EVALUATION OF CURRENT METHODS OF SOFT TISSUE REMOVAL FROM BONE

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The removal of soft tissues from skeletal remains (defleshing) is a common practice in many fields, however, no formal standards exist, even in forensic fields where small bony features and trauma marks must be preserved as evidence. Due to a lack of empirical research, little is known of the effects of defleshing methods on bone tissue or on trauma marks. This study evaluated the efficiency, effectiveness, and destructiveness of 6 common defleshing methods on white-tailed deer (*Odocoileus virgineanus*) hind limbs exhibiting cut and saw marks. Methods assessed were the use of dermestids, maceration, plain water boil, household bleach (Clorox®), sodium perborate, and enzymatic laundry detergent (Biz®). The 3 methods involving chemical or enzyme solutions were tested in low, medium, and high concentrations.

The dermestid samples were cleaned within a week, while maceration required over a month. A Kruskal-Wallis test compared the mean ranks of time-to-completion (TTC) for the heated treatments. The sodium perborate methods were significantly faster than the Biz® methods. There was no other significant difference in TTC. Maceration, plain boil, and Clorox® samples were completely cleaned of all soft tissues, but all 5 dermestid samples, 3 low concentration sodium perborate, and 4 Biz® (2 low, 1 medium, 1 high concentration) samples had remnant ligaments after processing.

No method altered the trauma marks, but damage in the form of holes through the bone was observed on 2 dermestid and 1 plain boil samples. Cortical bone exfoliation was observed

on 1 sample cleaned with a high concentration of Clorox®. Paired t-tests comparing pre- and post-processing values from the confined compression tests revealed that dermestids and high concentrations of Clorox® significantly decreased the stiffness of the bone and maceration significantly increased the compressibility of the bone.

This study found that bone tissue can be damaged macroscopically and altered microscopically by the defleshing method used. Therefore, one must consider the resulting effects on bone rather than just the ease of the method if skeletal remains are to be preserved for research or forensic evidence.

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PREFACE

The production of this dissertation has been a long journey which involved the valuable assistance and support of multiple individuals who deserve acknowledgement. I would first like to thank my undergraduate advisor, Dr. Dennis Dirkmaat, for initially spurring my interest in this particular topic, and for inspiring me to further pursue forensic anthropology. Also essential to my education and advancement within the field were my Master's advisor, Dr. Stephen Nawrocki, and my Doctoral advisors, Dr.s Michael Siegel and Margaret Judd; thank you all for assisting in my graduate education and supporting my interests in defleshing research.

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1.0 INTRODUCTION

The idiom, "there is more than one way to skin a cat," is typically used in a figurative sense, but is also applicable literally. A multitude of methods exist for removing the soft tissues from bone, or defleshing, for the preparation of a skeleton such that when one is presented with the task of defleshing a corpse, such as a cat, there are many options available. The preparator must choose a defleshing method that best suits the space, equipment, finances, and manual labor that are available to conduct the defleshing process, as well as consider the desired final appearance and condition of the skeletal remains. For example, different preparators require varying degrees of final bone quality, appearance, and ligamentary bone articulation depending on the intended purpose of the skeletal remains. This dissertation assesses the most common defleshing methods on variables important to consider when choosing a method. The results are pertinent to any field which requires soft tissue removal from bone, but the focus of this study is on variables important to consider when defleshing human remains in forensic contexts.

1.1 BACKGROUND: USES FOR DEFLESHING REMAINS

The preparation of skeletal remains from a fleshed corpse is a common practice in many fields where skeletal materials are desired for collection, display, research, or comparative purposes (Mairs et al. 2004). Museum curators prepare complete articulated skeletons for display as well

