A comparison of different maceration techniques on burnt remains

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ABSTRACT

Maceration is the process of removing soft tissue from bones so as to be able to study the skeletal elements. This is a necessary process in forensic anthropology because human skeletal remains can be used to create a biological profile that can aid in the identification of an unknown individual. The aim of this research was to determine the best method of maceration for burnt remains. Pigs' trotters were burnt in an open flame and then macerated using a variety of maceration techniques. After maceration, the results obtained were scored on a macroscopic scoring system to assess the damage caused by each of the techniques employed. The maceration technique that caused the least amount of exogenous damage to the skeletal elements was that using plain tap water at a temperature of 80°C. The addition of chemical or enzymatic agents, or an increase in the length of time that the remains were immersed in a solution, had a deleterious effect on the bones.

Keywords: maceration; defleshing; burnt remains; anthropology, skeletal

HIGHLIGHTS:

- A method for maceration of burnt remains using different maceration techniques.
- Burnt skeletal remains examined to determine which maceration technique incurs the least damage.
- Burnt remains immersed in plain tap water solutions at 80°C incur minimal damage.

Introduction

The analysis of skeletal remains is useful in many scientific disciplines (1). Human skeletal remains can reveal many aspects of a deceased person, such as their age-at-death, sex, ancestry, stature and if any physical trauma may have been incurred (2). By studying human skeletal remains, scientists can gather valuable information on the history and possible identity of a deceased individual.

Forensic anthropologists study human skeletal remains of unknown persons (acquired in a forensic context) in order to establish the life, death and post-life history of an individual (3). However, not all remains are fully skeletonized upon examination (4). To achieve skeletonization, the remaining soft tissue on the remains must be removed from the bones through the process of maceration (5;6).

There are many different maceration techniques, each with its own advantages and disadvantages (7;6). The most common techniques are: physical maceration, hot water or cooking maceration, cold and warm water bacterial maceration, chemical maceration, enzymatic maceration and invertebrate maceration (7). The effectiveness of a maceration technique may be defined by the adverse impact it can have on bone, the odour produced during the process, the time taken, the cost and the possible risks to the person performing the maceration (8).

In the case of burnt remains, some, if not all bones, are likely to be already damaged by fire, making the maceration process even more challenging. During the initial stages of bone

alteration due to fire, the bone loses water (dehydrates), resulting in bone shrinkage and bone deformation (3; 9). As the bones continue to dehydrate, cracks occur, and this may lead to fragmentation due to their fragile nature (3; 10). Currently, no formal standards for macerating burnt human remains exist. The aim of this study was to determine which technique of maceration causes the least amount of macroscopic damage to burnt skeletal remains.

Materials and Methods

The study was a prospective observational and descriptive study that compared several different maceration techniques to establish which technique was best suited for burnt remains. An ethics waiver letter was obtained through the animal ethics committee at the University of the Witwatersrand for this project (Triaca A: Waiver 17-04-2018-O). Porcine trotters (pigs' feet) (sus Scrofa) (Figure 1 – A) were purchased from a commercial butcher and no animal was killed for the specific purpose of providing material for this research. Figure 1 shows the hot water maceration process used to obtain a reference set of bones from an unburned porcine trotter.

Figure 1: Images showing the processing of an unburnt pig's trotter used for reference purposes. A: Fresh trotter as purchased (side and bottom views). B: Separation of all trotter bones after hot, plain water immersion. C: All reference trotter bones after final cleaning using physical maceration techniques.

To achieve the aim of this study, the porcine trotters were burned using a standardised test setup comprising a Cadac Skottelskaar Braai burner and simple support frame for the

trotters (Figure 2). The remains were then macerated using different maceration techniques, namely: cold and warm water bacterial maceration, hot water maceration, enzymatic maceration and chemical maceration.

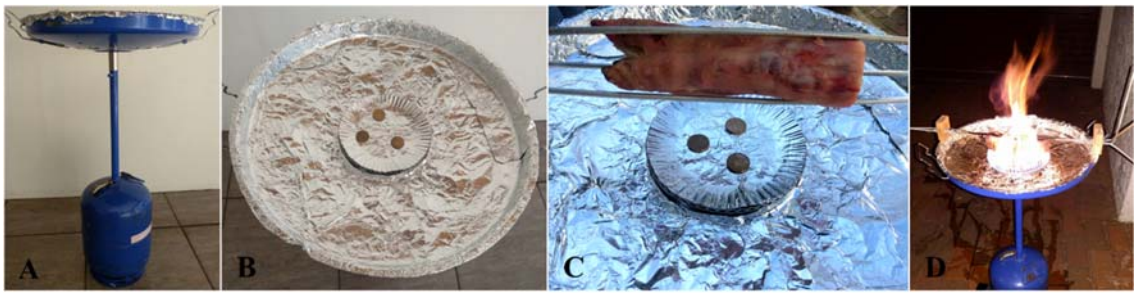

Figure 2: Image showing how a pig's trotter was burnt. A: The Cadac Skottelskaar Braai burner with 5kg LP gas cylinder. B: Tin foil used to protect the surface of the burner. C: A pig's trotter anchored between three support rods. D: The burning of the pig's trotter.

