Effects of soft tissue on the crystallographic changes to bone mineral upon heating

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Upon the recovery of burnt remains in a forensic or archaeological context, bone is often fragmented and comingled, making differentiation between human and nonhuman samples extremely challenging and subjective. Due to the thermal degradation of the organic component, biological techniques, such as DNA analysis, render futile and so attention is drawn to the final surviving component of bone, the mineral hydroxyapatite (HAp). The fundamental physical and chemical properties of HAp differ between species, although in its native, unheated state, these differences are difficult to detect. However, when heated, the mineral undergoes a number of significant changes and crystallographic differences between species become distinctly apparent [1, 2, 3].

Exploring the physicochemical modifications that occurs to HAp upon heating has shown promise in differentiating between species based on characteristic changes within its crystal lattice structure [1]. However, the effects soft tissue may have on the heat induced changes are not fully understood, yet are of paramount importance as most bodies are intact, not skeletonised, prior to a burning event. This study aims to explore the effect heating bone in conjunction with soft tissue has on the crystallography of HAp. It is important to understand the variation, if any, between defleshed and fleshed specimens to establish if current "dry" bone protocols can be reliably incorporated into forensic and archaeological investigations.

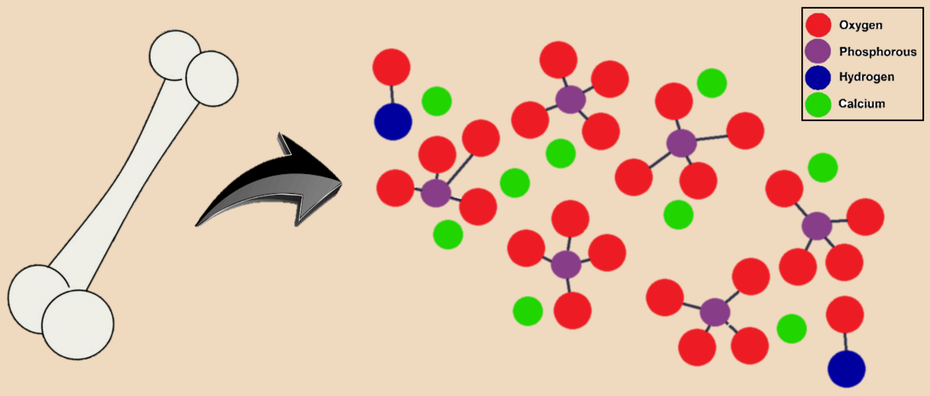
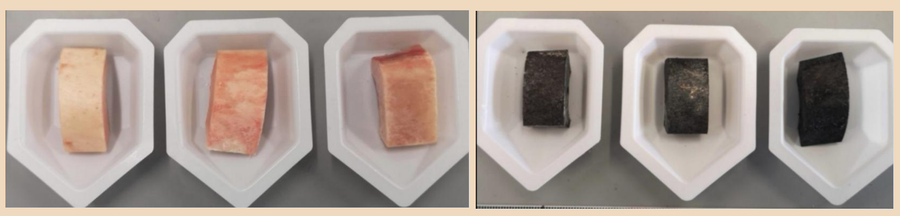


Fig. 1 – From the macroscale of bone to the nanoscale, depicted by the molecular structure of HAp

Figure 2 – The removed of soft tissue and trabecular bone from annulus before being sectioned into smaller specimens

Figure 3 – The addition of muscle, fat and skin

Figure 4 – Specimens before and after heating to 600°C



METHODOLOGY

Femoral cortical bone was taken from bovine specimens (Fig. 2). A food grade porcine joint was separated into varying soft tissue components. Varying weights of muscle, fat (5g, 7g and 10g) and one layer of skin (10g) were tested separately to understand their individual effect. Each soft tissue variant was measured and wrapped with an equal consistency around the bone specimen (Fig. 3). The samples were heated for 2 hours at either 400°C, 600°C or 900°C in aerobic conditions. The "crystallinity" of HAp was calculated by powder X-ray diffraction (pXRD) and Fourier Transform Infrared (FTIR) spectroscopy by measuring the Crystallinity Index and the relative amount of carbonate to phosphate. A Colorimeter was used to measure the colour differences between samples. Results were compared to control specimens which remained defleshed during heating.