as disarticulated skeletons for collections to facilitate comparative taxonomic research (Causey and Trimble 2005; Maiorana and Van Valen 1985), which involves "detailed examination and measuring" of the bones comprising the collections (Williams 1992, p.18). Similarly, zooarcheologists accumulate complete disarticulated vertebrate skeletons to compare with fragmentary faunal skeletal remains from archaeological sites for species identification (Causey and Trimble 2005). Physical anthropologists require collections of human skeletal remains for research purposes as well. These skeletal collections of known individuals are used to establish various metric and non-metric methods for generating a biological profile (age, sex, ancestry, stature) for unknown individuals. Forensic anthropologists and pathologists remove the soft tissues of deceased modern humans from crime scenes in order to expose the bones for analysis of trauma marks and assessment of the bony features which aid in the establishment of a biological profile. Researchers in a variety of clinical fields often require clean bones for their studies. For example, studies involving bone grafts, osteoporosis, genetics, biomechanics, and facial reconstruction may involve the use of fresh, defleshed bones. Soft tissue removal is also commonly practiced by professional taxidermists and even by the occasional amateur hobbyist or hunter.

Although soft tissue removal is a practice that is quite common and has a long history, methods vary greatly among and within fields, as no formal standards exist. Methods also vary based on the desired final product. For example, a ligamentary, or articulated, skeleton may be preferred over a disarticulated skeleton when working with small vertebrates or when preparing an entire skeleton for display. Additionally, some preparators prefer the cleaned bones to be grease-free and whitened to have an attractive appearance for display and handling, whereas others prefer their bones to have a more natural feel and appearance. Thus, major factors in

influencing the choice of a defleshing method are the expectations for the appearance and quality of the final skeletonized product within specific fields as well as personal preference.

1.2 DEFINING "DEFLESHING"

There is an inconsistency in terminology within and among fields when describing soft tissue removal from bones. Soft tissue removal from bone has been termed defleshing, skeletal preparation, and skeletal processing. The term, maceration, has also been used; however, it is not synonymous with the latter terms. Maceration is specifically "the process of rotting away tissues from skeletons placed in water, by bacterial action." (Hamon 1964, p.428). Despite this specific definition, "maceration" is often used to describe heated chemical and enzyme solutions for defleshing. In order to avoid confusion in this study, the term, maceration, will be used in the strict sense; it will refer only to the method of allowing remains to soak in plain water to be degraded by bacterial action, while the term, defleshing, will encompass all of the methods of soft tissue removal.

Common types of defleshing methods include: (1) bacterial maceration, (2) boiling remains in plain water, (3) the use of various chemicals such as household bleach or sodium perborate, (4) the use of proteolytic (protein-specific) enzymes such as papain, trypsin, or an enzyme-active laundry detergent, and (5) the use of carnivorous invertebrates such as dermestid beetles, to ingest soft tissues. Other defleshing methods exist, but are less commonly practiced. For example, a carcass may be buried and exhumed after enough time has passed for natural bacterial decay to occur (Davis and Payne 1992; Gobalet 2003; Hendry 1999; Mairs et al. 2004),

however, this is a lengthy process that takes approximately 2 years to achieve completely defleshed bones (Gobalet 2003).

1.3 BRIEF HISTORY OF DEFLESHING METHODS

Defleshing, or skeletonization, can occur naturally due to the processes associated with decomposition. Decomposition involves degradation of the body's soft tissues by means of autolysis, bacterial activity, and carnivorous insect activity (Clark et al. 1997; Galloway 1997). Bones typically endure once the soft tissues have decomposed, leaving the skeleton exposed and available for later retrieval. Natural decomposition is likely the earliest defleshing method used to achieve skeletons for study and collection, though there is no documented evidence of this practice (Olry 1998).

Though decomposition occurs naturally and involves no manual labor, a multitude of disadvantages exist if one plans to intentionally allow a corpse to decompose until skeletonization is completed. First, one must find a place to lay out a corpse that is not offensive to the public and is protected from scavenging animals. This disadvantage becomes even more difficult with human remains, unless the preparator has access to an official human decomposition facility. Bacterial action during decomposition produces strong, foul-smelling gasses which must be taken into consideration if utilizing this method for defleshing purposes. Additionally, this method can be a lengthy process, depending on the environmental conditions.

