The use of different detergents in skeletal preparations

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Highlights

- Skeletal preparation is an integral part of forensic anthropology, but can be a complex process.
- Detergent maceration is a reliable method but commercial detergent brands differ in ingredients.
- High temperatures and detergents with enzymes only was the best method tested for maceration.

Abstract

Skeletal preparation has become an integral component within the field of forensic anthropology. The aim of this study was to determine which commercial detergent was most effective and efficient for use in skeletal preparation. The hind limbs of 24 pigs (*Sus scrofa*) and five detergents with bleaching agents and enzymes (Surf and Ariel), only enzymes (OMO Auto and Sunlight powder) or only bleaching agents (Sunlight dishwashing liquid) were used. Specimens were skinned and immersed into a pre-heated 6 L detergent solution or tap water and macerated at either 45 °C, 50 °C, 55 °C and 60 °C. When maceration was deemed complete any remaining soft tissue was manually removed under running tap water and the remains left to dry. A scoring system was utilized to assess the effectiveness and efficiency of each detergent. OMO Auto specimens only required a single day to complete macerate regardless of the temperature and these specimens constantly scored better than the other detergents used, thus making it the most effective and efficient detergent tested.

Keywords: forensic anthropology; *Sus scrofa;* skeletal preparation; enzymatic maceration; detergent maceration

With the increasing demand of forensic anthropologists to assess, examine and interpret remains of varying stages of decomposition, knowledge of different maceration techniques are essential. Maceration is a common practice in many fields and disciplines, such as zoology, anatomy, museum conservation and taxidermy where skeletonized remains are required for research, teaching and display purposes [1], [2], [3]. Forensic anthropologists require clean skeletonized remains for anthropological assessment in medico-legal cases [4]. Maceration techniques employed in a forensic context should preserve both bone structure and integrity [3].

According to Fenton et al. [5], the best macerating technique is the one with little or no detrimental effects on the skeleton. However, Mann and Berryman [6] argue that methods need to differ to meet the criteria of the case being investigated in terms of urgency, as well as the state in which the remains are received. There a number of maceration techniques that have been described in literature, however these techniques are usually grouped into five categories namely: insect scavenging, chemical maceration, water maceration, enzymatic maceration and physical maceration [3], [4], [5], [6], [7], [8], [9], [10], [11], [12], [13]. The purpose of all of these techniques is to remove the remaining soft tissue from the bone to allow for a full skeletal analysis [3]. Therefore, these techniques can be used separately or more commonly in combination with each other. Even though there are a number of acceptable macerating techniques available [6], there are no universal standardized protocols which exists describing which technique or combination of techniques are best to use in a forensic setting [14]. Often universities or laboratories employ their own standardized methods and techniques according to their experience. The success of a macerating technique is determined by time efficiency, resources required and the desired result [4], [10].

Forensic anthropologists usually use literature to decide on the most appropriate method of maceration. Each method has its own advantages and disadvantages. Thus, forensic anthropologists choose methods based on the desired final appearance and condition of skeletal remains. However, in most cases, factors such as equipment available for use as well as the financial status of their institution limits the pool of options to choose from. Since there is no single maceration technique that fulfils the criteria of effectiveness, safety, bone and evidence preservation, one of the least expensive yet most effective method was chosen for this study – detergent maceration [15]. Detergent maceration is comparable to enzymatic maceration; however, it contains biological as opposed to synthetic enzymes which pose less health and safety risks [16]. A detergent is any substance that has assistive properties for the removal of dirt from substrates [17] and contains ingredients including but not limited to: surfactants, builders, silicate, anti-redisposition agents, optical brighteners, and enzymes. All commercial detergents have different proportions of the above-mentioned ingredients in varying amounts [18]. Enzymes found in detergents can quickly digest proteins and fats thus speeding up the maceration process [18]. Furthermore, detergents contain deodorants which eliminate foul odors [10], providing an alternatively safe and effective maceration process as opposed to other suggested maceration methods.