A total of 24 porcine trotters were utilized in this research. Each porcine trotter weighed between 400g and 500g, was not previously frozen, exhibited no physical, chemical or bacterial damage, and was subsequently burned within a maximum of three days after purchase. No physical maceration was done prior to the burning.

Each porcine trotter was individually burned. After being subjected to 15 minutes of burning on one side, the trotter was then rotated through 180° and burned for a further 15 minutes. This length of time allowed for most of the flesh to burn away from the bone, and aspects of the bone itself to be burned (Figure 3). Note, because some flesh remained, not all bone was equally burned. The extremities of the trotter experienced more severe burning, exhibiting calcined bone whereas the central aspect of the trotter remained unburned (Figure 3 images B and C). In accordance with the maceration protocols employed at the Johannesburg Forensic Pathology Service, each burnt trotter then had as much soft tissue as possible removed

by physical maceration (without causing damage to the bones). The entire trotter, together with all burnt fragments, was then subjected to the different maceration techniques (Figure 3).

Figure 3: Images showing the processing of a burnt pigs' trotter using plain hot water. A: Trotter immediately after burning showing degree of burning. B: Manually separated trotter bones after hot water immersion. C: All trotter bones after final cleaning using physical maceration techniques.

Maceration Techniques Undertaken

After burning, individual porcine trotters were then subjected to one of the following maceration techniques:

For hot water maceration (80°C) and warm water maceration (35°C), each individual burnt porcine trotter sample was placed in a 20*l* thermostatically controlled Aro™ urn already filled with 6*l* of plain tap water and pre-heated to a temperature of either 80°C or 35°C respectively. The water was then maintained at the desired temperature for a period of 24 hours.

For cold water bacterial maceration, chemical and enzymatic maceration, the individual burnt porcine trotter samples were placed into separate, 20l plastic buckets containing either 6l of plain tap water, 6l of a chemical solution, or 6l of an enzymatic solution. The buckets were sealed with a lid and stored in a closed, dark room, where the average ambient temperature was approximately 15°C (± 2°C).

Two chemical solutions were evaluated. The first comprised a ¼ cup of bleach, Aro™ Thin Bleach Lemon (sodium hypochlorite), added to 6*l* of plain tap water. The second chemical solution comprised ⅔ cup of Sunlight™ Lemon 100 (11) dishwashing liquid added to 6*l* of plain tap water.

The enzymatic solution comprised ⅔ cup of OMO™ Auto (12) powder detergent (containing enzymes) added to 6*l* of plain tap water.

After each 24-hour period, the remains were individually removed, examined, and then scored in accordance with the macroscopic scoring criteria specified in Table 1. If a score of 4 or more (for any of the macerating techniques) was recorded for the three criteria: 'disarticulation', 'ease of tissue removal' and 'ease of cleaning', without applying any physical maceration techniques, then the respective solutions (plain tap water, chemical or enzyme) were replaced with fresh solutions and the remains immersed back into the associated bucket or urn for another 24-hour period. However, if a score of between 1 and 3 was recorded for the same criteria, then the maceration process was stopped, and the remaining soft tissue was removed from the bones using running water and physical maceration methods (using brushes, scourers, tweezers and sponges).

Testing was stopped after seven weeks (49 days), as this length of time was deemed by the authors to be impractical for use in any medico-legal investigation.

Upon completion of the physical maceration process, the skeletal remains were placed onto paper towels and allowed to dry. Once dry, they were photographed and scored (by observation) in accordance with the macroscopic scoring criteria specified in Table 1, and the results tabulated in Table 2. Eight criteria were used when evaluating the condition of the skeletal remains:

- Disarticulation

Table 1: Table showing the macroscopic scoring criteria used to determine the best macerating technique for burnt remains.