The difficulties of odor and exposing the remains to the elements and public view can be alleviated by burial of the remains in soil (Gobalet 2003), sand (Mairs et al. 2004), or leaf mold

(Davis and Payne 1992). Natural decomposition still occurs, but is slowed so this method can take as long as 2 years or more (Gobalet 2003; Mairs et al. 2004). Furthermore, the bones can be lost in or discolored by the soil (Hamon 1964; Hendry 1999). The shortcomings of natural decomposition and burial methods have inspired preparators who require clean, display or research quality skeletons to develop processing methods that can be conducted in controlled indoor settings while also diminishing the odor.

Boiling remains in water works well to speed the defleshing process and to mitigate the odors that are typically generated during natural decomposition. This basic method of boiling remains in plain water is a common practice with a long history. During the Crusades, individuals, especially nobility, who died in distant lands were dismembered and boiled so that their clean bones could be easily transported to their home country for repatriation and proper burial (Brown 1981; Olry 1998; Walsh 1904). This method of treating the remains for repatriation became so common that it incited Pope Boniface VIII, who viewed this treatment as abuse of a corpse, to issue the papal bull of 1299, *Detestande feritatis* ("detested brutality"), which threatened excommunication to those who dismembered and boiled human remains for transportation purposes (Kornell 2000; Olry 1998; Walsh 1904). Boniface was so appalled at the brutal practice that he reissued the bull in 1300 (Brown 1981). Although this bull was not directed to the treatment of remains for the intended use of anatomical study, it influenced anatomists for a long time, as many were hesitant to perform dissections for fear of excommunication. This hesitation is represented in a letter in 1482 from an anatomist at the University of Tübingen (Germany) to Pope Sixtus IV to obtain permission for human dissection (Walsh 1904).

Boniface's bull did not deter all anatomists, however, as Andreas Vesalius clearly described boiling human remains to deflesh the bones for anatomical study in *De Humani Corporis Fabrica* in 1543. Vesalius' *Fabrica* provides the first published description of defleshing methods. He describes his preferred method of defleshing by boiling the remains in a large cauldron of plain water (Kornell 2000). The alternative method at the time was covering a corpse with lime in a perforated wood casket. This casket was then weighed down and placed in a stream of flowing water for several days. This alternative method left the ligaments intact, so that when the skeleton was removed from the stream, it could be positioned in the sun to dry as an articulated skeleton in that position (Olry 1998). However, Vesalius rejected this lime method "not only because it was 'troublesome, dirty, and difficult' but also because it rendered a skeleton unsuitable for instruction as the joints would be obscured by the dried and blackened ligaments." (Kornell 2000, p.98). The lime method also took several days to complete rather than the several hours that the boiling method would take. Both of these methods were illustrated in the initial letters of both the 1543 and the 1555 editions of the *Fabrica*. The boiling method is illustrated in the initial letter "O" and the lime method is illustrated in the initial letter "C" (Kornell 2000; Olry 1998).

The method of boiling remains in plain water has endured and is even currently used to deflesh human remains from forensic contexts in the prominent University of Florida C.A. Pound Human Identification Laboratory (Walsh-Haney et al. 2008). Despite the long life of the plain water boiling method, many other methods have been developed over the years to further speed the process, make soft tissue removal more efficient, and reduce the amount of manual labor involved. Other defleshing methods that have been employed include soaking the remains

in plain water for long periods of time, placing the remains in a heated solution with chemicals or enzymes, and even allowing flesh-eating insects or marine isopods to ingest the soft tissues.

1.4 DESCRIPTION OF COMMON DEFLESHING METHODS

1.4.1 Maceration

Maceration involves soaking the remains in a container of water for several weeks to months. This method facilitates bacterial growth and relies on the bacteria to act upon the soft tissues, loosening them from the bone (Gier 1951; Mairs et al. 2004). Maceration containers can be glass jars or aquariums for small to medium carcasses or a plastic water butt for larger animals (Adams 1980; Hendry 1999). The container should be fitted with a lid for the duration of the processes, except when changing water, to limit the amount of odor released from the vessel as the gases released during bacterial action are quite foul (Hamon 1964; Hubbell 1958; Searfoss 1995). Ordinary tap water is sufficient for maceration, as it works "as well as distilled water" (Hamon 1964, p.430). The water in the maceration container should be changed often so that mold does not form on the surface of the water; the bones could be damaged if they should come in contact with the mold (Hamon 1964). It is recommended that the maceration water be poured off and replaced daily (Hamon 1964) to every 4 days (Gier 1951).

