Forensic Science International 385 (2026) 112965

Contents lists available at ScienceDirect

Forensic Science International

journal homepage: www.elsevier.com/locate/forsciint

Thermogravimetric analysis: A new tool for dating human teeth

Leticia Rubio [a,b,*], Alba Delgado-Ruiz [a,b], Diego Lozano-Peral [b,c], Juan Suárez [a,b], Stella Martin-de-las-Heras [a,b]

[a] Departamento de Anatomía Humana, Medicina Legal e Historia de la Ciencia, Facultad de Medicina, Universidad de Málaga, Bulevar Louis Pasteur 32, Málaga 29071, Spain
[b] Instituto de Investigación Biomédica de Málaga (IBIMA), Plataforma-BIONAND, Calle Severo Ochoa 35, Málaga 29590, Spain
[c] Supercomputing and Bioinnovation Center, Servicios Centrales de Apoyo a la Investigación, University of Malaga, Malaga 29590, Spain

ARTICLE INFO

Keywords:
Human teeth
Thermogravimetric analysis
Postmortem Interval
Forensic odontology
Forensic anthropology
Forensic medicine

ABSTRACT

Dating human skeletal remains is a major challenge in forensic science. The aim of this study is to correlate dating intervals related to the time elapsed since tooth extraction and the mass loss of human teeth, measured using thermogravimetry analysis in two different atmospheres: air and nitrogen. Forty healthy human teeth were stored for different periods after extraction (0, 10, 25, 50 years; N = 10/group) under controlled laboratory conditions. Thermogravimetric parameters (total percentage of mass loss, and percentage of mass loss in Step 1, Step 2 and Step 3) and derivatives of thermogravimetric parameters (temperature of maximum mass loss at Peak 1, Peak 2 and Peak 3) were quantified. Binary logistic regression and receiver operating characteristic (ROC) analyses were applied to assess the ability of these parameters to discriminate among the 10-, 25-, and 50-year intervals. Dating accuracy was consistently higher in air than in nitrogen atmosphere. Using thermogravimetric parameters, predictive formulas capable of distinguishing the different dating intervals with high precision were developed. The method achieved excellent performance to estimate 10-, 25-, and 50-year intervals in the air atmosphere, with areas under the ROC curves (AUC) ranging from 0.95 to 1.00. The results highlight thermogravimetric analysis as a promising technique for accurately estimating the time elapsed since tooth extraction in human teeth stored under controlled conditions.

1. Introduction

Dating human skeletal remains a persistent challenge for forensic practitioners. Establishing the time since death is critical for legislative to investigative purposes, as it can be decisive in medico legal death investigation and humanitarian crises [1]. Despite decades of research, accurate and reliable approaches for estimating the late post-mortem interval (PMI) in skeletal remains are still lacking.

Tissue decomposition, the difficulty of visually assessing skeletal remains, and the influence of numerous environmental and endogenous factors all contribute to reducing the accuracy of late PMI estimation [2–4]. Late PMI approaches applied to date bone remains consist of several analytical techniques of quantitative biomarkers and macromorphoscopic markers [1] such as chemiluminescent reaction with luminol [5,6], radioisotopic measurements [7], X-ray diffraction [8], fluorescence analysis [9] and vibrational spectroscopy [10,11]. Despite evidence, further research is still needed to achieve reliable late PMI estimation in human skeletal remains [5].

Since skeletal remains (bones and teeth) contain both inorganic and organic components, thermogravimetric analysis (TGA), a technique sensitive to mass changes associated with the decomposition of these components, offers a promising approach for evaluating post-mortem changes [12]. In this regard, TGA has previously been performed to characterize the thermal decomposition of bone [12] and teeth [13] for forensic purposes. In teeth, thermal decomposition produced a total mass loss of approximately 25.2% across three well-defined steps: 1) Step 1, between 44 and 210 °C, corresponding to the loss of free water and reflecting the moisture content of enamel, dentin, and pulp; 2) Step 2, between 211 and 603 °C, associated with combustion of organic components, including protein degradation (sulfur dioxide release around 270 °C) and volatilization of DNA residues between 330 and 347 °C, with severe organic decomposition occurring between 150 and 200 °C; and 3) Step 3, between 604 and 940 °C, involving decomposition of inorganic components, particularly hydroxyapatite, which highlights

---

[*] Corresponding author at: Departamento de Anatomía Humana, Medicina Legal e Historia de la Ciencia, Facultad de Medicina, Universidad de Málaga, Bulevar Louis Pasteur 32, Málaga 29071, Spain.

E-mail address: lorubio@uma.es (L. Rubio).

https://doi.org/10.1016/j.forsciint.2026.112965
Received 21 July 2025; Received in revised form 7 April 2026; Accepted 8 April 2026

the predominance of calcium and phosphate in dental tissues [13].

Despite its potential, research-exploring TGA as a tool for late PMI estimation remains scarce. The earliest study applying thermogravimetry to PMI estimation focused on distinguishing fresh from old bones [14]. A subsequent study examined pig bones of varying post-mortem ages (up to 23 months) under air and nitrogen (N_2) atmospheres, revealing a progressive decrease in mass loss with increasing post-mortem age [15]. Although limited, these studies suggest that time-dependent changes in water, organic, and inorganic components of mineralized tissues may provide chronological information. Collectively, they support the potential of thermogravimetric analysis as a novel technique for dating human remains, including bones and dental tissues. However, TGA has never been used to investigate post-mortem changes in human teeth, despite the frequent recovery in forensic, anthropological, or archaeological settings.

In living individuals, tooth extraction involves the section of the periodontal ligament and the neurovascular bundle, leading to tooth death. Consequently, the time elapsed since tooth extraction can serve as a proxy for the time since tooth death. Moreover, other studies have used teeth extracted from living individuals to estimate the time elapsed since death. [11,16]. In this study, clinically extracted teeth stored under constant laboratory conditions were used to simulate long-term dating intervals (0, 10, 25, 50 years), isolated from the environmental factors typical of forensic scenarios. This pilot study aimed to assess whether dating-related changes in human teeth, using TGA under air and nitrogen atmosphere, can predict the time elapsed since tooth extraction, thereby providing a model of late PMI estimation in controlled conditions.

2. Materials and methods

2.1. Human teeth samples

A total of 40 healthy human teeth (molars and premolars) were obtained from the documented tooth collection of the Department of Forensic Medicine (University of Malaga, Spain). The longest storage group comprised human teeth collected between 1974 and 1975 and stored at the Department of Forensic Medicine (University of Malaga, Spain). Available records indicate that the specimens remained in long-term storage within the same institutional archive and had not undergone prior experimental manipulation before inclusion in the present study. The collection is maintained under institutional accession numbers, with complete catalogue entries and a centralized chain-of-custody registry documenting all transfers and handling events to ensure its reliability for subsequent forensic or anthropological analyses. For each specimen, anonymized donor ID, sex, age at the tooth extraction, collection date and clinical reason for extraction were recorded. All specimens were stored in sealed containers in a climate-controlled room at 21 °C and 65% relative humidity.

The sample included 20 females and 20 males aged 29–82 years (mean 60 ± 11.54 years). Teeth were originally extracted for clinical reasons (periodontal disease or orthodontic treatment) in public and private dental clinics in Malaga and Cádiz (Spain). All specimens were free of caries (Supplementary Table S1). Ethical approval was granted by the Research Ethics Committee of Malaga Province (approval reference: IDENTG-MS18; approval date: September 28, 2018), written informed consent was obtained from the donors at the time of extraction. All procedures complied with the Declaration of Helsinki and national data protection regulations.