RESULTS

As the temperature increases, HAp crystals become larger, more ordered, and have less strain, resulting in larger Crystallinity Index values with a smaller carbonate to phosphate ratio (Fig. 6 & 7). Overall, the addition of soft tissue has shown to contract this process. It had been hypothesised that the soft tissue creates an extrinsic energy source, increasing the thermal degradation of bone. However, the data indicates that the soft tissue may be providing a shielding effect to the bone by either slowing down the crystallisation of HAp, or by causing the crystallographic changes to occur at a higher temperature. Out of the three soft tissue types tested, muscle and skin have shown to be the variable with the largest disparity on the "crystallinity" of HAp. This may be due to its denser formation which is not "burnt off" as quickly as fat. There is a general trend within each soft tissue element in that larger quantities resort in a decrease in crystallographic changes. The results of the Colorimeter show that specimens heated with smaller quantities of tissue were lighter in appearance due to the heat induced calcification process (Fig. 8). This further supports the theory that soft tissue protects bone, reducing expected heat induced changes that are seen for defleshed specimens.

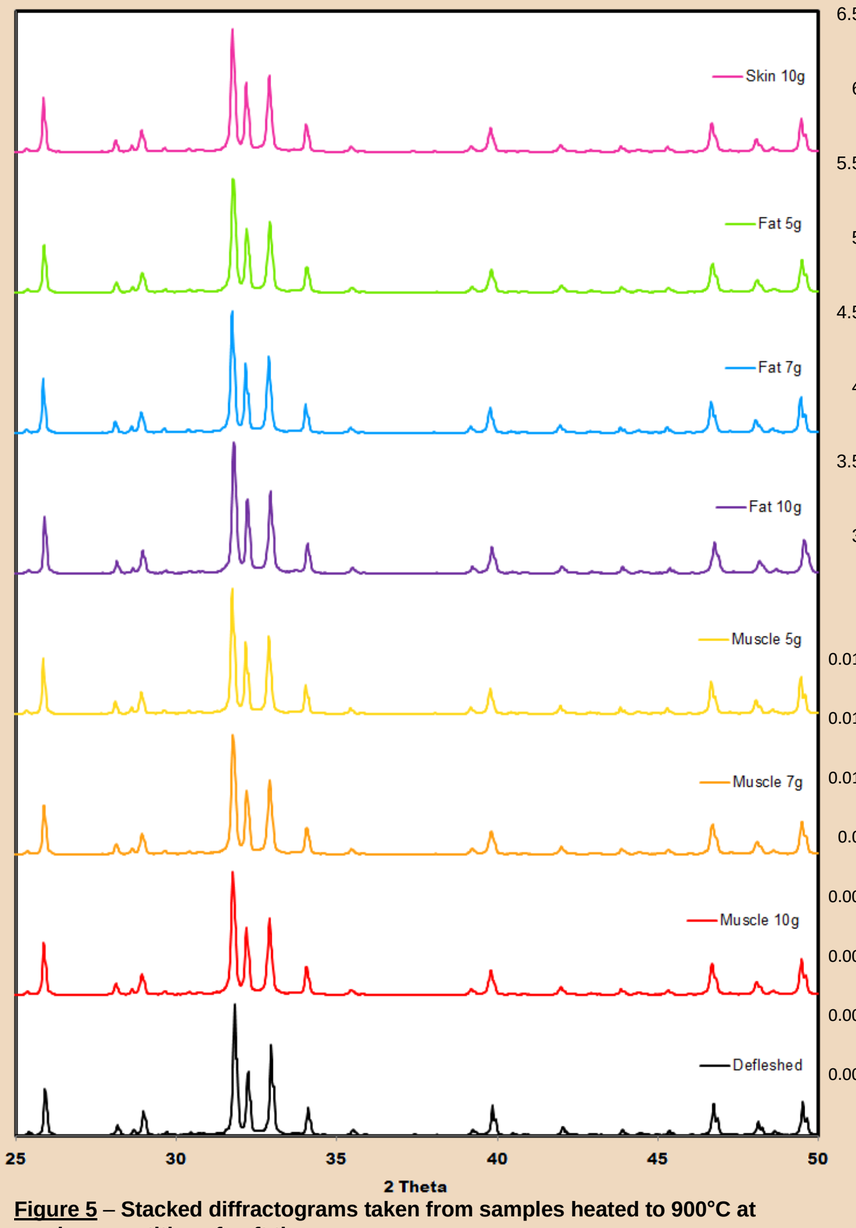


Figure 5 – Stacked diffractograms taken from samples heated to 900°C at varying quantities of soft tissue.