The maceration method is considered simple since the remains do not have to be continually tended (Anderson 1932; Hangay and Dingley 1985). Hamon (1964) asserts that despite the strong odors produced during the maceration process, the bones, once cleaned of soft tissues and rinsed in running water, have no foul odor. Although there are several advantages to

maceration and supporters of the method, many complain that maceration involves a great deal of labor in the form of manually picking off adhering tissues from the bones (Davis and Payne 1992; Hubbell 1958; Mann and Berryman 2012). The primary disadvantage of the maceration method, which is of concern in any laboratory, is the strong, unpleasant odor produced by the bacterial action that permeates from the maceration vessel (Hangay and Dingley 1985; Mori 1979). Even if a laboratory is equipped with a fume hood and a lid is placed on the maceration vessel, the fetid water must still be poured out and replaced regularly, which is an undesirable job for any preparator. Hurlin (1918) proposed a method of embedding intact remains into an agar solution to reduce the odor problem of maceration and to reduce the loss of small bones during processing. Agar is a semi-solid medium that supports bacterial growth and can absorb most of the odor as well as keeping small bones in anatomical position. This method is excellent for small animals which can be completely suspended in the gelatinous solution, but is impractical for an entire human body. Additionally, maceration may not be the most suitable defleshing method for all types of animal remains. Hill (1975) explains that though bacterial maceration works well on fishes, amphibians, and reptiles, it is slower and less efficient when used on birds and mammals.

Another disadvantage is that maceration is a relatively lengthy process. Many skeletal preparators, particularly forensic anthropologists who are under time constraints, believe that this process is too lengthy and, thus, have chosen a speedier method as an alternative (Hill 1975; Mori 1979). Maceration is also impractical if a constant flow of remains are expected to be defleshed (Brown and Twigg 1967) and the method involves significant health and safety concerns, especially in human forensic contexts, since the method facilitates bacterial growth and

includes no heat or chemical treatment to disinfect the remains of potentially hazardous diseases (Mairs et al. 2004).

1.4.2 **Heat treatment**

Heat has been applied to the maceration method to speed the process. A low heat provided by an incubator or aquarium heater accelerates bacterial action (Maltese 2001; Tompsett 1970). Once the temperature is increased further, the heat acts to denature the proteins of the soft tissues. Most proteins of muscle tissue begin to denature at 40° C; however, the fibrous proteins of muscles, tendons, and ligaments require higher temperatures to denature (Barham 2001). Collagen begins to denature at 60° C and at 70° C begins to gelatinize. Elastin and reticular fibers can only be denatured when heated for long periods of time at temperatures above 90° C (Barham 2001). Therefore, lower temperatures are able to denature muscle tissue, as seen in the browning process when cooking meat, but if the temperature is not increased, the dense connective tissues made primarily of collagen and other fibers, such as tendons and ligaments, will remain.

The effect of heated water on bone tissue has not been formally tested in the context of defleshing, though many consider heat to be a destructive agent to bone tissue (Mori 1979; Williams 1999; Williams and Smith 1995). Due to the lack of information on the effects of heated water on bone during the defleshing process, a disagreement exists as to whether it is safe to bring remains to a full boil or if lower temperatures should be used. It is known, however, that remains can be destroyed if overcooked and will scorch if left in direct contact on the bottom of a pot on a burner (Hangay and Dingley 1985; Mann and Berryman 2012). Microscopic changes in the bone tissue in the form of unpacking and fragmentation of the collagen fibrils can be

