9 hours), extended cooking times (27 hours) which might come about through cooking practices such as those documented in the Anglo-Saxon and Mediaeval periods (Tannahill 1988, Hagen 1992, Reynolds 1995) where bones were often left in a constant stockpot for days on end and repeatedly heated, or through the rendering of bones for grease, as observed in ethnographic studies (Brink 1997, Lupo & Schmitt 1997, Saint-Germain 1997), and a very extreme cooking time to provide an end member for the time series (81 hours). Experiments carried out on homogenised powders were used to determine key diagenetic parameters.

FTIR Spectroscopy

The bone powder is finely ground and pressed into a potassium bromide disk, which is scanned using Fourier Transform Infrared Spectroscopy (Stiner *et al.* 1995). The infrared splitting factor (SF) is determined as in Weiner and Bar-Yosef (1990). The splitting factor derived gives an indication of the stability of the bone crystals and is a function of both crystal size and the extent of the ordering within the crystal lattice (Stiner *et al.* 1995). Large, highly ordered crystals will give higher splitting factors than small, disordered crystals. The splitting factor varies from splitting factors of around SF2.7 ± 0.1 for fresh bone to values of SF≈7 for calcined or highly fossilised bone (Stiner *et al.*1995)

Collagen Extractions

Whole bone is crushed and ground to a fine powder in an agate pestle and mortar (for the test tube heated powders a Nitrogen-cooled spex mill was used before heating). Bone powders are demineralised in 0.6M HCl overnight at 4°C. The remaining acid insoluble fraction is rinsed three times in distilled water, then freeze-dried and weighed, the result given as a weight per cent insoluble fraction. For the experimental powders this insoluble fraction is presumed to be collagen, for archaeological bone a C:H:N analysis is carried out in order to confirm the residue's identity by its C:N ratio. For comparisons with archaeological data, in this paper the weight per cent insoluble fraction of the heated powders has been converted into a per cent nitrogen value. This is accomplished by using the simple formula [X * 0.185 = %N value] where X is the weight per cent insoluble fraction and 0.185 is representative of the nitrogen fraction in deamidated collagen. Using a standard value of 23% by weight for the insoluble fraction of modern bovine bone, the formula calculates a %N of 4.3 and an atomic C:N ratio of 3.2. This formula assumes that most

of the soluble proteins contained within the archaeological bone will have been leached out during burial or washed away during pre-treatment of the sample.

Nitrogen Analyses

In order to make the measurements, bone samples were homogenized to a powder; two analyses were provided on each sample. The analysis was carried out using a Carlo Erba 1106 Elemental Analyser in accordance with the manufacturers instructions, giving a per cent nitrogen content, which can be used as a proxy for the amount of collagen contained within a bone (Hedges *et al*. 1995).

Mercury Intrusion Porosimetry

Sections of bone between 400 mg and 1200 mg were cut using a hacksaw and dried (freeze-dried, or dried for 48 hours at 60°C) prior to analysis. HgIP measurements were made on a Micrometrics Autopore II 9220 at the University of Newcastle upon Tyne, with mercury being intruded into the bone under increasing pressures. The intrusion volumes are then density corrected using the measured bulk density of the sample, and plotted as a pore size distribution (PSD) graph. Data are reported as ml of Hg intruded per ml of bone and pore sizes are calculated assuming a contact angle between mercury and apatite of 163.1° (Joschek *et al*. 2000).

Histology

Sections of the bone are fixed in resin, and polished before examination under reflected light (Hedges *et al.* 1995). We have used the Histological Index of Hedges *et al.* (1995), which is a graded system from 0 to 5, with 0 representing no surviving histological structure, and 5 indicating near-perfect preservation of histology. Histological structure is mainly influenced by soil micro-organisms (Hackett 1981, Bell 1990, Jans, this volume).

RESULTS & DISCUSSION

Table 1 shows the diagenetic parameters as measured on the time series of boiled bone, as well as standard values for modern bovine bone and deproteinated bovine bone. Deproteination is achieved through hydrazine treatment to remove the collagen with minimal crystal alteration, following the method of Nielsen-Marsh (1997). Note the difference in values between the uncooked bovine rib and the modern bovine standard (tibia). Although within experimental error, the variance can also be attributed to the different skeletal elements that are being examined.