The use of detergents as an alternative method of maceration has previously been evaluated and compared with traditional maceration methods. It is known to be reliable, easy to learn and does not require a lot of laboratory space [19]. In this study, detergent maceration was selected as the different ingredients contained within commercial detergents may affect the duration, efficiency and effectiveness of maceration. The results of this study can help forensic anthropologists in determining which composition of ingredients found in common detergents are better to use for maceration, at what temperature to macerate and the ideal duration of maceration.

1. Materials and methods

This was a descriptive blind-test study which made use of a macroscopic scoring system. A total of 24 pig (*Sus scrofa*) hind limbs cut at the tarsocrural joint (anatomically homologous with the ankle of a human) were used to investigate the effectiveness and efficiency in the removal of soft tissue using five different commercial detergents: combination of bleaching agents and enzymes (Surf and Ariel), only enzymes (OMO Auto and Sunlight powder) or

only bleaching agents (Sunlight dishwashing liquid). The weight of each of the specimens ranged between 500–600 g and were purchased from a local butchery. An ethics waiver was obtained from the Animal Ethics Screening Committee at the University of the Witwatersrand, Johannesburg. Pig (*Sus scrofa*) were specifically selected in this study as an animal proxy because they are closely comparable to humans [20]. The five detergents selected for this study were selected as they are readily available on the market, but most importantly the ingredients and their proportions vary greatly. The four main ingredients per detergent are listed in Table 1.

Table 1. Showing the five most common ingredients in each of the five different detergents used in this study.

Empty Cell	**Surf**	**Ariel**	**Omo**	**Sunlight washing powder**	**Sunlight dishwashing liquid**
1	Sodium Carbonate	Alcohol Ethoxylte	Sodium Sulfate	Surfactants	Sodium Dodecylbenzene Sulfonate
2	Sodium Sulfate	Alkyl Ethoxy Sulfate	Sodium Carbonate	Builders	Sodium Lauryl Ether Sulfate
3	Sodium Dodecylbenzene Sulfonate	Amine Oxide	Sodium Dodecylbenzene Sulfonate	Silicates	Sodium Xylene Sulfonate
4	Sodium Silicate	Carboxymethyl Cellulose	Sodium Silicate	Anti-redeposition agents	Ethyl Alcohol
5	Zeolite	Citric Acid	Zeolite	Perfume	Cocamidopropyl betaine

1.1. Sample preparation

1.1.1. Pre-maceration

The specimens were divided into six groups of four. The detergents were labeled A–E and the control was labeled T. Each of the four specimens per solution was macerated at one of the following temperatures: 45 °C, 50 °C, 55 °C or 60 °C and were denoted by consecutive numerical values. The skin from each specimen was manually removed through the use of physical maceration techniques before being immersed into a pre-heated 20 L *Aro* urn. The urn either contained a detergent solution (240 ml of detergent mixed into 6 L of water or plain tap water for the control specimens) and macerated at the four different temperatures. The detergent solution or tap water was changed every 24 h and the condition of the specimens was scored using the criteria in Table 2.

Table 2. Showing the macroscopic scoring criteria and descriptions used to score each specimen.

Score	1	2	3	4	5
Criterion					
Ease of soft tissue removal	Soft tissue easily falls off as the specimen is being removed from the solution	Soft tissue is easily removed with fingertips	Soft tissue is moderately adhered to bone but can be removed using forceps. There is however remnants of soft tissue left behind.	Large amounts of soft tissue are adhered to the bone. The soft tissue is difficult to remove with the aid of scalpels and scissor leaving some soft tissue still attached to the bone.	All soft tissue is completely adhered to bone and it is impossible to remove even with the use of forceps, scissors and scalpels.
Extent of manual cleaning	Very easy, took less than 10 min	Easy, took about 15–20 min	Moderate, took about 30–45 min	Difficult, took close to 60 min	Very difficult, took close to 90 min
Bone cleanliness	No soft tissue	Few traces of soft tissue	There is moderate amount of soft tissue	There is a little bit of both soft tissue and cartilage	There is considerable amount of soft tissue, cartilage and tendons
Bone appearance	Normal bone texture and quality	Few bones have chalky edges	The cortical surface of five bones or less is chalky	The cortical surface of more than five bones is chalky	Bones are brittle, fragile and break easily