observed after only 1 hour of boiling via transmission electron microscope (TEM) (Koon et al. 2003). Additionally, boiling temperatures can denature DNA within bone (Arismendi et al. 2004) and extreme temperature fluctuations that occur with boiling and cooling the remains can cause the teeth to crack (Neves et al. 1995). Therefore, methods using heat require constant attention and should involve keeping the remains off of direct heat by using a strainer that fits within the cooking pot (Maltese 2001; Nawrocki 1997). Despite these hazards, the University of Florida C.A. Pound Human Identification Laboratory boils human forensic cases in plain water until soft tissue is removed. The preparators at this facility claim to obtain good results with this method as long as the dangers of overcooking are understood and care is taken during processing (Walsh-Haney et al. 2008).

1.4.3 **Chemical methods**

Chemicals have been added to heated water to speed the defleshing process even further, mitigate the odor, and to disinfect, degrease, and whiten the bones. Several chemicals have been suggested for use in removing soft tissue from bone, however, none have surfaced as obviously superior to the rest. Most of these chemicals work via the process of oxidation, which also functions to whiten the bone. The most common chemicals used in defleshing are those which are inexpensive and easily obtained, such as household bleach.

1.4.3.1 Household bleach

Household bleach is a popular chemical-based solution to use in defleshing due to its availability and low cost. It is typically a solution of the active ingredient, sodium hypochlorite (NaOCl), and a small amount of sodium hydroxide (NaOH). Clorox®, a brand name of household bleach, is

composed of 5-10% sodium hypochlorite (NaOCl) and less than 1% sodium hydroxide (NaOH) (Clorox Company 2009). Sodium hypochlorite is the active bleaching agent of the solution, as it is a potent oxidizing agent. This oxidizing characteristic makes the chemical an effective disinfectant since it denatures proteins of microorganisms, such as viruses, fungi, and bacteria (Coons 1978; Smith 1994). Sodium hydroxide, also known as lye or caustic soda, is added to household bleach solutions to stabilize the sodium hypochlorite by delaying its breakdown into sodium chloride and sodium chlorate (Smith 1994).

Defleshing methods using bleach can vary greatly. Some soak the remains in straight, undiluted bleach with no heat treatment (Gross and Gross 1966; Mann and Berryman 2012), while others use diluted solutions that are heated (Nawrocki 1997; Nawrocki 2008; Stephens 1979). A single formal protocol for soft tissue removal using bleach, with a specific chemical concentration level, has not been established.

Though this method has numerous advocates and is widely used in many fields, even in forensic anthropology, the effects of bleach on bone are greatly debated. Many researchers have claimed that bleach is safe enough for small, delicate bones (Gross and Gross 1966; Hamilton 1977; Konnerth 1965) or skulls (Hoffmeister and Lee 1963). Stephens (1979) explains that while other chemicals are harmful to bone tissue, bleach is safe enough for human forensic cases, even those exhibiting trauma marks. Mann and Berryman (2012) support this claim and have used bleach on human forensic cases with stab wounds and gunshot wounds on delicate ribs with no damage to the bone or trauma marks. Nawrocki (2008) intimates that, due to safety issues, the use of household bleach is necessary to sterilize biohazardous remains before they are brought into the main osteology laboratory to be handled for analysis. Despite this acclaim, there are several researchers who claim that bleach is too harsh and must never be used on bone

(Fenton et al. 2003; Mori 1979) and that the chemical can continue to act on the bone after processing, causing bone damage (Fenton et al. 2003; Nawrocki 1997). There has not been adequate empirical research to support these claims, so there is ongoing controversy over the use of bleach on bones (Mann and Berryman 2012).

One must also take care when using sodium hypochlorite because it reacts with other commonly used chemicals, such as ammonia and hydrogen peroxide, to produce hazardous chlorine gas and oxygen gas, respectively (Smith 1994). Furthermore, household bleach is known to lower the quality of DNA retrieval, so processing using this method should not be used in forensic cases unless samples for DNA analysis have already been collected from the remains (Lee et al. 2010).