As the bone is boiled, protein is lost and crystallinity increases, as does porosity. Histology remains unchanged. Figure 1 shows the correlation between nitrogen loss and splitting factor increase. There is a remarkable linearity shown, and the correlation is extremely high. Similar relationships have been noticed when examining archaeological bone, with bones low in nitrogen generally exhibiting higher crystallinity (Person et al. 1995, Sillen & Parkington 1996, Nielsen-Marsh & Hedges 2000), although the issue is rarely as clear-cut due to the many confounding factors in the burial environment. The main increase in porosity is in the range of pore sizes less than 0.1μm. The large peak that appears at ~0.02μm with extensive boiling is an indication of this (Figure 2). This peak is also observed in deproteinated bone, and has been attributed to the loss of collagen from sub-fibrillar size pores (Nielsen-Marsh 1999). There is also considerable mineral alteration shown by the

bones that display this peak, raising the possibility that some of this porosity is linked to the rearrangement of the crystal structure into a coarser configuration, as speculated on by Nielsen-Marsh & Hedges (2000). If the porosity in this region is plotted against protein content, we see a linear trend (R^2= 0.834, rising to 0.915 on addition of archaeological data) supporting a direct relationship (Figure 3). Furthermore, when the crystallinity of the same samples is plotted against small pore porosity, this correlation disappears, implying that crystal rearrangement has little or no influence on this feature.

Smith et al. (this vol.) reviews the archaeological data in figure 3 in more depth, documenting the diagenetic state of bones from a southern Italian mediaeval cemetery in Apigliano. These bones are curious because they exhibit extremely rapid collagen loss with no microbial attack. They share many characteristics with boiled bone, including the sharp peak in the small pore region, extensive mineral alteration and intact histology. This same peak is also exhibited by deproteinated bone, though the crystallinity is not as affected. That the traces for deproteinated bone and severely boiled bone are similar is to be expected, as collagen is lost through chemically mediated hydrolysis (Collins et al. 1995) in both cases, accelerated by different mechanisms (hydrazine hydrate and increased temperature). Finding such a correspondence in archaeological bone is surprising however, the more so considering their relatively young age. Given that these are human bones, from articulated burials, the likelihood of cooking is remote. It shows that the physico-chemical signature of boiling is not unique, and that diagenesis can mirror the effects of cooking. What may be more useful to archaeological science is the possibility that boiling can be an analogue of certain types of burial diagenesis, and with further study may facilitate greater understanding of these diagenetic processes. Boiling also increases porosity at larger pore sizes, which might have more even more severe ramifications in terms of burial diagenesis, as it has been shown that the larger pore sizes have a greater influence on the rate of bone dissolution than small pores (Pike et al. 2001).

In order to further clarify the relationship between the loss of collagen and the increase in crystallinity, heating experiments were carried out on bone powders at different temperatures as detailed above. Figure 4 shows the results of these experiments, superimposed on a background of archaeological data (samples from Elands Bay Cave analysed in Sillen & Parkington 1996, samples from Yarnton, Brean Down, Bercy and Poundbury analysed in Nielsen-Marsh 1997, and Pleistocene bones analysed in Nielsen-Marsh & Hedges 2000). It can be seen that not only does the relationship between collagen loss and mineral alteration hold, but also that it is time and temperature *independent*, at least until the vast majority of the collagen is lost from the bone. The archaeological data also shows this trend. The dataset consists of bones from different periods (Pleistocene through to Anglo-Saxon) and vastly differing environments.