After extraction, teeth were rinsed with distilled water and their external surfaces were cleaned with curettes to remove any extraneous material. Then, to model long-term dating intervals, the extracted teeth were stored under controlled conditions (21°C and 65% relative humidity) for 0, 10, 25 and 50 years. These intervals were used as a proxy for the time elapsed after tooth death.

2.2. Sample pulverization

The teeth were pulverized under cryogenic conditions using a 6770 Freezer Mill (SPEX CertiPrep Freezer Mill, Stanmore, London, UK). The resulting powder was collected in sterile containers and stored at −80 °C until TGA.

2.3. Thermogravimetric analysis

Thermogravimetric analysis (TGA) was analyzed using a Mettler-Toledo TGA/DSC1 thermogravimetric analyzer coupled to a Thermo-StarTM GSD 320 mass spectrometer (Pfeiffer Vacuum, ASslar, Germany). Approximately 15 mg of powdered sample was placed in a 70-μL alumina crucible. Each sample was analyzed in two atmospheres: air and nitrogen (N_2). The air flow rate was 20 mL min^{-1} and the N_2 flow rate was 150 mL min^{-1}. Heating was performed from 30 ℃ to 1000 ℃ at 10 ℃ min^{-1}.

The analyzer was calibrated using indium, aluminum, and gold standards. Baseline curves were generated under identical conditions. Each sample was analyzed in triplicate, and the mean curve was used for all calculations. Empty crucibles were run under identical conditions, and their curves were subtracted from sample curves.

Thermogravimetric (TG) curves were generated using $STAR^e$ v.16.00 software (Mettler-Toledo System). The TG curves plot the change in the mass of the samples against the temperature. Mass loss (Δm) was calculated as the difference between the initial mass (m_0) and the mass at a given temperature (m_t), expressed as a percentage of the initial mass: $\%\Delta m = (\Delta m/m0) \times 100$. The first derivative of the thermogravimetric curve (DTG) with respect to temperature was used to show the rate of mass change (mass loss per unit of temperature). The DTG curves were used to identify decomposition phases including desorption (loss of water), sublimation, vaporization, pyrolysis (breakdown of organic components), and oxidation (other residues). DTG peak temperatures corresponded to maximum mass-loss rates. From these curves, TG parameters (total percentage of mass loss, and percentage of mass loss in Step 1, Step 2 and Step 3) and DTG parameters (temperature of maximum mass loss at Peak 1, Peak 2 and Peak 3) were extracted.

2.4. Statistical analysis

All TG and DTG parameters were exported to Excel (Microsoft Corporation, Redmond, WA). GraphPad Prism 9.0 and IBM SPSS Statistics 26.0 were used for statistical analysis. Data are represented as the mean ± standard error of the mean (SEM) for each experimental group ($n = 10$). Normality was assessed using the Dallal–Wilkinson–Lillie corrected Kolmogorov–Smirnov test. Most variables followed a Gaussian distribution ($p > 0.1$), allowing the use of parametric tests. Homogeneity of variances was evaluated using Bartlett's test.

To assess the effect of dating intervals, atmosphere, and sex, an ANCOVA model was fitted with donor age as a covariate, followed by Tukey's post-hoc test for multiple comparisons. Sex was included as a potential discriminatory factor (see Supplementary Tables S2–7 for further information). Since the sample comprised posterior teeth, the tooth type factor was not included (see Supplementary Tables S8–12 for further information). Pearson correlations were used to explore associations between TG/DTG parameters that were stratified by experimental factors (dating intervals, atmosphere and sex).

Binary logistic regression models were developed to classify samples into 10-, 25-, and 50-year intervals. Backward elimination was performed within a bootstrap framework of 5000 successful resamples to identify stable predictor combinations. For each model, the area under the receiver operating characteristic (ROC) curve (AUC) and overall classification accuracy (percentage of correctly predicted cases) were estimated. The final model was defined as the predictor set achieving the highest discriminatory performance. Statistical significance was set at $p < 0.05$.

3. Results

TG and DTG spectra obtained from teeth exposed to thermal decomposition by thermogravimetric analysis were studied for quantitative differences of the TG and DTG parameters regarding dating intervals, atmosphere and sex (Fig. 1).

3.1. Effects of dating intervals on mass loss by thermogravimetric analysis in teeth

The mean values of the total mass loss percentage, the mass loss percentage in each of the three TG steps (Step 1, Step 2 and Step 3) and the mean temperature of the maximum mass loss at each of the three DTG peaks (Peak 1, Peak 2 and Peak 3) in both atmospheres (air and N_2) at 0, 10, 25, and 50 years of dating are presented in Table 1. The total percentage of mass loss in the air atmosphere was 25.28%, 24.65%, 24.47%, and 25.42%, at 0, 10, 25, and 50 years of dating, respectively. The total percentage of mass loss in the N_2 atmosphere was 25.32%, 24.81%, 24.9%, and 25.5% at 0, 10, 25, and 50 years of dating, respectively.

Significant differences in the total percentage of mass loss were observed when comparing the different dating intervals ($F_{3,72} = 5.09$; $p = 0.003$) (Fig. 2A). When the three thermogravimetric steps were analyzed separately, a significant effect of dating intervals on the percent mass loss was found in Step 1 ($F_{3,72} = 2.70$; p = 0.05) and Step 2 ($F_{3,72} = 4.43$; p = 0.006) (Figs. 2B, 2C) and a significant effect of the atmosphere was observed on mass loss in Step 2 ($F_{1,72} = 86.52$; $p < 0.0001$) and Step 3 ($F_{1,72} = 27.94$; $p < 0.0001$) (Figs. 2C, 2D).The percentage of mass loss was lower in Step 2 and higher in Step 3 under N_2 atmosphere compared to air atmosphere in either of the dating intervals analyzed ($^{\#\#/\#\#\#}p < 0.01/0.001$; Figs. 2C, 2D). In addition, an interaction between dating intervals and atmosphere was specifically found in Step 3 ($F_{3,72} = 2.85$; $p < 0.04$), suggesting that the percent mass loss in Step 3 under air and N_2 atmospheres is dating interval-dependent.

The analysis of the DTG spectra exhibited effects of dating intervals on the maximum mass loss temperatures at Peak 1 ($F_{3,72} = 13.89$; $p < 0.0001$) and Peak 3 ($F_{3,72} = 6.69$; $p < 0.001$). The temperatures of maximum mass loss at Peak 1 were higher in the 10-, 25- and 50-year intervals under air atmosphere, as well as in the 25- and 50-year intervals under N_2 atmosphere, compared to the respective 0-year ($^{*/**/***}p < 0.05/0.01/0.001$; Fig. 2E). On the contrary, the temperatures of maximum mass loss at Peak 3 were specifically lower in the 25- and 50-year intervals under an air atmosphere, but such a change was not observed under N_2 atmosphere, when compared to the respective 0-year ($^{*/**}p < 0.05/0.01$; Fig. 2G). A significant effect of atmosphere on the maximum mass loss temperatures was observed at Peak 1 ($F_{1,72} = 107.5$; $p < 0.0001$), Peak 2 ($F_{1,72} = 5.45$; $p = 0.02$) and Peak 3 ($F_{1,72} = 266.6$; $p < 0.0001$). The temperature of maximum mass loss was lower at Peak 1 and higher at Peaks 2 and 3 under N_2 atmosphere compared to air atmosphere in either of the dating intervals analyzed, excepting the 0 years at Peak 2 ($^{\#/\#\#/\#\#\#}p < 0.05/0.01/0.001$; Fig. 2E-G).

3.2. Sex-specific effects of dating intervals on mass loss by thermogravimetry in teeth

Sex-specific effects of dating intervals on mass loss by thermogravimetry in teeth.