1.4.3.2 Sodium perborate

Sodium perborate (SPB), which has the chemical formula $NaBO_3$, is a bleaching agent that is popular in the detergent industry but has also been used to deflesh remains. Sodium perborate is a more stable, powdered form of hydrogen peroxide. When sodium perborate is added to water, it dissociates into sodium metaborate and hydrogen peroxide. "This property of perborate makes it possible to incorporate hydrogen peroxide in a powder detergent without major interactions with other detergent components" (Dorfer and Lieser 1994, p.174). The hydrogen peroxide is a potent oxidizer, functioning similarly to sodium hypochlorite to denature proteins and whiten the bones. The additional borate that is formed acts as degreaser, which is helpful in the detergent industry as well as in defleshing carcasses.

Sodium perborate, though important in the detergent industry, is a less familiar chemical in the fields which deflesh remains to prepare skeletons. Methods for using sodium perborate for defleshing, thus, are not well known. There have only been a small number of articles published

describing the use of sodium perborate, and no reference of sodium perborate use on human remains. The first documented use of sodium perborate for defleshing purposes was by Roche (1954), who used the method at the Natural History Museum in Paris. The method then spread to the United Kingdom (Chapman and Chapman 1969; McDonald and Vaughan 1999) and the United States (Jakway et al. 1970), as evidenced by articles published on the topic.

Despite the limited amount of published methods, a variety of concentrations have been recommended for defleshing purposes. Table 1.1 displays the range of concentrations, and units of concentration, recommended in the publications using sodium perborate.

The method of using sodium perborate must be followed closely as to avoid an unwanted and messy reaction. A boiling temperature aids in quickly dissociating the oxygen from the chemical, as seen as a frothy, bubbly reaction (Chapman and Chapman 1969). But, if the boiling temperature is maintained, the reaction is so strong that the foam created will overflow from the pot (Chapman and Chapman 1969; Jakway et al. 1970; McDonald and Vaughan 1999). Conversely, if the solution cools too much or the concentration of sodium perborate is too high, the chemical will crystallize and become ineffective (Chapman and Chapman 1969). In order to diminish the risk of either of these reactions occurring during processing, Jakway and colleagues (1970) recommend that the water be brought to a boil before placing the remains and the sodium perborate into the pot. Once the remains and SPB are added, the pot is removed from the heat source and left to cool to room temperature for several hours. During this time, the chemical acts to degrade the soft tissues from the bones (Jakway et al. 1970). The remains may also be incubated in a sodium perborate solution at 60°C for several days, as proposed by McDondald and Vaughan (1999).

Table 1.1: List of references for sodium perborate (SPB) use.

Reference	**Concentration of SPB Recommended**
Roche 1954	60-70g/L for small animals
	70-100g/L for large animals
Chapman & Chapman 1969*****	2.5% weight volume solution for small to large animals (birds, snakes, and mammals ranging in size from shrews to horses)
Jakway et al. 1970	8 to 1 for small animals
Hiller (cited in de Wet et al. 1990)	60-100g/L (animal type not specified)
de Wet et al. 1990*****	85g/20L (animal type not specified)
McDonald & Vaughan 1999	15g/150ml for Stoat or Weasel (skull, femurs, baculum)
	55g/800ml for Brown Hare (skull, lumbar spine, tibia)

*****Only used SPB after boiling the remains until much of the soft tissue was removed (thus, only used as a whitener/degreaser)

Users of sodium perborate praise the chemical for its ability to deflesh effectively as well as whiten and degrease bones to such a degree that post-processing bleaching or degreasing steps

are not required for this method (Chapman and Chapman 1969; McDonald and Vaughan 1999). More importantly, many claim that sodium perborate is not damaging to bone, even small, fragile bones and teeth (Chapman and Chapman 1969; Jakway et al. 1970; McDonald and Vaughan 1999). Despite this praise, some still claim that the chemical is too harsh for use on bone and may cause the bone to become “soft and chalky” if not properly controlled (Davis and Payne 1992, p.101).

