H10 The Effects of Papain and EDTA on Bone in the Processing of Forensic Remains

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The goal of this presentation is to assess the effects of papain and EDTA on bone as well as determine an effective, cost-efficient concentration of papain for the removal of soft tissue from bone.

This presentation will impact the forensic community by providing a method for soft tissue removal that is quick, efficient, and nondestructive to bone.

It is essential in forensic anthropology to employ a defleshing method that is both efficient and nondestructive to bone tissue or fine trauma marks, while working within time constraints imposed by law enforcement. Results from a previous study (Kemp et al. 2008) suggested that papain solutions fulfill these conditions, representing a viable method which can be utilized in forensic contexts. However, other studies have found that papain can be destructive to bone.

Papain is a proteolytic enzyme (protease) derived from the unripened papaya fruit (*Carica papaya*). Proteases are specific enzymes that induce protein decomposition (proteolysis) by promoting hydrolysis of the peptide bonds that link amino acids. Therefore, these enzymes would primarily target the protein content of muscle and connective tissue (ligaments, tendons, and cartilage), rather than the mineral or tightly packaged organic matrix of the bone. Other benefits of the enzyme are that optimal activity occurs at low temperatures (45°-65°C), thus remains need not be subjected to boiling, and it works so effectively that no harsh instrument is required to remove soft tissues.

Papain can be inactivated by metal ions, so commercial papain solutions normally contain ethylenediamine tetra-acetic acid (EDTA), as a chelating agent. EDTA binds to metal ions, including calcium, forming metal complexes with very high stability constants (pK-values). Thus, EDTA can bind to and remove calcium from bone tissue. Due to this property, EDTA is commonly used to decalcify bone for the preparation of histological slides or the retrieval of DNA. Therefore, it is proposed that the destruction of bone tissues reported in other studies may in fact be attributed to EDTA rather than papain.

The present study addresses three primary questions: (1) What is the most effective, cost-efficient concentration of papain for soft tissue removal? (2) Is papain destructive to bone at this concentration? and (3) Is EDTA destructive to bone?

A sample (n=16) of New Zealand white rabbits (*Oryctolagus cuniculus*) was obtained from a colony housed at the University of Pittsburgh (all individuals in the study came from previously approved protocols). The skulls, forelimbs, and hindlimbs of each rabbit were removed following standardized procedures. All remains were weighed prior to and following processing, recording any differences in wet or dry weights. The subsequent steps of the study were conducted in two phases. The first phase of the study focused on the effectiveness of papain and its effect on bone, while the second focused on the effect of EDTA on bone.

Phase 1 samples (12 forelimbs, 15 hindlimbs, and 11 skulls) were placed in stainless steel pots in solutions consisting of 10g, 8g, 6g, 4g, 2g or 0g (control group) of papain in 3.8 10^3 ml (1gal) of distilled water. Solutions were heated on electric burners, then heated to and maintained at 55°-65° C. The condition of the remains was documented every 30 minutes. Results suggested that the 4g solution was the most efficient and cost-effective, based on variables such as the time-to-completion, final dry weight of the bones, and efficacy of the enzyme on varying tissue types. Hence, this concentration was employed in the EDTA solutions analyzed in Phase 2 of the study.

Phase 2 samples (7 forelimbs and 6 hindlimbs) were placed in solutions consisting of 3.8 10^3 ml (1 gal) of distilled water, 4g papain, and 10g, 6g, or 2g of EDTA. The same procedures were followed as described above in Phase 1.

Gross bone destruction was not noted in any of the Phase 1 samples, whereas severe destruction was observed on several bones in the Phase 2 EDTA samples. The thin bone of most of the scapulae was eroded through, and some subchondral bone was also destroyed. These results strongly suggest that EDTA, rather than papain, is highly destructive to bone and should be avoided in defleshing protocols. The developed protocol reduces the amount of free metal ions in the solution by using distilled water rather than tap water and stainless steel pots rather than aluminum, therefore, EDTA is not required for proper activity of papain. The enzyme was found to work quickly and effectively with the proposed protocol, without EDTA significantly affecting the time-to- completion.

Reference:

[1] Kemp BJ, Cabo-Perez LM, Matia J, Dirkmaat DC. 2008. The effectiveness of papain in the processing of remains. Poster presented at the 60th annual meeting of the American Academy of Forensic Sciences.

Maceration, Papain, EDTA

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