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A study of storage conditions and treatments for forensic bone specimens using thermogravimetric analysis

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Abstract Bone provides an important source of forensic evidence. The storage conditions of bone have been recognised as a factor in maintaining the integrity of such evidence. Thermogravimetric analysis (TG) has been employed to examine the effects of storage environments and preparation methods on the structural properties of pig bones. A comparison of oven and freeze drying has been made to study the effect of storage conditions. A comparison has also been made of ground bone specimens with cut specimens. Freeze-dried hand ground specimens provided the most consistent results and, thus, this is the recommended method of preparation of bone specimens for TG analysis.

Keywords Bone · Forensic · Thermogravimetric analysis

Introduction

Bone is an important form of evidence for forensic examiners. The age and state of bone fragments or decomposed skeletal remains can provide valuable information about the manner of death. However, the structural nature of bone and the complex nature of the interaction of the material with the environment provide a challenge for those seeking such information [1–3].

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As forensic bone specimens are archived as evidence, a question arises as to whether the means of preservation and the storage environment may have an effect on the information provided by the bones at a later time. There are alternative approaches to the preparation of bones for long term storage, including techniques such as oven drying, freeze drying or freezing. Little work has been reported on the effect of storage conditions on the structure of bones. One reported study has examined the effect of freezing bones on the histology of bones using microscopic techniques and reported minimal change [4]. However, no studies have been carried out on the changes to the molecular structure of bone as a result of storage conditions.

Another factor to be considered when examining the structure of bones is the physical form. Different analytical techniques may require the sample to be in a particular state. For instance, examination of bone specimens by X-ray diffraction [5] or infrared spectroscopy [6, 7] requires the bone to be in powdered form, while a technique such as electron microscopy involves studying the specimen in bulk form [6, 8]. There is the possibility that transforming bone from its natural structural form into a powder may affect the interpretation of data.

Thermogravimetric analysis (TG) has been successfully employed as a technique for forensic bone characterisation [9–18]. Some success has been achieved in the correlation of TG data with the age of pig bone specimens up to 5 years of age through mass loss determination [11]. The mechanisms of decomposition of the pig bone have also been found to be sensitive to the type of burial environment (e.g. soil burial, surface deposit) [10]. The storage environment and preservation methods are predicted to be observed as artefacts in the thermal data, especially given the sensitivity of thermal methods to sample type and geometry.

For this study, the effect of storage conditions and preparation methods on the TG data of forensic bones was investigated. In particular, the effect of drying conditions and the sample state (cut segments versus hand ground bone) have been investigated.

Materials and methods

Bone specimens with post-mortem ages ranging from 3 to 20 months were obtained from pigs. All samples were flat rib bones from female pigs (*Sus scrofa*) sourced from the same farm with an identical diet and weighing 40–45 kg. The carcasses were buried in soil at 60 cm below the surface for the designated amount of time. The average ambient temperature was 25 °C and a soil pH 5. After exhumation the bones were stored in sealed plastic bags at 4 °C prior to analysis. The bones were mechanically sliced using a Buehler IsoMet low speed diamond saw and the cortical bone was chosen for analysis.

Four sample preparation methods were used for the bone specimens:

1. Oven dried in a vacuum oven at 50 °C for 2.5 h in lump form (OD-L);
2. Oven dried in a vacuum oven at 50 °C for 2.5 h and ground using a ring mill (Rocklab) for 30–45 s (OD-R-P);
3. Freeze dried (Christ Alpha 2-4 LD Plus) at −90 °C and a vacuum of 0.0010 mbar for 24 h and hand ground in an agate mortar and pestle (FD-H-P);
4. Freeze dried at −90 °C and a vacuum of 0.0010 mbar for 24 h and ground using a ring mill for 30–45 s (FD-R-P).

TG was carried out on a TA Instruments SDT 2960. Samples (3–5 mg; determined by the bulk density of the sample) were placed in a platinum crucible, using an empty platinum crucible as a reference and heated in a flowing air atmosphere (20 mL min^{-1}) from 30 to 1000 °C at a rate of 10 °C min^{-1}.

Results and discussion

The TG measurements reveal that the manner in which the bone specimens were prepared does affect the results obtained. The TG data for 3-month samples prepared by four sampling methods and heated in an air atmosphere are shown in Fig. 1. Significant differences are observed in the mass loss data that can be attributed to the method of sample preparation. The water loss region (30–200 °C) is grouped into oven-dried and freeze-dried specimens, where the freeze drying process is observed to have removed less of the total water. Oven drying removed more water and this was not significantly affected by the state of the sample (lump or powder).