When sex was included as a discriminating factor, a sex effect on total percentage of mass loss was observed ($F_{1144} = 24.76$; $p < 0.0001$), with females showing lower mass loss, being significant at 0 years of dating under N_2 atmosphere ($^{\$}p < 0.05$; Fig. 3A). When the three TG steps were analyzed separately, sex effects on the percent mass loss were specifically found in Step 1 ($F_{1144} = 12.97$; $p = 0.0004$) and Step 2 ($F_{1144} = 26.94$; $p < 0.0001$), with females showing lower mass loss, being

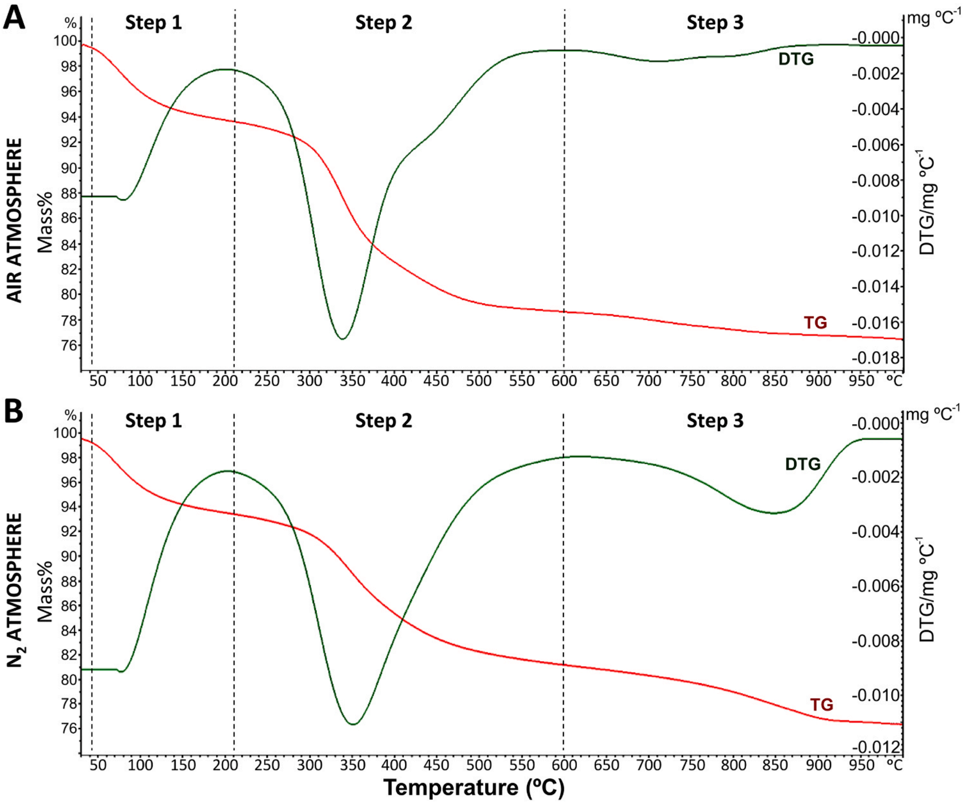

Fig. 1. Representative TG (red line) and DTG (green line) spectra that depict mass change (%) when the teeth are exposed to increasing temperatures (°C°) under air (A) and N_2 (B) atmospheres. DTG spectra were used to identify the different decomposition phases along temperature divided into Step 1 (44–210 °C), Step 2 (211–603 °C) and Step 3 (604–940 °C). The peaks observed in the DTG spectra are located at the temperature where the rate of mass loss is at its maximum.

Table 1
Mean values of percent mass loss and maximum mass loss temperature in air and N_2 atmospheres in the dating intervals (DI) of 0, 10, 25 and 50 years.

DI (years)	Step 1 (%)	Step 2 (%)	Step 3 (%)	TOTAL (%)	Peak 1 (°C)	Peak 2 (°C)	Peak 3 (°C)
AIR ATMOSPHERE (mean ± SD)							
0	7.02 ± 0.17	16.54 ± 0.41	1.88 ± 0.06	25.28 ± 0.60	75.34 ± 0.99	331.56 ± 10.06	712.70 ± 8.03
10	6.81 ± 0.17	16.21 ± 0.42	2.02 ± 0.14	24.65 ± 0.37	77.98 ± 0.36	345.41 ± 0.29	720.63 ± 1.11
25	7.04 ± 0.14	15.84 ± 0.57	1.84 ± 0.01	24.74 ± 0.70	79.52 ± 0.33	345.61 ± 0.29	743.32 ± 6.04
50	6.65 ± 0.18	16.86 ± 0.37	1.89 ± 0.04	25.42 ± 0.45	79.85 ± 0.48	345.55 ± 0.38	736.28 ± 3.16
N_2 ATMOSPHERE (mean ± SD)							
0	6.70 ± 0.15	14.04 ± 0.53	4.57 ± 0.06	25.32 ± 0.67	72.04 ± 0.78	349.17 ± 15.42	811.22 ± 22.25
10	6.62 ± 0.20	13.72 ± 0.36	4.47 ± 0.05	24.81 ± 0.53	73.45 ± 0.70	350.89 ± 0.49	827.40 ± 3.73
25	6.76 ± 0.14	13.49 ± 0.25	4.64 ± 0.07	24.90 ± 0.45	74.35 ± 0.42	351.89 ± 0.32	851.40 ± 9.90
50	6.74 ± 0.16	14.01 ± 0.28	4.74 ± 0.06	25.50 ± 0.36	74.66 ± 0.61	352.33 ± 0.56	838.44 ± 3.99

