H51 The Effectiveness of Papain in the Processing of Remains

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The goal of this presentation is to demonstrate the potential of the papain enzyme in the process of soft tissue removal.

This presentation will impact the forensic community by demonstrating a simple, non-aggressive method of soft tissue removal, with applications in both forensic investigations and osteological collection curation.

The removal of soft tissue to allow for direct examination of bone poses an important challenge to forensic anthropologists. The researcher is confronted with the issues of using a nondestructive method while remaining within time constraints. At present, the main alternatives available are based on maceration in either plain water or in solutions of chemicals or enzymes. True maceration in plain water is slow and infamously odiferous. Bleach or detergents serve both to speed the process and to mitigate the odor, but are much more aggressive to the bone tissue, requiring close monitoring. Heat treatment can be added to both types of maceration to increase reaction speed, but at the cost of significantly increasing their aggressiveness.

The present study tests the utility and efficiency of papain for soft tissue removal in forensic settings. Papain is a proteolytic enzyme (protease) derived from the papaya fruit (*Carica papaya*). Proteases are highly 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. Papain offers the advantage of being a versatile enzyme with a multitude of uses, including medical applications in the removal of scar tissue or herniated discs, or as a component of household meat tenderizers. This translates in a widespread commercial availability and, importantly, in a moderate cost.

This experimental design was constructed to: (1) test the efficiency of a pure papain solution against those of other alternative solutions and a blank control, in terms of time and bone integrity, (2) assess the minimal effective concentration of papain required for significant tissue removal (an important factor in relation to the cost/efficacy ratio of the method), and (3) estimate the effect of papain concentration and time on bone integrity.

Nonfrozen store-bought chicken (*Gallus gallus*) legs were used as an animal proxy in this study. Chicken legs were selected for study due to their uniformity and availability, allowing for more effective randomization and larger sample sizes. Additionally, the lower density and relative wall thickness of avian bones, as compared to mammals, was expected to result in higher ratios of organic to mineral bone content, and therefore higher rates of enzymatic bone destruction. This confers a necessary conservative approach to the bone destruction estimates, in comparison to those expected in human remains for the same concentrations of the enzyme.

The chicken limbs were randomly assigned to different solutions of equal volumes of distilled water and varying concentrations of papain BioChemika powder from Sigma Aldrich, a solution of household meat tenderizer, and a control of water. All solutions were kept at a constant heat of 50°C. Soft tissues were manually removed at fixed times, avoiding the use of any aiding or surgical instrument, and the wet weight of the removed and remaining tissue (including bone) was recorded. Wet weight was also recorded before treatment, and wet and dry weights after treatment, in order to control for bone degradation in terms of weight loses.

Differences in bone degradation were tested through a repeated meas- urements ANOVA design, with initial wet and final dry weights as dependent variables. Time differences were assessed through a one-way ANOVA analysis of the times required for complete tissue removal. Finally, linear regression techniques served to assess the influence of papain concentration on time for complete tissue removals.

The results strongly suggest that papain maceration is a viable and efficient method for tissue removal, significantly reducing processing time and the risk of bone damage, deserving further research and application in forensic and curation contexts. The concentrations of papain necessary to obtain useful results place the method within a reasonable rank of cost-effec- tiveness, especially in those cases where bone integrity and time constraints play a significant role. Pending a more detailed study, a preliminary yet reliable processing protocol for papain is provided.

Papain, Maceration, Enzyme

 ** Presenting Author*