1.1.2. Post-maceration

Maceration was deemed complete when the soft tissue and most of the joints were loose or when the duration of maceration totaled 3 consecutive days. The time taken, in days, for maceration to complete was recorded. Following the completion of maceration, the remnants of soft tissue still adherent to the bone was manually removed and cleaned under running tap water. During this process, the ease of soft tissue removal (Table 2) and the extent of manual cleaning (Table 2) were assessed and scored. The bones were then placed into a stainless steel tray and left to dry at room temperature for several days. Thereafter, bone cleanliness (Table 2) and bone appearance (Table 2) were assessed and scored. Each specimen was photographed throughout the different stages of maceration to document the various changes observed (Fig. 1). For each criterion assessed and scored the average and mean composite score was calculated. This study investigated the efficiency and effectiveness of five different commercial detergents. The efficiency of the detergents was measured as duration of maceration and qualitatively evaluated as descriptions of ease of soft tissue removal and extent of manual cleaning.

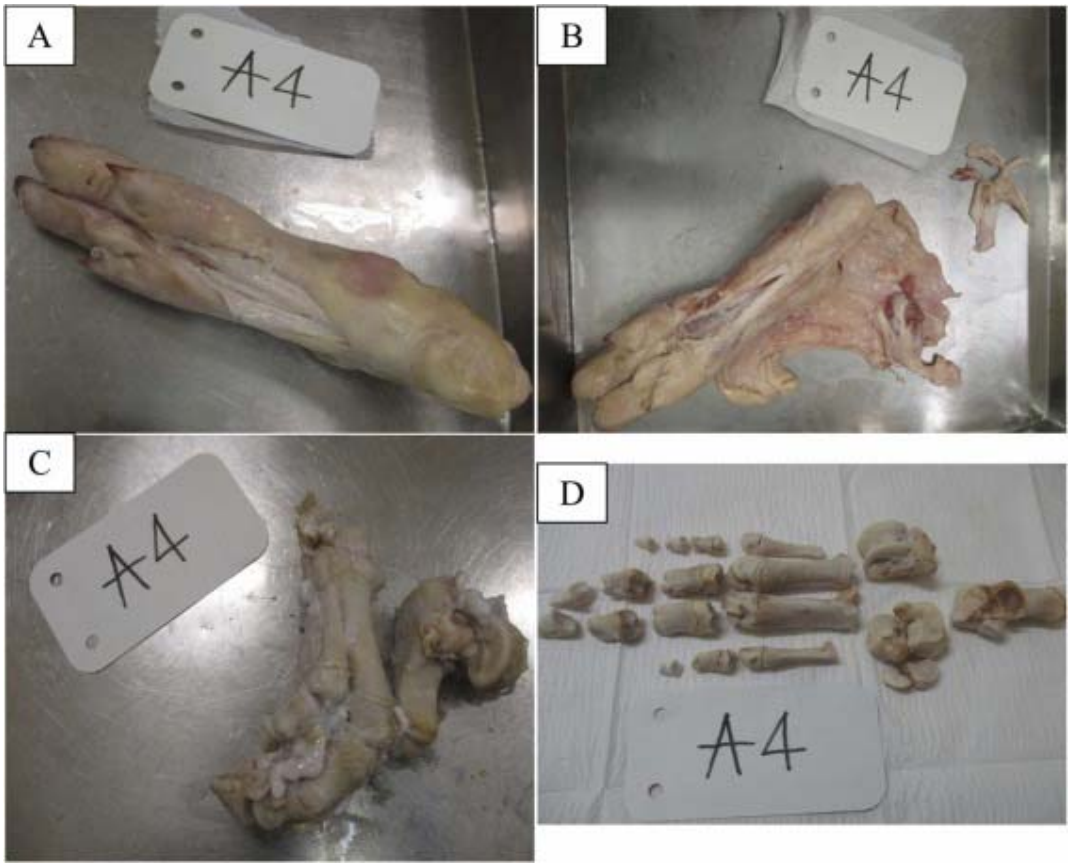

Fig. 1. Shows changes observed during the process of maceration. A – condition of the specimen before skinning. B – specimen after the removal of skin. C – condition of the specimen after maceration. D – condition of the bones after drying.