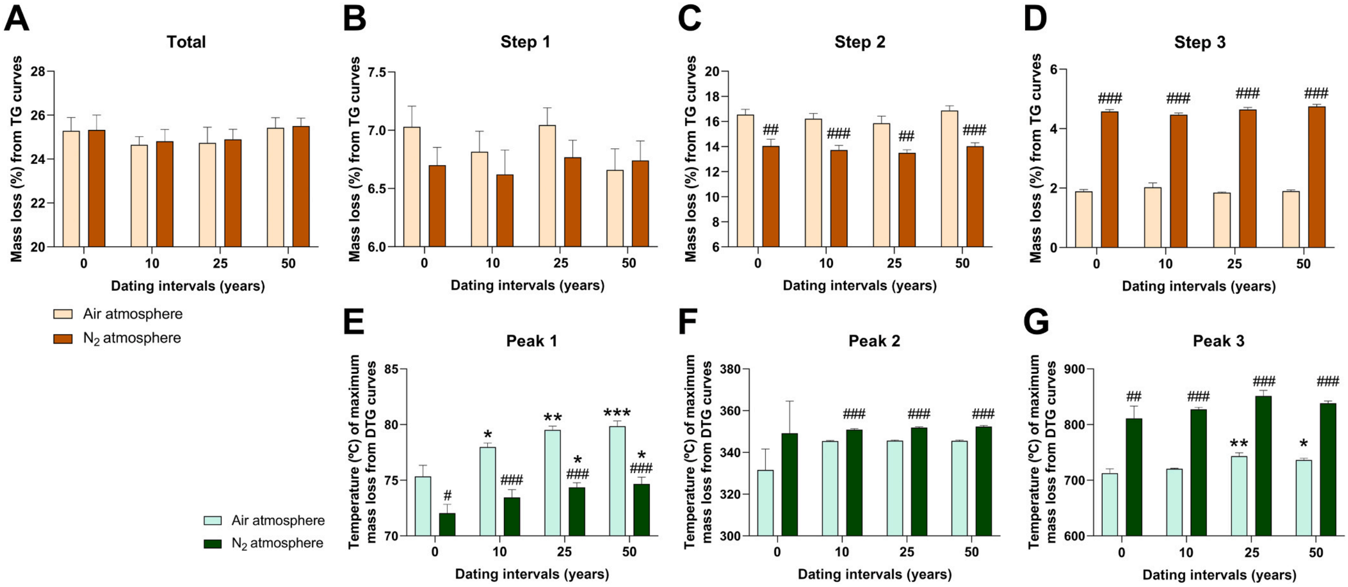

Fig. 2. Effect of dating intervals (0, 10, 25, and 50 years) on TG (percentage of mass loss) and DTG (temperature of maximum mass loss) parameters. (A): Effect of dating intervals on mass loss in total TG spectra. (B): Effect of dating intervals on mass loss in Step 1. (C): Effect of dating intervals on mass loss in Step 2. (D): Effect of dating intervals on mass loss in Step 3. (E): Effect of dating intervals on temperature of maximum mass loss in Peak 1. (F): Effect of dating intervals on temperature of maximum mass loss in Peak 2. (G): Effect of dating intervals on temperature of maximum mass loss in Peak 3. Histograms represent means ± SEM (n = 10). $^{\#/\#\#/\#\#\#}p < 0.05/0.01/0.001$ *versus* air group of the same dating interval; $^{*/**/***}p < 0.05/0.01/0.001$ *versus* 0-year interval group of the same atmosphere.

significant at 0 years of dating in Step 2 under N_2 atmosphere ($^{\$}p < 0.05$; Fig. 3B-D). Following sex as a factor, a significant effect on the total percentage of mass loss was observed ($F_{3144} = 4.18$; $p = 0.009$) when the different dating intervals were compared (Fig. 3A), resulting in a specific dating effect on the percent mass loss in Step 2 ($F_{3144} = 2.98$; $p = 0.03$) (Fig. 3C). No significant differences in the total percentage of mass loss were observed when comparing air and N_2 atmospheres in both sexes (Fig. 3A), but significant effects of atmosphere on the percent mass loss in Step 1 ($F_{1144} = 4.139$; $p = 0.04$), Step 2 ($F_{1144} = 166.7$; $p < 0.0001$) and Step 3 ($F_{1144} = 4865$; $p < 0.0001$) were detected (Fig. 3B-D). Interestingly, the percentage of mass loss of both sexes was lower in Step 2 and higher in Step 3 under N_2 atmosphere, compared to air atmosphere, in either of the dating intervals analyzed, excepting for 0-year interval in males and 25-year interval in females at Step 2 ($^{\#/\#\#/\#\#\#}p < 0.05/0.01/0.001$; Fig. 3C,D). Following sex as a factor, an interaction between dating intervals and atmosphere was specifically found in Step 3 ($F_{3,1144} = 5.37$; $p = 0.0016$), suggesting that the effect of air and N_2 atmospheres on the percent mass loss in Step 3 depend on dating intervals, but not sex. These results demonstrate that, in both sexes, the combustion of organic matter is higher in the air atmosphere and that the decomposition of inorganic matter is higher in the N_2 atmosphere in a sex-independent manner.

Regarding the analysis of the DTG spectra by sex, we observed effects of dating intervals ($F_{3144} > 10.85$; $p < 0.0001$), atmosphere ($F_{1144} = 198$; $p < 0.0001$) and sex ($F_{1144} = 4.088$; $p = 0.04$) on the temperatures of maximum mass loss at Peak 1 and Peak 3, as well as effects of atmosphere ($F_{1144} = 11.54$; $p < 0.001$) and sex ($F_{1144} = 11.11$; $p = 0.001$) on the temperatures at Peak 2 (Fig. 3E-G). Increasing dating interval was specifically associated with higher temperatures at Peak 1, being significant at 10, 25 and 50 years of dating in the male teeth under air atmosphere ($^{**/***}p < 0.01/0.001$; Fig. 3E). In addition, the temperatures of maximum mass loss were lower at Peak 1 and higher at Peak 2 and Peak 3 in the dating intervals of both sexes under N_2 atmosphere, compared to the respective temperatures in air atmosphere ($^{\#\#/\#\#\#}p < 0.01/0.001$; Fig. 3E-G). In general, female teeth showed lower temperatures at Peak 1, Peak 2 and Peak 3, being specifically significant in the 25-year interval at Peak 1 under air atmosphere ($^{\$}p < 0.05$; Fig. 3E). Interactions between dating intervals and sex were observed at Peak 2 ($F_{3144} = 11.22$; $p < 0.0001$) and Peak 3 ($F_{3144} = 2.92$; $p = 0.0359$), suggesting that the temperatures of maximum mass loss at Peak 2 and Peak 3 when the teeth are exposed to 0-, 10-, 25-, and 50-year intervals are sex-dependent.

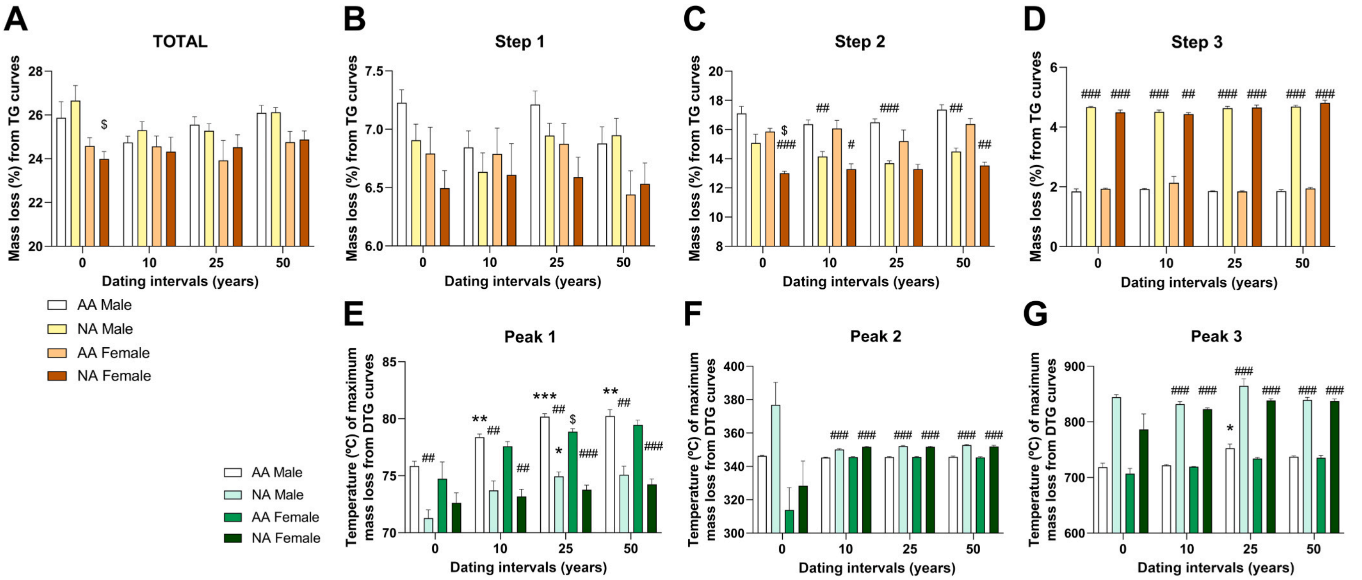

Fig. 3. Sex-specific effects of dating intervals (0, 10, 25, and 50 years) on TG (percentage of mass loss) and DTG (temperature of maximum mass loss) parameters. (A): Sex-specific effects of dating intervals on mass loss in total TG spectra. (B): Sex-specific effects of dating interval on mass loss in Step 1. (C): Sex-specific effects of dating interval on mass loss in Step 2. (D): Sex-specific effects of dating interval on mass loss in Step 3. (E): Sex-specific effects of dating intervals on temperature of maximum mass loss in Peak 1. (F): Sex-specific effects of dating intervals on temperature of maximum mass loss in Peak 2. (G): Sex-specific effects of dating intervals on temperature of maximum mass loss in Peak 3. Histograms represent means ± SEM (n = 5). $^{\#/\#\#/\#\#\#}$ $p < 0.05/0.01/0.001$ *versus* air group of the same dating intervals and sex; $^{*/**/***}$ $p < 0.05/0.01/0.001$ *versus* 0-year interval group of the same atmosphere and sex; $^{\$}$ $p < 0.05$ *versus* male group of the same dating interval and atmosphere.