Collagen is gelatinised more rapidly at higher temperatures, and its loss displays a high correspondence with changing crystallinity. The relationship between collagen loss and crystallinty is echoed in archaeological samples. The relationship between mineral and organic is fundamental to bone architecture, and bone survival. Experiments have previously demonstrated the presence of an organic 'diffusional barrier' within the bone matrix that has to be removed before any ion exchange affecting the bone mineral can take place (Walsh *et al*. 1994). In our experiments, the rate of mineral alteration is linked to the rate of gelatinisation. A decrease in mechanical strength has previously been noted in both archaeological bone and cooked bone (Pearce & Luff 1994, Turner-Walker & Parry 1995,) and is often cited as a key factor in determining bone preservation. Boiling, in disrupting the mineral-organic interface and increasing porosity might have an important influence on bone survival, but this will be entirely dependent on the duration of cooking.

CONCLUSIONS

In mirroring the patterns shown by archaeological bone we can conclude that the boiling process is to some extent an analogue of diagenesis, with similar processes occurring (loss of collagen, increasing crystallinity, increasing porosity). We have also shown, however, that it requires boiling for extensive periods to fashion these changes, with what could be considered conventional boiling times (1 – 9 hours) having little or no physico-chemical effects. This might explain the difficulties experienced by previous researchers in distinguishing cooked from uncooked bone. None of the bones utilised in burial experiments were cooked for longer than an hour (Bell et al. 1996, Nicholson 1996, 1998), and the bones used in fracturing experiments were also not heated for any considerable length of time (Alhaique 1997).

What our experiments cannot tell us is the fate of cooked bone over archaeological timescales. Bone which has been severely boiled is unlikely to survive, due to its high porosity and reduced mechanical strength, although it also has little organic content to attract further scavenging damage or microbial attack, and its altered, more stable mineral organisation may be less reactive in the burial environment.

There is a possibility that the effects of boiling, although not detectable by our analyses, might cause accelerated diagenesis through a kind of 'loosening' of the bone structure. Once in the ground, if boiling has altered the bone, mineral alteration and microbial attack might proceed more rapidly. Increased porosity might have a severe effect, with a greater surface area available for dissolution processes (Trueman 1999, Pike et al. 2000). Some indications of the possible effects of cooking are given by differential scanning calorimetry analysis of bone. Demineralised collagen from a bone cooked for 7 hours showed a significant decrease in its gelatinisation temperature (Nielsen Marsh et al. 2000), although other diagenetic parameters were not distinguishable from modern bone (Roberts, unpubl. data). A weakened collagen helix would imply that the association between the collagen and the mineral is also weakened (Walsh *et al.* 1994, Kronick & Cook 1996), which is likely to cause accelerated diagenesis.

Cooking can be considered an analogue for burial diagenesis, with similar patterns obtained as are observed in archaeological samples. With further study this may lead to an increased understanding of the diagenetic processes, and the varied chemical interactions involved in the deterioration of bone. In terms of identifying cooked bone in the archaeological record, we have discovered no singular feature that is unique to boiling. However, from our initial results we would expect boiled bone to be less resistant to all forms of diagenetic alteration, including microbial attack.