2. Results

The effectiveness was assessed as bone cleanliness and appearance post-maceration. The scoring system ranged from 1 to 5, with one being most effective and five being the least effective. Thus, the commercial detergent with the highest composite score was deemed to be, in this study, the least efficient and effective detergent to use for skeletal preparation.

The time taken to complete maceration at high temperatures (60 °C and 55 °C) was one day for all the specimens of the five different detergents and the control specimens (Fig. 2). At 50 °C, the control specimen took 2 days to macerate while all the detergent specimens took a day. At 45 °C the control and Sunlight Dishwashing Liquid specimens macerated for 3 days, while Surf, Ariel and Sunlight powder detergent specimens took 2 days. OMO Auto was the most efficient detergent as the duration of maceration took a single day for all specimens at the varying temperatures tested (Fig. 2).

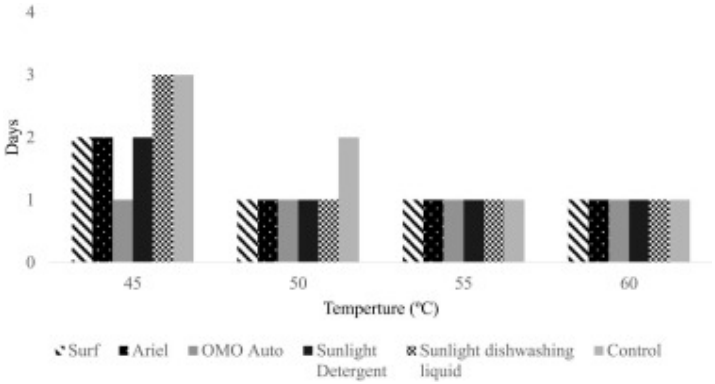

Fig. 2. A bar graph showing the duration of maceration (in days) for the various temperatures used of all the detergents and control specimens.

OMO Auto produced the most successful results regarding ease of soft tissue removal with an average score of 2. While the Sunlight dishwashing liquid specimens were the most difficult to clean with scores ranging from 2 to 5. Surf, Ariel and Sunlight powder detergent specimens were the second best, with moderately loosened soft tissue which could be removed when using forceps. The ease of tissue removal scores for the control specimens were notably higher compared to the OMO Auto specimens (Table 3). However, they were much easier to clean when compared to the Sunlight dishwashing liquid specimens. In addition, specimens macerated at high temperatures were much easier to clean compared to those macerated at low temperatures.

Table 3. Scores of the detergent and control specimens at all the tested temperatures.

Temperature (°C)	Ease of tissue removal	Extent of manual cleaning	Bone cleanliness	Bone appearance	Composite score
Surf					
45	3	4	2	2	11
50	3	3	2	2	10
55	2	2	1	2	7
60	2	2	1	2	7
Average	2.5	2.8	1.5	2.0	8.8
Ariel					
45	3	4	2	3	12
50	3	3	2	1	9
55	2	2	1	2	7
60	2	2	1	2	7
Average	2.5	2.8	1.5	2.0	8.8
OMO Auto					
45	3	4	2	2	11
50	2	3	2	2	9
55	2	2	1	2	7
60	1	1	1	2	5
Average	2.0	2.5	1.5	2.0	8