3.3. Correlation between thermogravimetric parameters is affected by atmosphere and dating intervals

Pearson's correlation analysis of thermogravimetric parameters was performed for each dating interval separately. The Supplementary Tables S13–16 show the complete Pearson’s correlation analysis for each dating interval. The results suggest that the reduction in mass loss associated with increasing age of the tooth donor was less significant as dating intervals increased. In 0-year interval (Fig. 4A), donor age was negatively correlated with total and Step 1 % mass losses and Peak 2 maximum mass loss temperature under air atmosphere ($*p < 0.05$), as well as total, Step 1, Step 2, and Step 3 % mass losses under N_2 atmosphere ($*p < 0.05$), being more significant than 10-, 25-, and 50-year intervals (Fig. 4A-D).

Stronger positive correlations between thermogravimetric parameters were observed in the 0-year interval compared to other dating groups (Fig. 4A-D), particularly for percent mass loss in Step 1, which showed more correlations with other parameters in both atmospheres (Fig. 4A). This suggests that increased mass loss in Step 1 is significantly associated with losses in Steps 2 and 3 and with maximum mass loss temperatures (Peaks 1, 2, and 3), especially in the 0-year interval, and that these changes are atmosphere-dependent. Additionally, under air, Step 2 mass loss showed significant correlations with other parameters in 0- and 10-year intervals, unlike under N_2 or in 25- and 50-year intervals (Fig. 4A-D). When comparing air and N_2 atmospheres, all parameters—except Step 3 mass loss—showed positive correlations across 0-, 10-, 25-, and 50-year intervals (Fig. 4A-D). Notably, no significant correlations between atmospheres were found for Peak 3 temperature in the 10-, 25-, and 50-year intervals (Fig. 4B-D).

The Supplementary Tables S17–19 show the complete Pearson’s correlation analysis in total sample and sample segregated by sex. Sex-specific correlations between percent mass loss and maximum mass loss temperature in teeth are shown in Supplementary Figure S1 and described as Supplementary Results.

3.4. Predictive analysis of dating intervals using thermogravimetric parameters

A binary logistic regression was performed using the TG and DTG (Table 2). The binary logistic regression analysis selected several models that could predict the dating intervals for each atmosphere, obtaining the following formula:

$$\text{loglog}\quad (p/1-p)\quad = a + b1 * V1 + b2 * V2 + b3 * V3$$

where a is the constant (intercept), bn are the coefficients associated with the explanatory variables, and Vn are the variables that apply to each of the models (Step 1, Step 2, Step 3, Peak 1, Peak 2 or/and Peak 3). See Supplementary Tables S20-29 and Supplementary Figure S2 for the complete regression analysis described in the Supplementary Results.

Regarding the samples exposed to the air atmosphere (Table 2), the model that predicted the 10-year interval showed an overall success rate of 88.9% (ROC-AUC = 0.95, 95% CI = 0.85–1.00, Sensitivity = 0.87 and Specificity = 0.90), and the models that predicted the 25-and 50-year intervals showed an overall success rate of 100% (ROC-AUC = 1.0, 95% CI = 1.00–1.00, Sensitivity = 1.0 and Specificity = 1.0). Analysis of the predictive probabilities for teeth exposed to air atmosphere during TG (Fig. 5A-C) indicated that the means were different only for 10-year compared to 0-year interval (10-year: $t_{16} = 6.27$, $p < 0.0001$).

The best-predicted model in the N_2 atmosphere was the 25-year interval, showing an overall success rate of 94.1% (ROC-AUC = 0.93, 95% CI = 0.78–1.00, Sensitivity = 1.0 and Specificity = 0.86), followed closely by the 50-year interval with an overall success rate of 94.1% (ROC-AUC = 0.89, 95% CI = 0.67–1.00, Sensitivity = 1.0 and Specificity = 0.86) and, finally, the model that predicted the 10-year interval showed an overall success rate of 70.6% (ROC-AUC = 0.76, 95% CI = 0.51–1.00, Sensitivity = 0.80 and Specificity = 0.57) (Table 2). Analysis of the predictive probabilities for teeth exposed to N_2 atmosphere during TG (Fig. 5D-F) indicated that the means were different for10-year, 25-year and 50-year intervals compared to 0-year (10-years: $t_{15} = 2.18$, $p = 0.045$; 25-year: $t_{15} = 4.70$, $p = 0.0003$; 50-year: $t_{15} = 4.69$, $p = 0.0003$).