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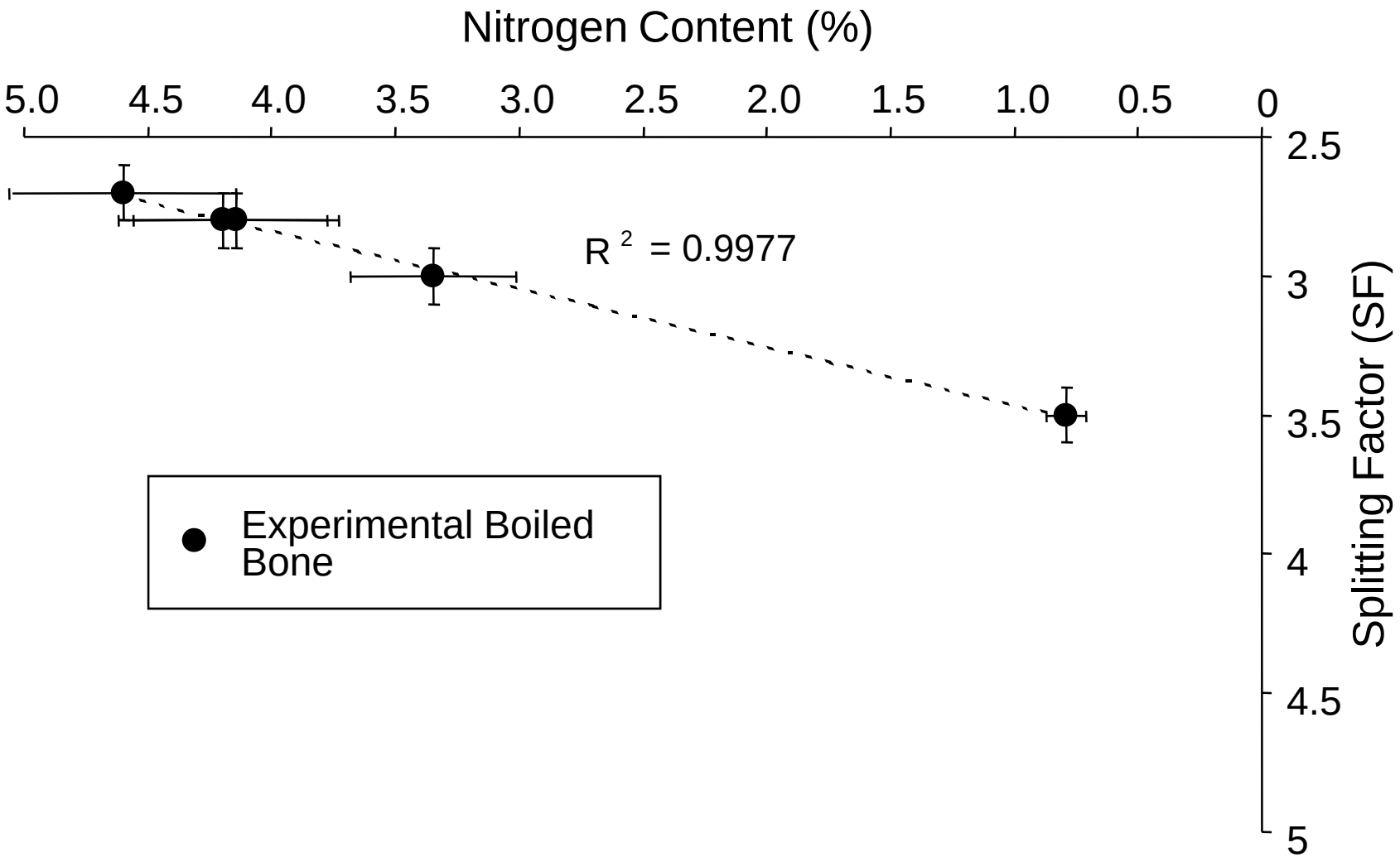
FIGURE LEGENDS

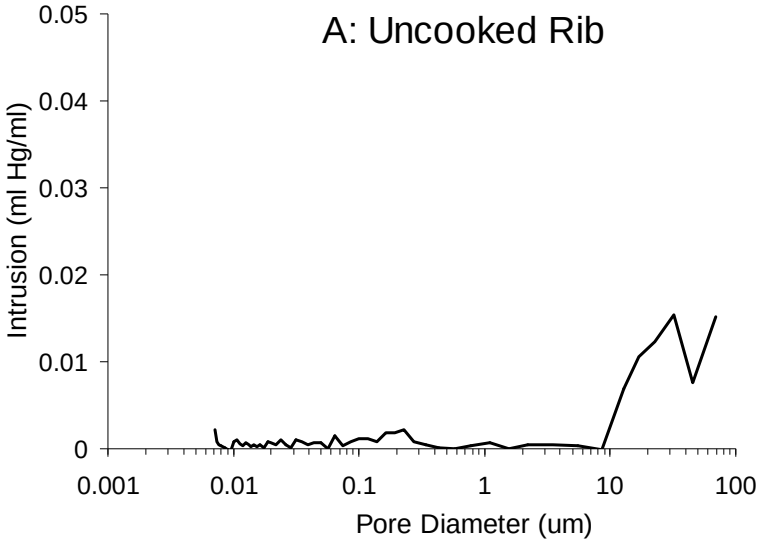
Figure 1: Correlation between protein loss, manifested by change in N% and mineral alteration, manifested as a splitting factor increase, in boiled bovine rib bones.