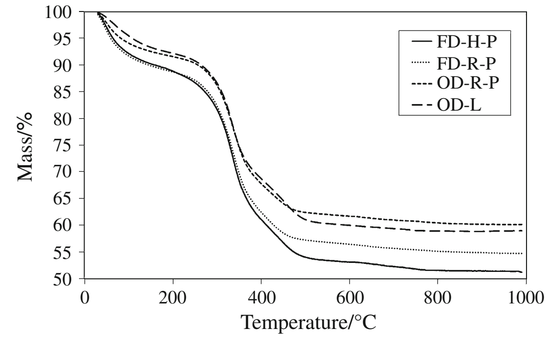

Fig. 1 TG data for 3 month samples in an air atmosphere

Differences were also observed in the organic decomposition region (200–600 °C). In this region, the differences were based on whether or not the specimens were ring ground. A greater mass loss was observed for the oven dried lumps than the ring ground oven dried powder. Similarly, a greater mass loss in this region was observed for the hand ground than the ring ground freeze-dried specimens. These observations suggest that ring grinding imparts a significant amount of energy into the sample resulting in some degradation and volatilisation of the organic phase.

As little control of the amount of water in the specimen during drying was possible, the water content (Fig. 2) and organic component content (Fig. 3) of the bones, plotted as a function of the burial time were examined in terms of the residue mass. The water content was observed to vary significantly for all the different sample preparation methods, making water content an unreliable method of ageing. The data obtained for the region associated with loss of organic matter also show a significant degree of variability with age. The hand ground freeze-dried bone samples, however, showed a consistent reduction of approximately 0.6% per month in organic content with ageing time. This correlation

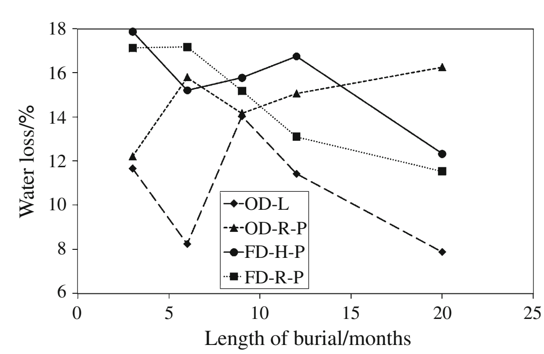

Fig. 2 Water loss as a percentage of final mass versus bone age

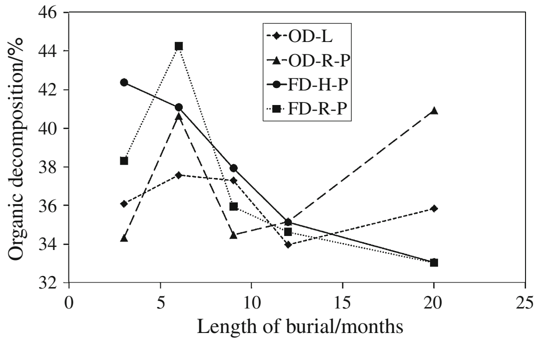

Fig. 3 Organic mass loss as a percentage of final mass versus bone age

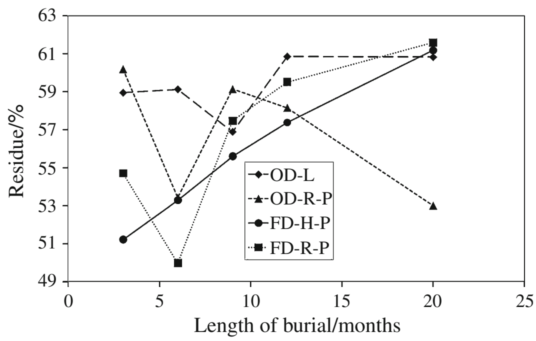

Fig. 4 Residue as a percentage of initial mass versus bone age

may be due to the minimal amount of damage applied to the sample during sample preparation for analysis.

Figure 4 shows the residue as a percentage of the initial mass versus bone age. The residue appears fairly variable, except for the hand ground freeze-dried bone samples. This is due to the fact that any variation in mass loss due to water content is small in comparison to the change in mass loss due to the organic content. As a consequence, the residue mass shows a relationship with the burial age.

Conclusions

This investigation of bone specimens has demonstrated the importance of careful sample handling techniques in data acquisition using thermal methods. For the case of the bone specimens, which contain both water and organic material, the method of removal of water and the energy imparted during the preparation of a homogeneous powder specimen significantly influences the TG data. Ill considered preparation methods, therefore, introduce artefacts. These artefacts may be introduced from a lack of sample preparation, such as in the case of the lump specimens, or from the transfer of excessive amounts of energy (ring grinding) in the preparation of more homogeneous powder specimens, resulting in sample degradation. The conditions of freeze drying followed by the minimal energy imparted to the specimens by hand grinding were the only conditions that produced consistent change in the mass loss with burial age. It is these preparation conditions that are recommended for the analysis of bone specimens by TG, allowing this technique to be applied to the determination of burial duration.

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