



# Sex Determination Using Enamel Peptides After Simulated Forensic Heating

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## INTRODUCTION

In forensics, where extreme thermal conditions may be involved (ie, fires or explosions), conventional methods of identification, such as DNA analysis, are often severely compromised and thus ineffective. Tooth enamel, however, is the hardest tissue in the human body and is highly resilient to extreme environmental conditions. Enamel amelogenin peptides derived from the AMELX and AMELY genes can determine biological sex and are retained long after DNA is no longer detectable.

Recent advancements in proteomic analysis, especially through nanoLC-MS/MS, facilitate the observation of such peptides even from scanty or deteriorated specimens. This approach holds considerable promise, but knowledge of the effect of elevated temperatures on the integrity and detectability of such peptides is scarce.

This study focused on the feasibility and reliability of amelogenin peptides obtained from enamel as a potential sex-identification tool following high-temperature exposure. So the aim of this study is to investigate how peptide detection and overall accuracy are influenced by increased temperature and exposure time, and whether this is applicable in a real forensic scenario where remains are severely compromised.

## METHODS

This is an experimental in vitro study using extracted human permanent and primary teeth to mimic the forensic conditions of high-temperature trauma or fire. The experimental groups were subjected to heat exposure at controlled temperatures of 100° C, 300° C, 500° C, and 800° C for 15, 30, and 60 minutes in a dental porcelain furnace. All the samples were cooled after heat treatment and then processed. The only patient information collected included sex, Axium number, and age. No identifying information will be collected at any time. The only extracted teeth are systematically grouped into labeled containers, organized by biological sex (male/female).

### Inclusion Criteria

- Adult patients.
- Pediatric patients.
- Extraction is a treatment planned and consented to before collection, as outlined in the protocol.

Teeth must have an adequate amount of visible enamel. This may involve excluding teeth that are worn, extensively restored, or grossly carious.

### Exclusion Criteria

- Teeth with extensive restorations, full crowns, or large carious lesions that significantly compromise enamel.

Teeth with insufficient exposed enamel for safe drilling and sampling.

Teeth that have been stored in formalin or unknown chemical fixatives.

### Sample Selection: 182

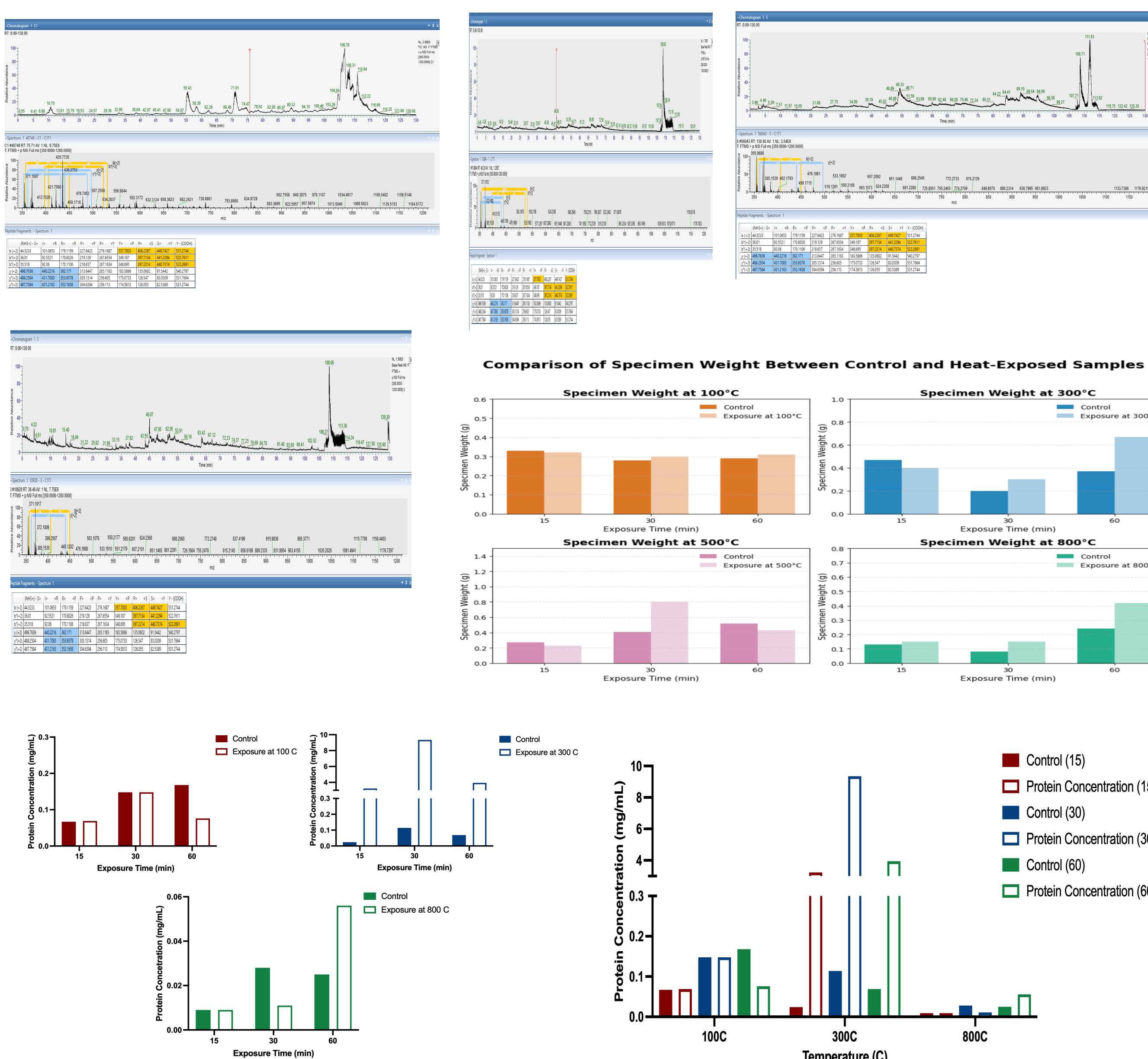
### Data Collection and Blinding

- This study will follow a single-blind protocol to reduce bias during laboratory analysis

## METHODS



## RESULTS



## RESULTS

- Successful extraction and detection of enamel-derived proteins using the developed protocol.
- nanoLC-MS/MS confirmed detection of the AMELX peptide in control samples and confirmed biological sex.
- Detectable protein concentrations were obtained at all evaluated temperatures, including 800° C.
- Higher protein concentrations were observed in some samples exposed at 300° C for 15, 30, and 60 minutes.
- Statistical analysis showed that specimen weight was not a confounding variable between the same patients at different time points and different temperatures.
- Heat-treated samples were successfully preserved for future nanoLC-MS/MS analysis.

## CONCLUSION

- The study successfully validated the enamel protein extraction protocol.
- Enamel proteins remained detectable after thermal exposure, including up to 800° C.
- Preliminary findings indicate that the existing enamel proteins hold under simulated forensic conditions.
- Specimen weight analysis showed that tooth mass did not significantly affect protein concentration results.

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## ACKNOWLEDGEMENTS

- We acknowledge the support of Howard University College of Dentistry, the Department of Oral Pathology and Research, Dr. Tyler, Dr. Gu, Dr. Xiaowu Pang, Dr. Latoya Barham, Dr. Esther Childers, Marzia Mustamand, Jyothirmai Simhadri, Andrey Ivanov, and Sarah Garcia.