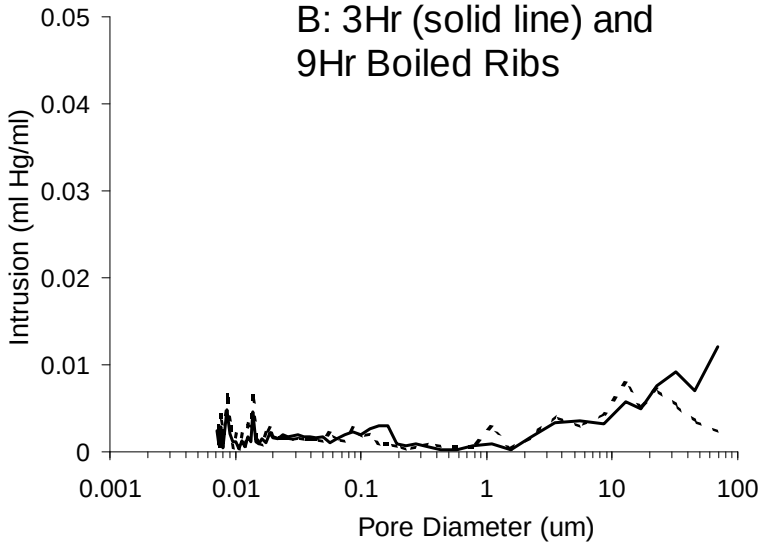
Figure 2: Pore size distribution graphs for boiled bovine rib bones. Severe alteration to the bone is not significant up until 9 hours boiling. At 27 hours a large peak is observed in the small pore region that becomes accentuated upon further boiling.

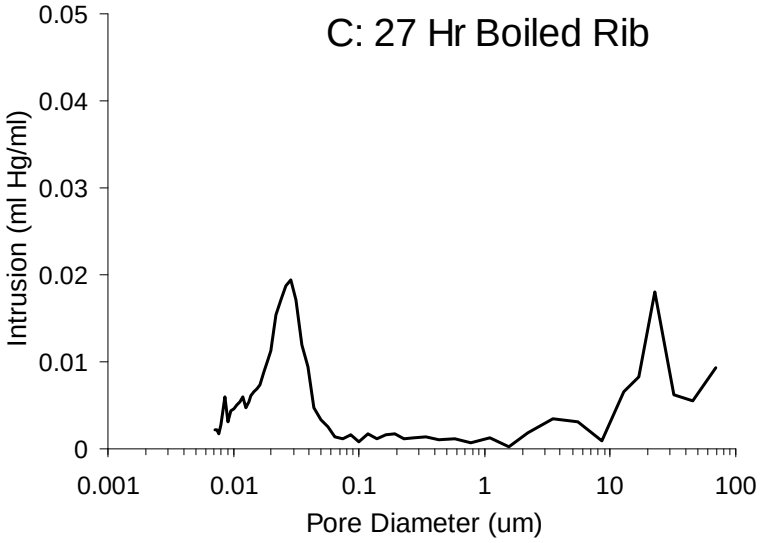
Figure 3: Small pore porosity (<0.1μm) plotted against protein content, manifested by change in N%, for a variety of bones. The Apiligiano data is published in full elsewhere (Smith, this vol.), these bones are those with histology ratings of 4 or 5 (i.e. those unaffected by microbial attack).