1.4.4 **Enzymatic methods**

Proteolytic enzymes, or proteases, are highly specific enzymes that induce protein decomposition (proteolysis), by promoting hydrolysis of the peptide bonds that link the amino acids that form proteins. Therefore, these enzymes primarily target the protein content of muscle and soft connective tissues (ligaments, tendons, and cartilage), rather than the mineral or tightly packaged organic bone matrix. Most of these enzymes require the addition of heat for activation, but high temperatures will denature and deactivate the enzyme, so it is important to know the optimal temperature of the specific enzyme being used. Maintaining the optimal pH value for a specific enzyme during defleshing will also augment the effectiveness of the method.

Several researchers believe that the use of proteolytic enzymes to remove soft tissue is the most rapid and efficient defleshing method (Hill 1975; Mairs et al. 2004; Subrahmanyan et al. 1939). Yet not all praise this method; others find enzymes to be too destructive to the bone tissue as they may, like the chemicals, destroy the bone if left in the solution too long or continue to work on the organic matrix of the bone tissue after the defleshing process is complete (Fenton et al. 2003; Mori 1979).

The proteolytic enzymes papain, pepsin, pancreatin, and trypsin have been proposed to deflesh remains. Papain is a protease derived from the unripened papaya fruit (*Carica papaya*), which works in approximately neutral conditions (pH 6-7). Papain was first published as a defleshing agent by Luther (1949). Luther claimed that this method was so fast that 20 or more small mammal skulls could be defleshed in a single day and that even the small auditory ossicles were left clean and intact. Since Luther's publication, other researchers have described papain as destructive to bone (Fenton et al. 2003; Hill 1975; Mori 1979; Steadman et al. 2006). However, some of these researchers (Hill 1975; Steadman et al. 2006) have used the chelating agent,

ethylenediamine tetra-acetic acid (EDTA), in combination with the papain. Kemp et al. (2009) found that the damage to bone tissue observed by these researchers was likely a result of the EDTA, due to its ability to decalcify bone, rather than the papain. No macroscopic evidence of bone destruction has been observed in Kemp and colleagues' (2008; 2009) studies of pure papain solutions containing no EDTA, even in solutions with a high concentration of papain. Despite the quick action on muscle tissue, papain is not as effective on collagen-dense tissues such as tendons and ligaments, so Kemp et al. (2009) suggest a protease combination of papain and a collagenase to increase the effectiveness on all soft tissues.

Pepsin, trypsin, and pancreatin are enzymes that are produced naturally by the body's digestive system in order to hydrolyze specific ingested foods. Pepsin is a protease produced by the stomach and, thus, works best in acidic conditions (1.5-2.5 pH). The acidic conditions required for optimal action of pepsin would, however, potentially be destructive to bone tissue (Hangay and Dingley 1985). Trypsin and pancreatin are produced by the pancreas and secreted into the small intestine. Like papain, these enzymes work under approximately neutral conditions (pH 6-7). Trypsin is a protease, while pancreatin is a combination of three different enzymes. Pancreatin includes amylopsin, which digests polysaccharides, pancreatic lipase, which digests fat, and the protease, trypsin (Shelton and Buckley 1990). Both pancreatin and isolated trypsin have been used in defleshing remains, but these enzymes have been found to be less effective than papain (Subrahmanyan et al. 1939). Pancreatin has been recommended for use in producing ligamentary skeletons since the enzyme does not digest the ligaments efficiently (Rowley 1925).

The benefits of using enzymatic defleshing methods are that less soft tissue removal using a scalpel is required prior to processing, temperatures below boiling are used (typically 55

- 65˚C), and the time to completion is greatly reduced as they typically work in a matter of hours rather than days or weeks. However, these enzymes tend to be quite expensive and need to be purchased through a scientific supply company, which deters many researchers from using them (Chapman and Chapman 1969; Hill 1975; Sandstrom 1969). Many investigators have also complained of a strong chemical odor to some of the enzymes, especially of papain (Davis and Payne 1992; Fenton et al. 2003; Harris 1959; Mairs et al. 2004). These enzymes also have an added health risk, as they can be irritating, or even damaging, to the respiratory mucosa if inhaled in their powdered form (Mairs et al. 2004; Simonsen et al. 2011). In order to reduce this health risk, the enzymes can be ordered in an aqueous solution and kept refrigerated until needed (Mairs et al. 2004), but this method may be impractical for laboratories with limited or no refrigerator space.