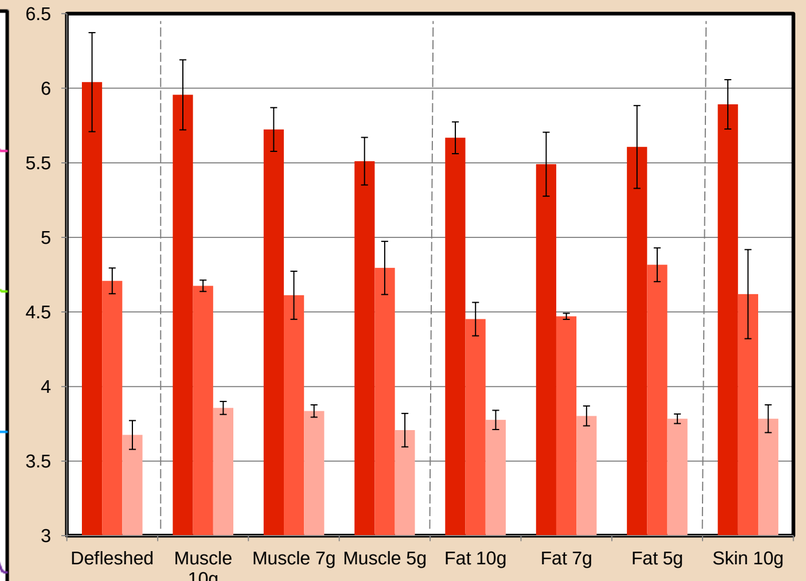


Figure 6 – The average Crystallinity Index values calculated from each specimen (■ 900°C ■ 600°C ■ 400°C)

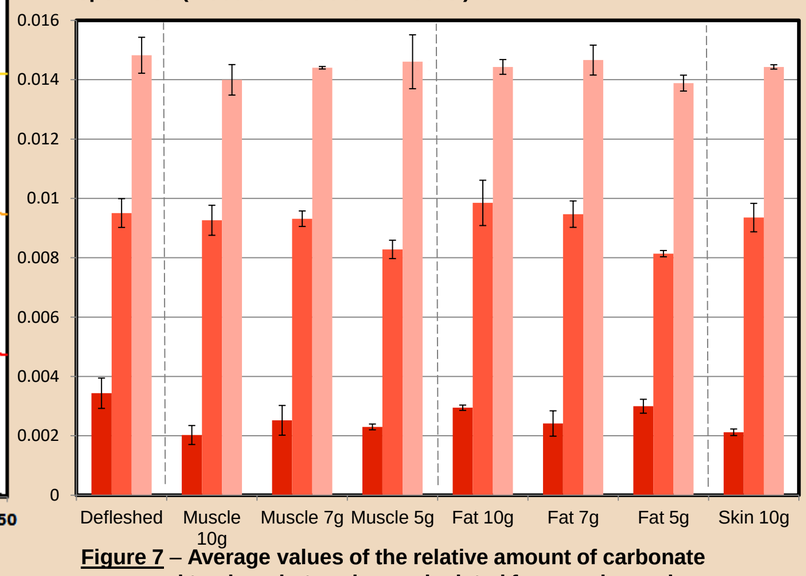


Figure 7 – Average values of the relative amount of carbonate compared to phosphate values calculated from each specimen (■ 900°C ■ 600°C ■ 400°C)

	600°C		400°C	
Muscle 10g	Defleshed		Defleshed	
Muscle 7g				
Muscle 5g				
Fat 10g				
Fat 7g				
Fat 5g				

Figure 8 - The average Colorimeter values calculated for specimens heated with varying quantities of muscle and fat at either 400°C or 600°C.

CONCLUSION

The current findings suggest that fleshed bone crystallises either at a slower rate, or at a later temperature, compared to specimens that are defleshed. This is likely due to the soft tissue acting as a protective layer, shielding the bone from heat induced changes. These findings should therefore be taken into consideration when applying data from studies which solely utilise dry bone when attempting to identify the species of origin based on the crystallographic changes to HAp.

References:

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[3] Londoño-Restrepo SM, Jeronimo-Cruz R, Millán-Malo BM, Rivera-Muñoz EM, RodriguezGarcía ME. "Effect of the Nano Crystal Size on the X-ray Diffraction Patterns of Biogenic Hydroxyapatite from Human, Bovine, and Porcine Bones". Scientific Reports. 2019;9(5915):1-12.