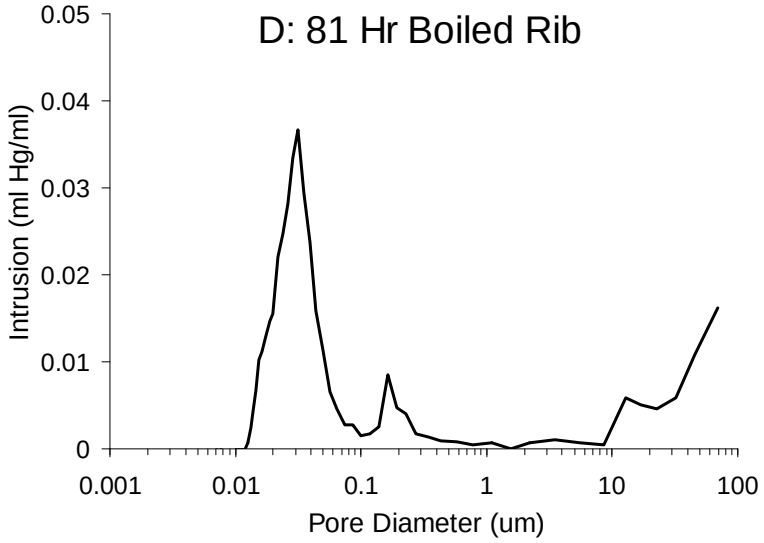
Figure 4: Mineral alteration plotted as a function of protein loss for the experimentally heated powders and bones. Also shown is a background of archaeological data showing the similar relationship observed. (Archaeological data drawn from Sillen & Parkington 1996, Nielsen-Marsh 1997, and Nielsen-Marsh et al. 2000)