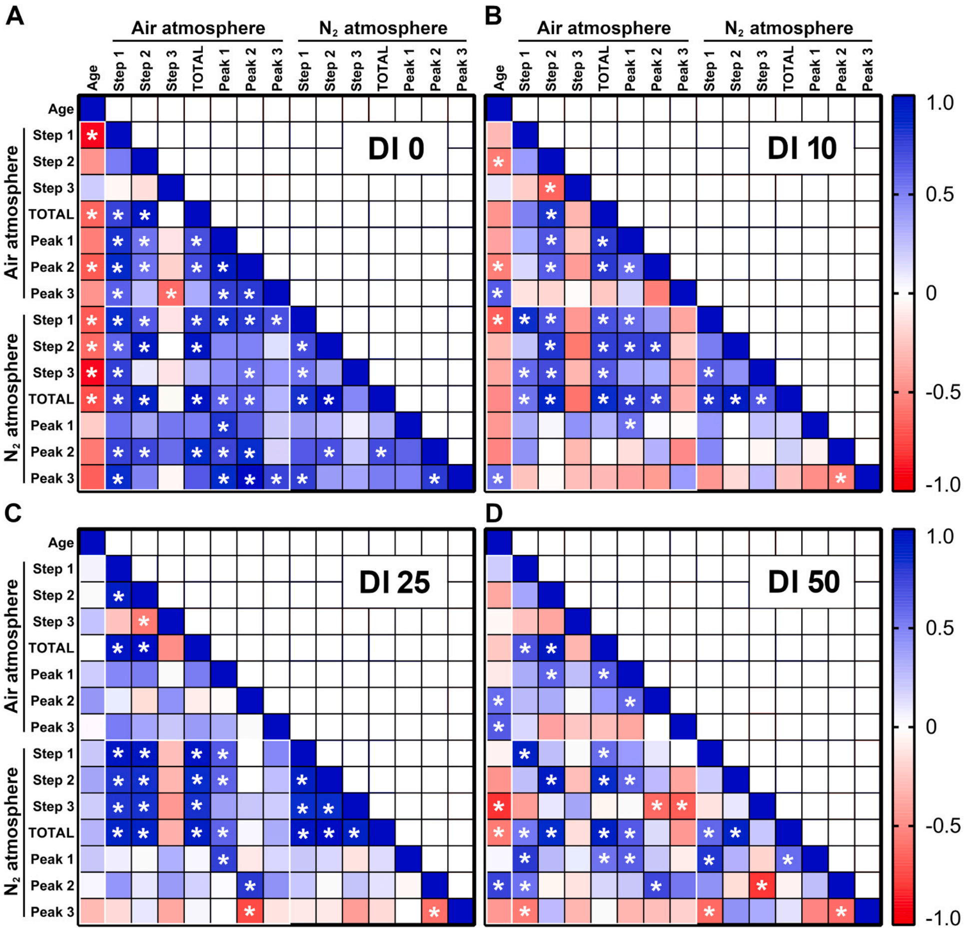

Fig. 4. Correlation analysis between age and thermogravimetric parameters stratified by dating intervals (0, 10, 25, and 50 years) and atmospheres (air and N_2). (A): Correlation analysis between age and thermogravimetric parameters in 0-year interval samples stratified by air and N_2 atmospheres. (B): Correlation analysis between age and thermogravimetric parameters in 10-year interval samples stratified by air and N_2 atmospheres. (C): Correlation analysis between age and thermogravimetric parameters in 25-year interval samples stratified by air and N_2 atmospheres. (D): Correlation analysis between age and thermogravimetric parameters in 50-year interval samples stratified by air and N_2 atmospheres. (*) represent significant correlation between parameters.

Table 2
Summary of binary logistic regression analysis and ROC values.

Atmosphere	DI* (years)	Model	Accuracy (%)	AUC	SE	95% CI	*ROC* *P* value	Sensitivity	Specificity
Air	0 vs 10	Step 1 Step 2 Peak 1 Peak 3	88.9	0.953	0.053	0.844–1.000	***0.0014***	0.875	0.900
	0 vs 25	Step 2 Peak 1 Peak 3	100	1.000	0.000	1.000–1.000	***0.0002***	1.000	1.000
	0 vs 50	Peak 1 Peak 3	100	1.000	0.000	1.000–1.000	***0.0002***	1.000	1.000
N_2	0 vs 10	Step 3 Peak 1	70.6	0.757	0.126	0.509–1.000	0.079	0.800	0.571
	0 vs 25	Step 1 Peak 1 Peak 3	94.1	0.929	0.073	0.785–1.000	***0.0034***	1.000	0.857
	0 vs 50	Step 1 Step 3 Peak 1	94.1	0.885	0.108	0.672–1.000	***0.0084***	1.000	0.857
Air vs N_2 atmosphere		Step 3	100	1.000	0.000	1.000–1.000	***< 0.0001***	1.000	1.000

DI *: Dating Intervals

4. Discussion

Estimating the time elapsed after death is essential in forensic science but remains one of the most complex forensic challenges, particularly in cases involving badly decomposed corpses and skeletal remains. This pilot study stands out as one of the few that have used TGA and, to the

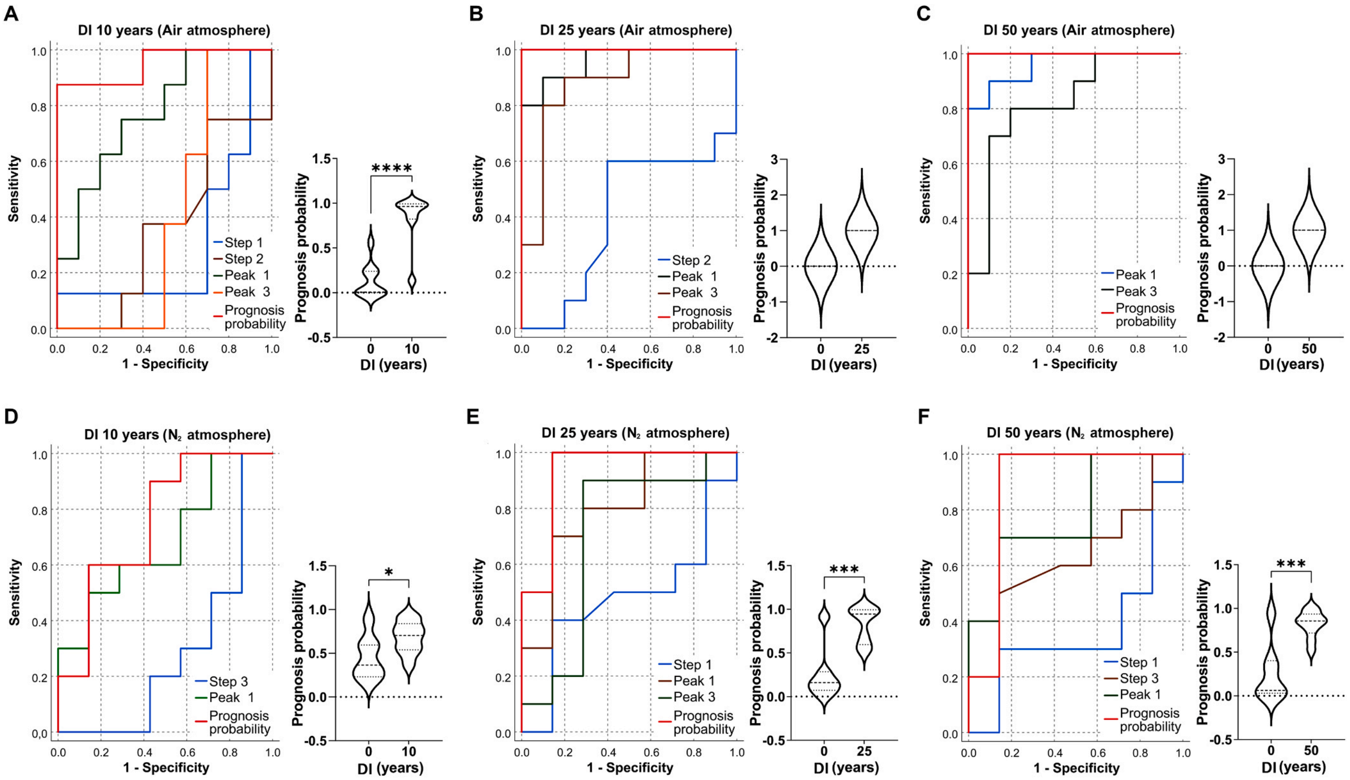

Fig. 5. Receiver operating curve (ROC) and prognostic probability analysis predicting dating intervals (0 *versus* 10, 25, and 50 years) in human teeth analyzed by thermogravimetry in air and N_2 atmospheres. (A): ROC curve and prognosis probability of 10-year interval in air atmosphere. (B): ROC curve and prognosis probability of 25-year interval in air atmosphere. (C): ROC curve and prognosis probability of 50-year interval in air atmosphere. (D): ROC curve and prognosis probability of 10-year interval in N_2 atmosphere. (E): ROC curve and prognosis probability of 25-year interval in N_2 atmosphere. (F): ROC curve and prognosis probability of 50-year interval in N_2 atmosphere. */***/**** $p < 0.05/0.001/0.0001$ *versus* 0-year interval group.

best of our knowledge, the only one in human teeth samples, providing a potential tool to accurate estimate the time elapsed after the death of the tooth (dating intervals of 0, 10, 25 and 50 years) under controlled laboratory conditions. Nevertheless, these findings are exploratory and its applicability to forensic or anthropological context requires further validation.