Score	Disarticulation	Ease of Tissue Removal	Bone Flaking	Damage to Surface of the Bone	Chalkiness	Ease of cleaning	Cartilage Remaining	Fragmentation
1	Completely disarticulated in water	Soft tissue falls off in water	No bone flaking present	No damage to the bone	No chalkiness	No physical cleaning	No cartilage remaining	All fragments intact
2	Mostly disarticulated in water, needs minor physical maceration to disarticulate completely	Most of the soft tissue falls off in water, easy to remove remaining tissue with fingers	Minor bone flaking present on less than 20% of the bone	Minor damage to the bone surface such as bone pitting in small areas	Minor bone chalkiness present on less than 20% of the bone	Minor physical cleaning (10 minutes)	Some cartilage remains on small bones	Some fragments damaged
3	Needs physical maceration to disarticulate all joints	Some soft tissue remaining but easy to remove using fingers and forceps	Bone flaking present on 20-50% of the bone	Damage to many areas of the bone surface	Bone chalkiness present on 20-50% of the bone	Some physical cleaning (30 minutes)	Some cartilage remains on more than 30% of bones	More than 50% of fragments disintegrating
4	Extremely difficult to disarticulate the joints, some unable to disarticulate	Difficult to remove remaining soft tissue, scissors and scalpels are required	Bone flaking off on 50-70% of the bone	Severely compromised bone surface	Large amounts of bone chalkiness on 50-70% of the bone	Lots of physical cleaning (30-60 minutes)	Cartilage unable to be removed on more than 60% of bones	More than 70% of fragments disintegrating
5	Unable to disarticulate most of the joint	Most soft tissue cannot be removed by any means	Bone flaking off on 70-100% of the bone	Very severely damaged bone, unable to distinguish between maceration damage and prior damage	Large amounts of bone chalkiness on 70-100% of the bone	Unable to completely physically clean	Unable to remove cartilage on 60-100% of the bones	All bone fragments disintegrate

Table 2: Scores assigned to the burnt pigs' trotters after the completion of hot, warm and cold water maceration techniques. (Avg. = the sum of the scores from group 1 and group 2 divided by two.)

Macerating Technique		Disarticulation			Ease of Tissue Removal			Bone Flaking			Damage to Surface of Bone (excl. Burning)			Chalkiness			Ease of Cleaning			Cartilage Remaining			Fragmentation			Total Composite Score of Average values
Burnt Groups		1	2	Avg.	1	2	Avg.	1	2	Avg.	1	2	Avg.	1	2	Avg.	1	2	Avg.	1	2	Avg.	1	2	Avg.	
Hot Solution	OMO Auto	1	1	1	1	1	1	3	3	3	3	3	3	5	3	4	1	1	1	1	1	1	3	3	3	17.00
	Bleach	2	2	2	3	3	3	3	2	2.5	3	3	3	4	4	4	3	3	3	2	2	2	3	3	3	22.50
	Sunlight Liquid	2	2	2	3	3	3	2	2	2	2	2	2	2	2	2	3	3	3	3	3	3	2	3	2.5	19.50
	Plain Water	2	2	2	2	1	1.5	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	1	1	1	11.50
Warm Solution	OMO Auto	3	4	3.5	3	4	3.5	2	3	2.5	2	3	2.5	2	2	2	3	4	3.5	3	3	3	3	3	3	23.50
	Bleach	3	3	3	2	3	2.5	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	4	4	4	24.50
	Sunlight Liquid	4	4	4	4	4	4	2	2	2	2	2	2	2	2	2	3	3	3	5	5	5	4	4	4	26.00
	Plain Water	3	4	3.5	4	4	4	2	2	2	1	1	1	1	1	1	4	3	3.5	4	4	4	3	3	3	22.00
Cold Solution	OMO Auto	3	4	3.5	3	3	3	3	3	3	3	3	3	3	2	2.5	3	3	3	4	3	3.5	3	4	3.5	25.00
	Bleach	5	4	4.5	4	4	4	4	3	3.5	4	3	3.5	4	3	3.5	5	4	4.5	5	4	4.5	5	5	5	33.00
	Sunlight Liquid	3	4	3.5	3	4	3.5	2	1	1.5	2	2	2	1	1	1	3	4	3.5	4	4	4	4	4	4	23.00
	Plain Water	3	3	3	4	4	4	1	1	1	2	2	2	1	1	1	4	4	4	3	4	3.5	4	4	4	22.50

- Ease of tissue removal
- Bone flaking
- Damage to the surface of the bone
- Chalkiness
- Ease of Cleaning
- The amount of cartilage remaining
- Bone fragmentation

The results from both the initial and repeated experiments were then averaged in each of the maceration techniques used. The average score for each criterion was then added together to obtain a total composite score of average values. The lower the composite score, the better the maceration technique.