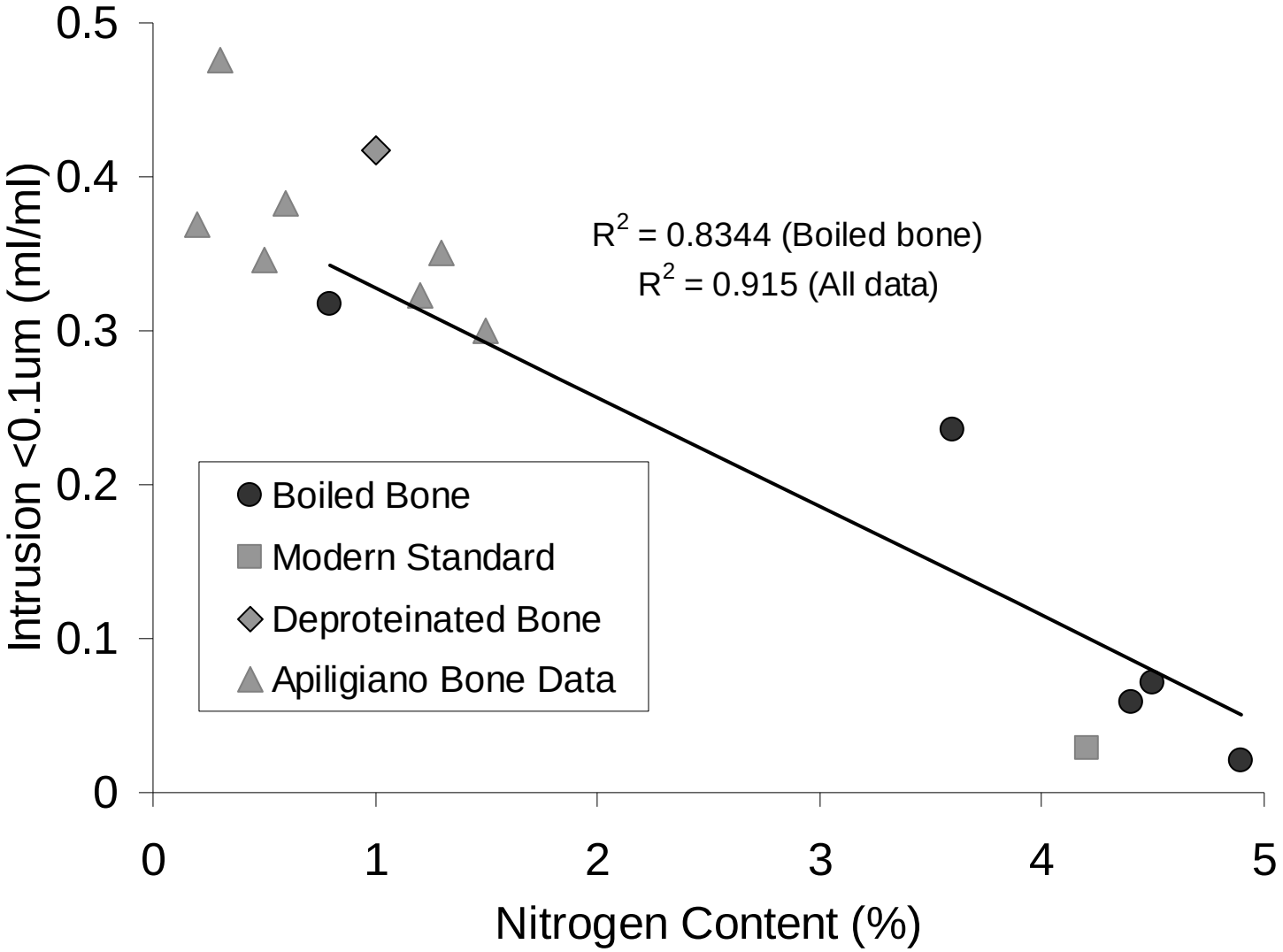

Nitrogen Content (%)
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
2.5
3
3.5
4
4.5
5
Splitting Factor (SF)
R 2 = 0.9977
Experimental Boiled Bone