A diagnostic test is considered “highly accurate” with an AUC value of > 0.9, “useful for some purposes” with a value of 0.7–0.9 and “poor” with a value of 0.5–0.7 [17]. The present study has developed models based on thermogravimetric parameters with AUC values > 0.9 in air atmosphere, indicating that this method can be considered highly accurate to estimate 10-, 25-, and 50-year dating intervals. In a N2 atmosphere, this method is highly accurate for estimating 25-year and useful for this purpose at both 10- and 50-year dating intervals. Consequently, thermogravimetry models in the air atmosphere can be used for dating human teeth stored under controlled conditions. For example, applying the predictive formula described in the results section to a tooth in air atmosphere with specific TGA values (7.39 in Step 1, 16.96, in Step 2, 1.83 in Step 3, 80.97 in Peak 1, 346.04 in Peak 2 and 752.52 in Peak 3) yielded probability of 51.9% for a 10-year interval (AUC = 0.95; Sensitivity = 0.87; Specificity = 0.9), 100% for a 25-year interval (AUC = 1.00; Sensitivity = 1.0; Specificity = 1.0), and 0.0001% for a 50-year interval (AUC = 1.00; Sensitivity = 1.0; Specificity = 1.0). However, these results should be interpreted cautiously. Despite the use of bootstrap resampling to improve robustness of the study, the combination of small sample size and model selection procedures increases the risk of overfitting. The observed perfect discrimination (AUC = 1.00) might not reflect the true out-of-sample performance. Accordingly, these models should be considered exploratory and hypothesis-generating. Further validation in larger, independent cohorts is required to determine their reproducibility, stability, and potential practical relevance.

Thermogravimetric analysis provides insight into the organic-inorganic composition of teeth through temperature-specific mass-loss reactions [13]. In our study, mass loss occurred across all three TGA steps for every dating interval, and elapsed time since tooth death significantly influenced the percentage of mass loss. Comparisons with previous research are limited by the scarcity of TGA-based PMI studies and the absence of research on human teeth. One of these studies showed that thermogravimetric analysis could differentiate between fresh human bones and old bones [14]. A decrease in organic matter in archaeological bones compared with new bones was also confirmed [18]. Another study used thermogravimetry to examine pig ribs bones of different postmortem ages (3 months to 7 years) in air and N2 atmospheres [15]. Their results showed a decrease in total mass loss with time up to 23 months postmortem. Above this postmortem age, mass loss did not significantly change [15]. Discrepancies with our results on mass loss and postmortem age can be explained for different reasons. First, the type of sample used: pig bones vs human teeth. Despite the similarities in physiology and anatomy between pigs and humans, substantial differences can greatly affect the decomposition process [1,19] Second, the differences in the composition of bones and teeth, being the latter the most mineralized tissue in the human body with lower organic content and porosity [20–22]. Furthermore, the dating intervals in our study were longer (10, 25 and 50 versus 3 months to 7 years) and the experimental designs differed.

In the present study, the percent of mass loss in Step 3 varied according to dating intervals in both atmospheres, with consistently higher values in the nitrogen atmosphere. This suggests that inorganic components of mineralized samples may be more susceptible to degradation in the N_2 environment, particularly in samples with longer dating intervals. Previous work on bones exposed to nitrogen reported more complex decomposition patterns and no clear relationship between mass loss and postmortem age [15]. To date, no comparable studies have examined teeth under nitrogen conditions.

Age and sex have previously been reported as potential modifiers of PMI estimation [1,23]. We observed a reduction in mass loss with increasing donor age, being less significant as dating intervals increase. This may be explained by the higher water and organic content in younger individuals [24]. Sex also influenced several TGA parameters (Step 1 and 2, and Peaks 2 and 3), although the sample size did not allow sex-specific predictive models of dating intervals. Future research with larger samples may clarify the effect of sex. Moreover, because our study focused on long-term intervals, sex determination in real forensic cases (especially during skeletonization), may not always be feasible without genetic analysis.

In our study, samples comprised posterior teeth. Molars and premolars do not differ substantially in enamel/dentine ratios, pulp volume and surface area. Those factors may influence water retention and organic/inorganic composition and, therefore, TG/DTG parameters. Our results indicate that pooling posterior teeth did not increase sampling heterogeneity or interfere with true effects on TGA differences. Further studies involving different types of teeth are warranted.

Changes in taphonomic processes are critical considerations in forensic anthropology. Dental tissues can be affected by diagenetic and environmental factors [21], and decomposition is influenced by burial conditions, flora, fauna, ambient temperature, soil chemistry, humidity, rainfall, and individual characteristics such as age and sex [25]. However, teeth possess a distinctive composition that renders them more resilient than bones to postmortem degradation, aging, and external environment, attributable to their high inorganic content and protection within the jawbones [11,26,27]. A key limitation of this study is that all samples were stored under controlled laboratory conditions, preventing assessment of environmental variability typical of real forensic scenarios. Thus, while preliminary, our findings indicate that TGA holds promise as a future tool for dating human teeth.

As with other biochemical and structural analysis of mineralized samples, TGA requires destructive sampling [26], a standard practice in many human identification laboratories. Furthermore, techniques such as X-ray diffraction [8,11] and vibrational spectroscopy [10,11] also require specialized laboratories, limiting their routine forensic or anthropological use. Additionally, intra- and inter-individual variability, such as differences between tooth types (anterior *versus* posterior) or between healthy and unhealthy teeth, may influence thermogravimetric profiles. Further research is therefore needed to establish the reliability and accuracy of TGA to estimate postmortem tooth aging, taking into consideration factors such as environmental conditions, diagenetic changes and individual variability.

5. Conclusions

Thermogravimetric analysis revealed a significant association between total mass loss in human teeth and the dating intervals analyzed (0, 10, 25 and 50 years). Both age and sex of the individuals influenced the total mass loss. The thermogravimetric parameters in the air atmosphere obtained from human teeth stored under controlled laboratory conditions enabled the development of predictive models capable of distinguishing 10-, 25-, and 50-year intervals. Although these parameters effectively discriminated among simulated storage intervals in a controlled environment, these findings should be considered preliminary. Extensive validation using teeth with known forensic PMI and exposure to diverse environmental and taphonomic conditions is required before TGA can be recommended as a tool for routine PMI estimation in real forensic or anthropological contexts. Overall, these findings underscore the potential of thermogravimetric analysis as a novel and potentially reliable technique for dating human teeth and support the need for further investigations to confirm its applicability in real forensic scenarios.

Fundings

This research was supported by IBIMA Plataforma-BIONAND, Grants Numbers: PIE25–01 and EMG21–001, European Starting Grant: The Social Life of Dead Bodies (SOLID; GA. 101163677) and by the University of Malaga, Spain, CTS1073. Funding for open access charge: Universidad de Málaga / CBUA.

CRediT authorship contribution statement

Leticia Rubio: Writing – review & editing, Writing – original draft, Project administration, Methodology, Funding acquisition, Conceptualization. **Stella Martin-de-las-Heras:** Writing – review & editing, Project administration, Methodology, Funding acquisition, Conceptualization. **Juan Suárez:** Writing – original draft, Formal analysis, Data curation. **Diego Lozano-Peral:** Investigation, Formal analysis. **Alba Delgado-Ruiz:** Writing – original draft, Investigation, Data curation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

Professor Leticia Rubio and Professor Stella Martin-de-las-Heras are members of the European COST ACTION CA-22106- Migrant Disaster Victim Identification (MDVI).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.forsciint.2026.112965.

Data Availability Statement

The data that support the findings of this study are available on reasonable request from the corresponding author.

References

[1] K.E. Weisensee, M.M. Atwell, Human decomposition and time since death: persistent challenges and future directions of postmortem interval estimation in forensic anthropology, Am. J. Biol. Anthr. 186 (2024) e70011, https://doi.org/10.1002/ajpa.70011.