Temperature (°C)	Ease of tissue removal	Extent of manual cleaning	Bone cleanliness	Bone appearance	Composite score
Sunlight					
45	3	3	1	2	9
50	3	3	2	2	10
55	2	3	1	2	8
60	1	1	1	2	5
Average	2.3	2.5	1.3	2.0	8.1
Sunlight dishwashing liquid					
45	5	5	5	1	16
50	3	4	2	1	10
55	3	3	2	2	10
60	2	3	2	2	9
Average	3.3	3.8	2.8	1.5	11.4
Control					
45	4.	4	2	2	12
50	3	3	2	2	10
55	3	3	2	2	10
60	2	3	1	2	8
Average	3.0	3.3	1.8	2.0	10.1

The extent of manual cleaning required after maceration of the specimens varied greatly. The specimens macerated at low temperatures (50 °C and 45 °C) had a higher score compared to those macerated at higher temperatures (60 °C and 55 °C). Sunlight powdered detergent produced the best results with scores ranging between 3 at 45 °C and 1 at 60 °C. However, it had the same average score of 2.5 as OMO Auto, which scored 4 at 45 °C and 1 at 60 °C. Sunlight dishwashing liquid specimens were moderately difficult to clean compared to the other detergent macerated specimens, thus the process of manually removing soft tissue post maceration took longer.

The specimens macerated at 60 °C and 55 °C had few or no traces of soft tissue present while those macerated at low temperatures (50 °C and 45 °C) had moderate amounts of both soft tissue and cartilage. The bone cleanliness of Sunlight powder detergent scored better at 45 °C compared to all the other detergents and the control. It was also the most effective detergent at all temperatures regarding bone cleanliness. Surf, Ariel and OMO Auto had similar scores at all temperatures. Specimens macerated at 60 °C and 55 °C scored 1 while those macerated at 50 °C and 45 °C scored 2. Sunlight dishwashing liquid was the least effective detergent; specimens macerated at 60, 55 and 50 °C had few traces of soft tissue, thus scored 2. While the specimen macerated at 45 °C, for this detergent, had large amounts of soft tissue, cartilage and tendons adhering to the bone, which held some bones together, even after manual cleaning post-maceration. Although the Sunlight dishwashing liquid and control specimen were macerated under the same temperature conditions and duration, the control specimen had considerably fewer amounts of soft tissue compared to the Sunlight dishwashing liquid specimen, thus scored lower.

Sunlight dishwashing liquid specimens scored lower for bone appearance compared to the specimens macerated with the powdered detergents. The specimens macerated at low

temperatures (50 °C and 45 °C) scored 1 and those macerated at high temperatures (60 °C and 55 °C) scored 2. The Ariel specimen macerated at 45 °C scored higher when compared to all the other specimens macerated at the same temperature (Table 2). However, the Ariel specimen macerated at 50 °C scored lower compared to those of the other powdered detergents macerated under the same temperature conditions. Surf, OMO Auto, Sunlight powder detergent and control specimens scored a 2 at all the different temperatures tested.

Generally, the specimens macerated at high temperatures scored lower than those macerated at low temperature. All specimens macerated at 60 °C and 55 °C had a composite score lower than 10 (Fig. 3 and Table 3). OMO Auto and Sunlight powder detergent specimens each scored a composite value of 5 at 60 °C which is by far better than the composite score achieved by the control and Sunlight dishwashing liquid specimens. At 50 °C, OMO Auto and Ariel powder detergent specimens were the only specimens to score a composite score of less than 10. Overall, OMO Auto showed to be the most effective detergent to use in skeletal preparation, with an average composite score of 8 (Fig. 4 and Table 3). Specimens macerated with detergents containing either or both enzymes and bleaching agents scored better than the control and Sunlight dishwashing liquid specimens.