Results

Overall, the burnt remains macerated in hot water solutions scored consistently lower than the burnt remains macerated in either warm or cold-water solutions, regardless of the chemical or enzymatic agents added (Figure 4).

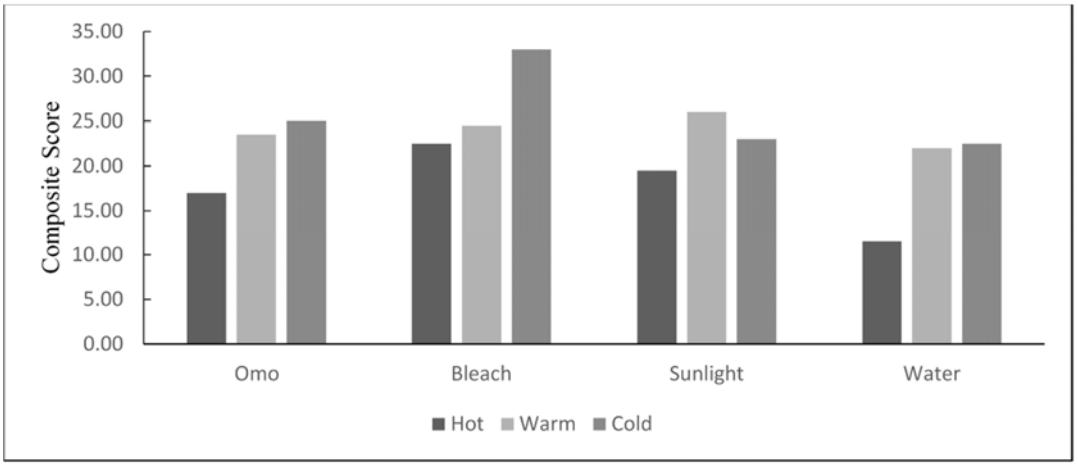

Figure 4: Bar graph showing Overall composite score obtained for different maceration techniques.

It was noted that, when a low score was assigned to a particular specimen for disarticulation, a low value was also assigned to that specimen for the criteria ‘ease of tissue removal’ and ‘ease of cleaning’. None of the specimens macerated in warm or cold water were able to be completely cleaned i.e. all the flesh removed (Table 2).

The burnt specimens immersed in plain tap water solutions at 80°C were the only remains that did not incur any exogenous damage on both the unburned and burned aspects of the bone. They also exhibited the least amount of fragmentation.

Discussion

The hot water maceration technique was seen to be the most effective maceration technique when analysing the criteria ‘disarticulation’, ‘ease of tissue removal’, and ‘ease of cleaning’ because these specimens scored the lowest for these criteria.

On the more comprehensively burned parts of the pigs trotter, a hard layer of charred flesh (the black coating visible on the larger bones shown in Figure 3 – B), that was not able to be removed by physical maceration, remained. This layer of hardened, charred flesh was subsequently removed during the hot water maceration process in a similar manner to any residual unburned flesh (Figure – 3B). Hot water is known to effectively remove the soft tissue from bone through the process of cooking the soft tissue (7;13). The high temperatures denature the protein present in the soft tissue, thereby loosening its connection to the bone (7;14;15). Although this cannot be said for those areas of bone that were comprehensively burned, the hot water maceration technique yielded the best results in removal of the remaining covering of hardened, charred flesh, with minimal damage to the underlying bone.

The specimens immersed in all cold-water solutions took the longest period of time to macerate (7) and was deemed too long to be considered feasible in a forensic setting.

Burning the remains in an open flame caused some of the bone to splinter and fragment off (figure 2). When remains are burned in this manner, the composition and crystal structures of the bone are altered and the bone becomes more fragile and lighter (2;9). Collagen, the organic component of bone, gives the bone its flexibility. Collagen is destroyed during the burning process and this causes the remains to become more fragile (2;16). Due to the fragility of the burnt remains, the addition of chemicals and enzymes can result in more damage. This was observed in the study, where only those burnt remains that were exposed to hot, plain tap water solutions, did not disintegrate.

It is extremely difficult to successfully macerate burnt remains due to their very fragile composition and variability. Best results are obtained by minimising the amount of time that the specimens are exposed to macerating solutions. The best macerating technique determined from this study is that using a plain tap water solution, heated to a temperature of 80°C for a period of 24 hours.

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