[2] Shrestha, R.; Kanchan, T.; Krishan, K. Methods of Estimation of Time Since Death. [Updated May 30, 2023]. In StatPearls Publishing: Treasure Island, FL, USA, 2023. Available online: ⟨https://www.ncbi.nlm.nih.gov/books/NBK549867/⟩ (accessed on March 2025).

[3] N. Akbulut, S. Çetin, B. Bilecenoğlu, A. Altan, S. Akbulut, M. Ocak, K. Orhan, The micro-CT evaluation of enamel-cement thickness, abrasion, and mineral density in teeth in the postmortem interval (PMI): new parameters for the determination of PMI, Int. J. Leg. Med. 134 (2020) 645–653, https://doi.org/10.1007/s00414-019-02104-2.

[4] S.C. Zapico, J. Adserias-Garriga, Postmortem interval estimation: new approaches by the analysis of human tissues and microbial communities' changes, Forensic Sci. 2 (2022) 163–174, https://doi.org/10.3390/forensicsci2010013.

[5] F. Ramsthaler, K. Kreutz, K. Zipp, M.A. Verhoff, Dating skeletal remains with luminol-chemiluminescence. Validity, intra and interobserver error, Forensic Sci. Int. 187 (2009) 47–50, https://doi.org/10.1016/j.forsciint.2009.02.015.

[6] C. Ermida, J. Rosa, E. Cunha, M.T. Ferreira, Postmortem interval estimation of human skeletonized remains through luminol chemiluminescence, Int. J. Leg. Med. 13 (2025) 429–440, https://doi.org/10.1007/s00414-024-03343-8.

[7] D.H. Ubelaker, B.A. Buchholz, J.E. Stewart, Analysis of artificial radiocarbon in different skeletal and dental tissue types to evaluate date of death, J. Forensic Sci. 51 (3) (2006) 484–488, https://doi.org/10.1111/j.1556-4029.2006.00125.x.

[8] G. Piga, A. Malgosa, T.J.U. Thompson, S. Enzo, A new calibration of the XRD technique for the study of archaeological burned human remains, J. Archaeol. Sci. 35 (8) (2008) 2171–2178, https://doi.org/10.1016/j.jas.2008.02.003.

[9] N. Hoke, A. Grigat, G. Grupe, M. Harbeck, Reconsideration of bone postmortem interval estimation by UV-induced autofluorescence, Forensic Sci. Int. 228 (2013) 176.e1–176.e6, https://doi.org/10.1016/j.forsciint.2013.03.013.

[10] Q. Wang, Y. Zhang, H. Lin, S. Zha, R. Fang, X. Wei, S. Fan, Z. Wang, Estimation of the late postmortem interval using FTIR spectroscopy and chemometrics in human skeletal remains, Forensic Sci. Int. 281 (2017) 113–120, https://doi.org/10.1016/j.forsciint.2017.10.033.

[11] L. Rubio, J. Suárez, S. Martin-de-Las-Heras, S.C. Zapico, Partners in postmortem interval estimation: X-ray diffraction and fourier transform spectroscopy, Int. J. Mol. Sci. 24 (2023) 6793, https://doi.org/10.3390/ijms24076793.

[12] A. Onishi, P.S. Thomas, B.H. Stuart, J.P. Guerbois, S.L. Forbes, TG-MS analysis of the thermal decomposition of pig bone for forensic applications, J. Therm. Anal. Calor. 92 (2008) 87–90, https://doi.org/10.1007/s10973-007-8741-0.

[13] D. Lozano-Peral, A. Arango-Díaz, S. Martín-de-las-Heras, L. Rubio, Thermogravimetric analysis of teeth for forensic purposes, J. Therm. Anal. Calor. 139 (2020) 1121–1129, https://doi.org/10.1007/s10973-019-08441-z.

[14] P.E. Villanueva, F. Girela, M. Castellanos, The application of differential thermal analysis and thermogravimetric analysis to dating bone remains, J. Forensic Sci. 21 (1976) 822–830.

[15] S. Raja, P.S. Thomas, B.H. Stuart, J.P. Guerbois, C.O. Brian, The estimation of pig bone age for forensic application using thermogravimetric analysis, J. Therm. Anal. Calor. 98 (2009) 173–176, https://doi.org/10.1007/s10973-009-0124-2.

[16] N. Ishikawa, Y. Nakamura, K. Kitamura, H. Kuroda, K. Ono, M. Hashimoto, A method for estimating time since death through analysis of substances deposited on the surface of dental enamel in a body immersed in freshwater, J. Forensic Leg. Med 92 (2022) 102447, https://doi.org/10.1016/j.jflm.2022.102447.

[17] J.A. Swets, Measuring the accuracy of diagnostic systems, Science 240 (1998) 1285–1293, https://doi.org/10.1126/science.3287615.

[18] T. Devièse, M.P. Colombini, M. Regert, B.H. Stuart, J.P. Guerbois, TGMS analysis of archaeological bone from burials of the late Roman period, J. Therm. Anal. Calor. 99 (2010) 811–813, https://doi.org/10.1007/s10973-009-0497.

[19] J.M. DeBruyn, K.M. Hoeland, L.S. Taylor, J.D. Stevens, M.A. Moats, S. Bandopadhyay, S.P. Dearth, H.F. Castro, K.K. Hewitt, S.R. Campagna, A. M. Dautartas, G.M. Vidoli, A.Z. Mundorff, D.W. Steadman, Comparative decomposition of humans and pigs: soil biogeochemistry, microbial activity and metabolomic profiles, Front Microbiol 11 (2021) 608856, https://doi.org/10.3389/fmicb.2020.608856.

[20] K. Sarna-Boś, P. Boguta, K. Skic, D. Wiącek, P. Maksymiuk, J. Sobieszczański, R. Chałas, Physicochemical properties and surface characteristics of ground human teeth, Molecules 27 (2022) 5852, https://doi.org/10.3390/molecules27185852.

[21] M. Lebon, A. Zazzo, I. Reiche, Screening in situ bone and teeth preservation by ATR-FTIR mapping, Palaeogeogr. Palaeoclimatol. Palaeoecol. 416 (2014) 110–119, https://doi.org/10.1016/j.palaeo.2014.08.001.

[22] J.D.D. Teruel, A. Alcolea, A. Hernández, A.J.O. Ruiz, Comparison of chemical composition of enamel and dentine in human, bovine, porcine and ovine teeth, Arch. Oral. Biol. 60 (2015) 768–775, https://doi.org/10.1016/j.archoralbio.2015.01.014.

[23] L. Franceschetti, A. Amadasi, V. Bugelli, G. Bolsi, M. Tsokos, Estimation of late postmortem interval: where do we stand? a literature review, Biology 12 (2023) 783, https://doi.org/10.3390/biology1206078.

[24] Shedge, R., Krishan, K., Warrier, V., Kanchan, T. Postmortem Changes. [Updated 2023 Jul 24]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025. Jan-.Available from: 〈https://pubmed.ncbi.nlm.nih.gov/30969563/〉.

[25] A. Baptista, M. Pedrosa, F. Curate, M.T. Ferreira, M.P.M. Marques, Estimation of the post-mortem interval in human bones by infrared spectroscopy, Int. J. Leg. Med. 136 (2022) 309–317, https://doi.org/10.1007/s00414-021-02641-9.

[26] D. Higgins, J.J. Austin, Teeth as a source of DNA for forensic identification of human remains: A review, Sci. Justice 53 (2013) 433–441, https://doi.org/10.1016/j.scijus.2013.06.001.

[27] P. Shah, P.R. Velani, L. Lakade, S. Dukle, Teeth in forensics: a review, Indian J. Dent. Res. 30 (2019) 291–299, https://doi.org/10.4103/ijdr.IJDR_9_17.