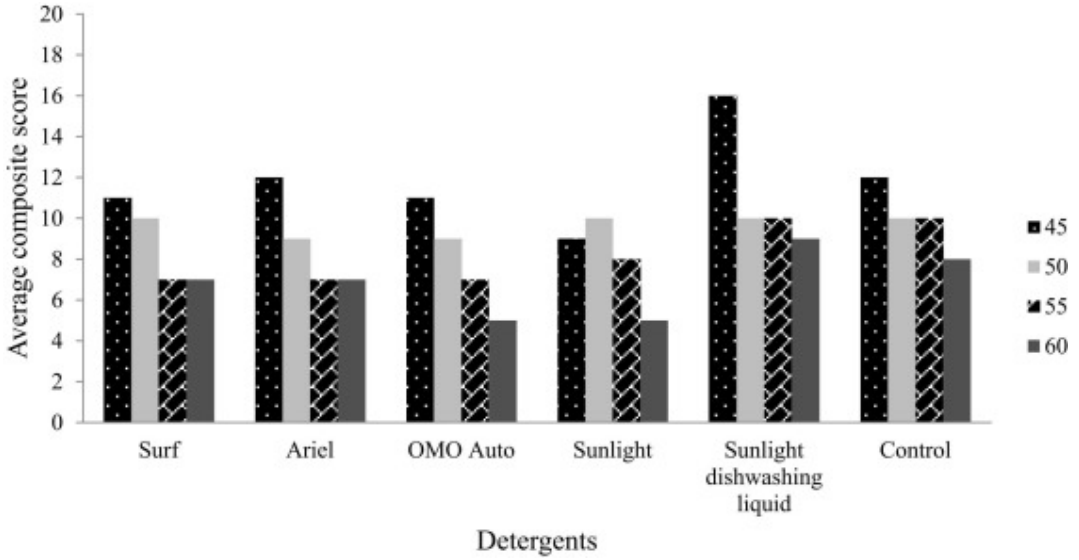

Fig. 3. A bar graph showing the average composite score of all the detergents and control specimens at four different temperatures.

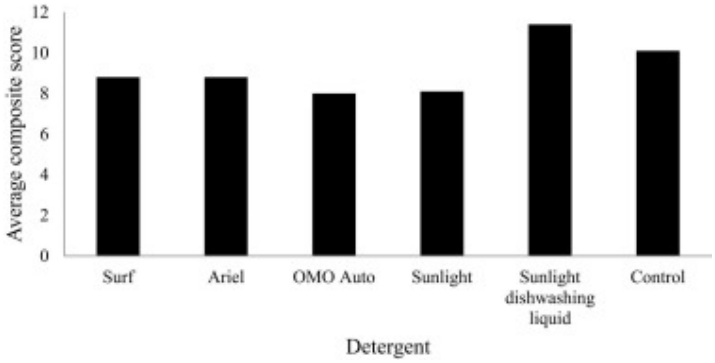

Fig. 4. A bar graph showing the average composite score achieved by each detergent and the control.

3. Discussion

The ideal maceration technique employed in a forensic setting must be time conducive and preserve bone structure and integrity. The aim of this study was to determine the most efficient and effective detergent for skeletal preparation. Detergents contain ingredients such as enzymes and bleaching agents, which increase the rate of soft tissue breakdown [16], [18]. They also provide a safer and more effective alternative to work with as opposed to using concentrated household bleach [10]. Bleaching agents attack and oxidizes protein bonds within soft tissues, causing these bonds to break down [6], while enzymes digest these soft tissues [10]. In addition, simmering in water softens muscles, tendons and ligaments, so that they can be easily removed [6]. Hot water maceration effectively and quickly removes soft tissue and as heat increase the rate of tissue breakdown also increases [4], [10]. Hence at considerably high temperatures (60 °C and 55 °C), the duration of maceration was shorter (one day), compared to the lengthy 3 days seen at low temperatures. Additionally, the use of detergents with bleaching agents and enzymes (Surf and Ariel) or only enzymes (OMO Auto and Sunlight powder) sped up the maceration process due to the increase in soft tissue breakdown.

