A16 Collagen Structural Changes and Decomposition in Burnt Bone and Their Significance for Forensic Anthropology — New Insights Via Amino Acid Racemization

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After attending this presentation, attendees will better understand the changes bone collagen undergoes when subjected to heating and the implications these changes have on subsequent forensic analyses.

This presentation will impact the forensic science community by shedding new light on the thermal stability of bone collagen, allowing for an improved prediction of the success rate of collagen extraction for subsequent analyses, such as dating or isotope analyses.

All amino acids found in proteins exist as one of two possible stereoisomers, known as L-amino acids and D-amino acids. With the exception of glycine, all amino acids are L-amino acids, which have the ability to change into the D-form over time. The process of reaching an equilibrium between the L and the D form is called racemization. Amino Acid Racemization (AAR) is a time- and temperature-dependent process that has found application in the dating of materials, paleothermometry, and age-at-death estimation. Currently, there is no clear consensus in the literature regarding the thermal stability of collagen, an issue on which this research sheds more light.

Sheep (*Ovis aries*) ribs were cut into 4cm-long pieces and burnt at temperatures between 100°C and 1,000°C in 50°C increments for 45 minutes. All samples were weighed pre- and post-burning. Four demineralization samples were suspended in 0.5 M HCl, which was exchanged every two days. After ten days, the HCl was removed and replaced by distilled water until a solution of pH 3 was obtained. Samples were heated at 70°C for 48 hours and subsequently filtered. The extracted collagen was frozen at -20°C and 250µL of solution from each sample were placed in a sterile glass vial, adding 100µL 7 M HCl per sample. The vials were flushed with nitrogen, heated at 110°C for 18 hours, and subsequently dried under vacuum in a centrifugal evaporator. Samples were rehydrated for analysis. The sample's amino acid composition was analyzed by reverse-phase High-Performance Liquid Chromatography (HPLC) using fluorescent detection. Then 2µL of sample was injected and mixed with 2.2µL derivitizing reagent. The amino acids were separated on a C18 HyperSil™ BDS column (5mm * 250mm) at 25°C using a gradient elution of three solvents: sodium acetate buffer, methanol, and acetonitrile. The fluorescence detector uses a xenon-arc flash lamp at a frequency of 55Hz with a 280nm cut-off filter, an excitation wavelength of 230nm, and emission wavelength of 445nm.

The D and L isomers of 13 amino acids could be analyzed, namely serine, L-threonine, L-histidine, glycine, L-arginine, alanine, tyrosine, valine, phenylalanine, leucine, and isoleucine, as well as aspartic acid and glutamic acid. The amino acid concentration rapidly decreases from 250°C onward, being below reliable detection levels from 400°C. Up to temperatures of 250°C, only aspartic acid racemizes, reaching a D/L ratio of 0.3 at 250°C. From 300°C onward, the other amino acids commence racemization. From 400°C onward, the total amino acid concentration is too low to accurately depict D/L ratios. The composition of amino acids was dominated by collagen up to 400°C.

The findings illustrate the thermal degradation of bone collagen. Up to 250°C, virtually no amino acid racemization takes place, with the exception of aspartic acid, which is one of the only amino acids which can racemize while still internally bound. Collagen, when heated, begins to locally unravel its triple helix, releasing the collagen-

stabilizing H-bonded water, which leads to a gradual collapse of the triple helical structure at approximately 150°C. At temperatures between 250°C -300°C, a sudden drop in total amino acid concentration as well as the commencing of racemization of all other amino acids can be observed, indicating a catastrophic breakdown of collagen. The now-free amino acids continue racemization until their complete combustion from approximately 400°C onward.

Successful collagen extraction is the basis for a multitude of forensically relevant analyses, such as stable isotope analysis, radio carbon dating, or genetic profiling. Being able to accurately determine the point at which collagen denaturizes and amino acids are lost is therefore of paramount importance for forensic analyses.

Burnt Bone, Collagen, Amino Acid Racemization