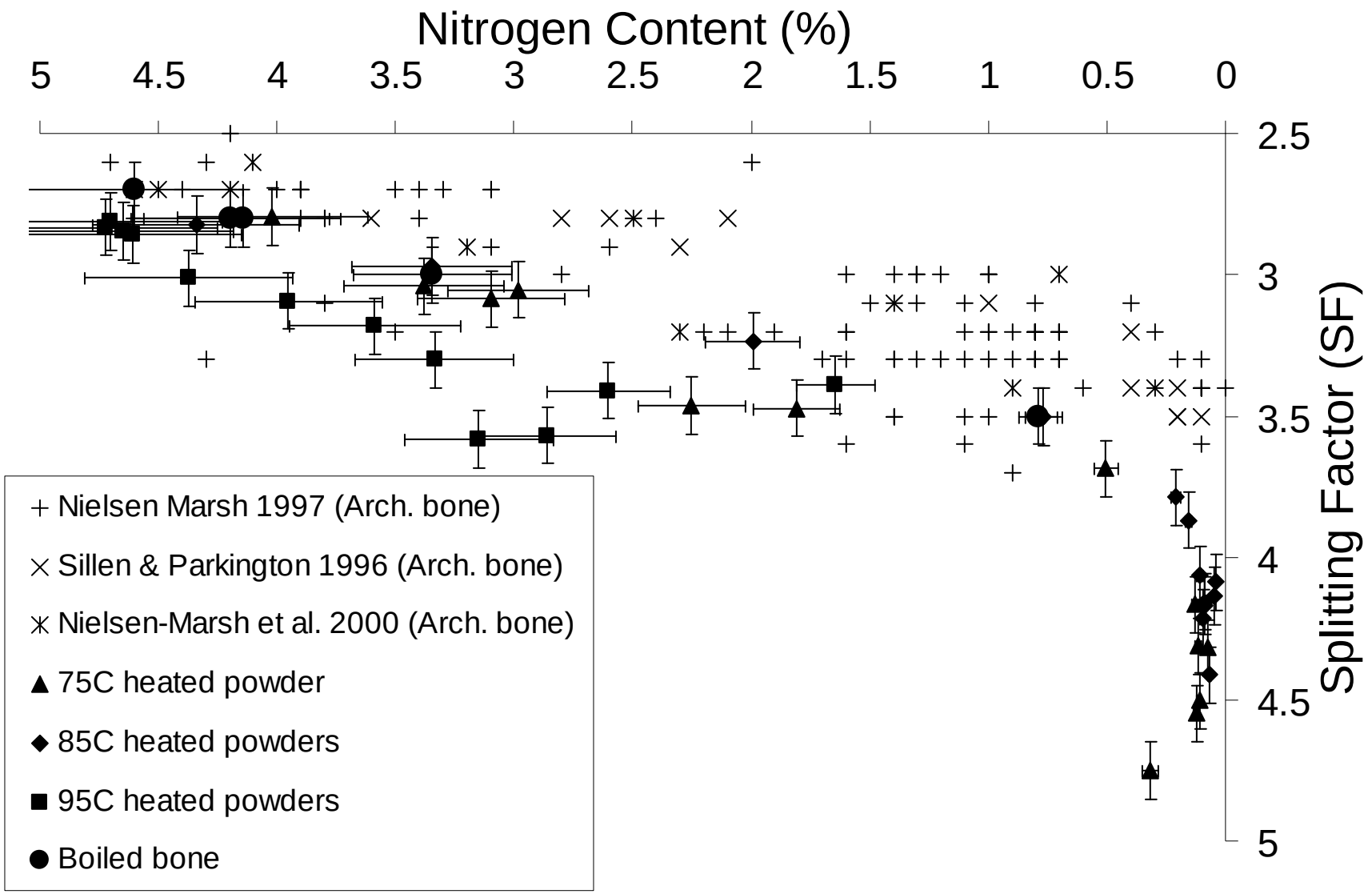

A: Uncooked Rib
Intrusion (ml Hg/ml)
0.05
0.04
0.03
0.02
0.01
0
0.001
0.01
0.1
1
10
100
Pore Diameter (um)


B: 3Hr (solid line) and
9Hr Boiled Ribs
Intrusion (ml Hg/ml)
0.05
0.04
0.03
0.02
0.01
0
0.001
0.01
0.1
1
10
100
Pore Diameter (um)


C: 27 Hr Boiled Rib
Intrusion (ml Hg/ml)
0.05
0.04
0.03
0.02
0.01
0
0.001
0.01
0.1
1
10
100
Pore Diameter (um)


D: 81 Hr Boiled Rib
Intrusion (ml Hg/ml)
0.05
0.04
0.03
0.02
0.01
0
0.001
0.01
0.1
1
10
100
Pore Diameter (um)


0.5
0.4
0.3
0.2
0.1
0
Intrusion <0.1um (ml/ml)
0
1
2
3
4
5
Nitrogen Content (%)
R2 = 0.8344 (Boiled bone)
R2 = 0.915 (All data)
Boiled Bone
Modern Standard
Deproteinated Bone
Apiligiano Bone Data


Nitrogen Content (%)
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
2.5
3
3.5
4
4.5
5
Splitting Factor (SF)
Nielsen Marsh 1997 (Arch. bone)
Sillen & Parkington 1996 (Arch. bone)
Nielsen-Marsh et al. 2000 (Arch. bone)
75C heated powder
85C heated powders
95C heated powders
Boiled bone

TABLES

Table 1: Values of diagenetic parameters measured on boiled bovine rib bones, with the standard values of diagenetic parameters measured on fresh and hydrazine deproteinated bovine bone. The values for hydrazine deproteinated bone are taken from Nielsen-Marsh (1997). Porosity values in brackets represent porosity in pores less than 0.1μm in diameter.

Bone Samples (all bovine)	**Splitting Factor (SF) [±0.1]**	**Nitrogen Content (%) [±10%]**	**Porosity [ml/ml] (*In pores <0.1μm*)**	**Bulk Density [±0.1]**	**Skeletal Density [±0.1]**	**Histology**
Uncooked Rib	2.7 ± 0.1	4.9	0.0999 (*0.0203*)	1.7	1.9	5
3 Hr Boiled Rib	2.8 ± 0.1	4.5	0.1272 (*0.0713*)	1.8	2.2	5
9 Hr Boiled Rib	2.8 ± 0.1	4.4	0.1303 (*0.0582*)	1.8	2.1	5
27 Hr Boiled Rib	3.0 ± 0.1	3.6	0.3133 (*0.2357*)	1.4	2.2	5
81 Hr Boiled Rib	3.5 ± 0.1	0.8	0.3958 (*0.3171*)	1.3	2.2	5
Standard Modern Bone (tibia)	**2.8 ± 0.1**	**4.3**	**0.0708 (*0.0287*)**	**1.9**	**2.1**	**5**
Deproteinated Bone (femur)	**3.2 ± 0.1**	**0. 3**	**0.4622 (*0.4178*)**	**1.3**	**2.5**	**5**