Specimens macerated over a period of 3 days were expected to take less time to clean post-maceration compared to those macerated for a day or two. The extent of manual cleaning was dependent on weight reduction and ease of soft tissue removal. Thus, specimens macerated with detergents were noted to only have scanty remnants of soft tissue remaining and these specimens could be cleaned in less than 60 min. The Sunlight powder detergent specimen took less time to clean compared to the Surf and Ariel specimens which underwent the same duration of maceration at 45 °C. It must be noted that the Sunlight powder specimen macerated at this temperature did show signs of early decomposition and as a result this could have influenced the findings in this study. Refrigeration time was difficult to keep consistent due to the maceration of active forensic cases occurring during this study which limited the number of available urns; however no specimen was kept longer than three consecutive days in a refrigerator. It is recommended by the authors that future research on maceration should consider refrigeration time as a factor.

Sunlight dishwashing liquid was the least effective detergent regarding bone cleanliness, with large amounts of residual tissue which held some bones together even after the manual removal of soft tissue post-maceration. Although Sunlight Dishwashing Liquid lacks ingredients such as enzymes and it does contain lemon extracts which are known to have bleaching effects [21]. Overall the Sunlight Dishwashing Liquid specimens scored better for bone appearance when compared to the other detergent and control specimens. Detergents contain enzymes and bleaching agents which have a degreasing effect [2], [15], and optical brighteners which have fluorescent components resulting in clean and white bones [22]. However, these harsh and aggressive chemicals remove all the fat from bone and according to Steadman and colleagues [4] can cause the bone to have a chalky appearance and become brittle which is undesirable in a forensic setting [4], [6]. Even though the cortical surfaces of most bones were chalky, the bones were not brittle and could be handled appropriately without them breaking. Heat fixes fat in bone, which then migrates to the surface resulting in a dull and greasy appearance of the specimens [13]. Chemicals such as ammonia have degreasing effects, which can be used to remove excess fat and improve bone appearance [4]. However, degreasing is not a usual norm in forensics unless the skeletal remains require long term preservation, facial approximation or skull superimposition [15]. Both the Sunlight

Dishwashing Liquid and control specimens had a dull and greasy appearance post-maceration due to no harsh chemicals contained within these detergents.

Although detergents contain ingredients such as bleach and enzymes, which are known to have destructive effects on bone [15], [18], detergents tested in this study seemed to have no direct effects on bone appearance. The chalkiness seen on the bones with most of the specimens can be attributed to the temperatures tested. Since the control specimens, in this study, had the same scores for bone appearance as the powder detergent specimens. To test this effect, further studies can be done using cold water detergent solution. Additionally, for higher temperatures, the duration of maceration could perhaps be decreased to prevent bone damages; however more research regarding this is recommended [4].

The most effective and efficient detergent to use in skeletal preparation, found in this study, was OMO Auto washing powder. It produced satisfactory results in one day and specimens were easier to clean post-maceration when compared to the other detergents understudy in this research. OMO Auto does not contain aggressive ingredients such as bleaching agents which are known to have destructive effects on bone resulting in them becoming brittle or fragile. The ideal temperature tested was 60 °C, at this temperature, the duration of maceration was significantly shorter and there was the greatest amount of soft tissue loss observed for all specimens.

There is limited research focusing on macerating skeletal remains using commercial detergents and establishing a protocol or standard operating procedure for skeletal preparation in any field. Consequently, the decision on what method to use is usually based on the experience of the Preparator, resources available to them, the ease of method selected or desired final product [14]. Detergents are easy to use, readily available and produce effective results as indicated above. They also contain ingredients such as bleaching agents and enzymes in small but satisfactory amounts. Using detergents for skeletal preparation are also not as destructive as immersing a specimen directly into undiluted bleach or very invasive, unpredictable and difficult to handle as synthetic enzymes. As a result, detergents used in skeletal processing are as effective as any other maceration method, and they are also known to have less detrimental effects on bone structure and integrity [15].

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CRediT authorship contribution statement

Trisha - Jean Mahon (MSc (Med)) – drafted manuscript for submission, research topic idea and main supervisor of project. Nqobile Maboke (BHSc HONS) – conducted practical component of research and contributed minor edits to manuscript draft. Jolandie Myburgh (PhD) – contributed major edits to manuscript and co-supervisor of project.

Conflict of interest

None declared.

Presentation of results

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