As a resolution to these disadvantages, Ossian (1970) proposed the use of commercially sold enzyme-based laundry detergent for skeletal preparation and claimed that the method works well at defleshing remains without damaging the bones. Laundry detergent is inexpensive, easily acquired, includes deodorants to reduce odors, and has less health hazards with use than pure powdered enzymes (Mairs et al. 2004). Enzyme-active laundry detergents have since been used on humans in forensic contexts with good results (Austin and Fulginiti 2008; Mairs et al. 2004; Stephens 1979). Mairs and colleagues (2004) used an enzymatic detergent on two human forensic cases with knife and saw marks. The cleaning in these cases was effective and not destructive to the fine trauma marks, as evidenced by analysis with an environmental scanning electron microscope (ESEM); they claimed to find no “apparent erosive effect” on the marks (Mairs et al. 2004, p.281).

Commercially available enzyme-active laundry detergents contain a mix of enzymes and other ingredients to be able to treat a wide array of laundry stains. The specific enzymes and other ingredients are not disclosed due to proprietary knowledge, but most are composed of a mix of proteases, lipases, additives, bleaching agents, and corrosion inhibitors (Simonsen et al. 2011). Sodium perborate may be included in some detergents to aid in degreasing and whitening laundry (Hendry 1999; Mooney et al. 1982). Some of the ingredients, such as sodium perborate and the lipases, function to degrease and whiten bones while defleshing such that no further bleaching or degreasing steps are necessary (Mairs et al. 2004; Mooney et al. 1982). Several brands of enzyme-active detergents have been found to be effective, but Biz® is available in the United States and the most commonly published laundry detergent for defleshing purposes (Mooney et al. 1982).

Despite numerous listed advantages and the claims that enzymatic detergent is not destructive to bone, severe damage has been documented on fish bones cleaned by this method. Shelton and Buckley (1990) describe the condition of the Ossian osteological collection, which is comprised mostly of fish, but also of amphibian, reptile, and mammal remains. In 1970, Ossian claimed that she used Biz® laundry detergent to deflesh over 800 animal remains and found no damage on the cleaned bones (Ossian 1970). However, an assessment of this same collection in 1988 found 82.3% of the fish remains exhibited some degree of bone damage (Shelton and Buckley 1990). Though the other taxa did not demonstrate the same types of bone damage, the damaged fish bones indicate that enzymatic laundry detergent may have long-term effects on small, delicate bones. It is difficult to know exactly what caused this damage, the enzymes or some other component, since the ingredients of the detergent are unknown. Shelton and Buckley

(1990) advise that the added costs of using pure enzymes are worthwhile since it is possible to regulate the type and amount of enzymes being used on the bones.

1.4.5 **Dermestid beetles**

The final class of defleshing methods involves the use of necrophagous (carrion-eating) microfauna. Necrophagous microfauna, such as insects and crustaceans, are part of the natural decomposition process and can be observed feeding on carcasses in natural settings (Catts and Haskell 1990; Haskell et al. 1997). Researchers have learned from these observations and utilized various species of insects and other living creatures, such as fly larvae (Majeed 2009), clothes moth larvae (Banta 1961), meal worms (Allen and Neill 1950), fire ants (Crawford and Atkinson 1975), pill bugs (Maiorana and Van Valen 1985); crayfish (Sealander and Leonard 1954), and marine microfauna (Bolin 1935; Friedman 1973; Packard 1959), for defleshing bones in controlled settings. However, no necrophagous organisms are as easily contained and accessible world-wide than dermestid beetles.

Dermestid beetles, or dermestids, are a family of beetles that feed on carrion (Catts and Haskell 1990). The genus, *Dermestes*, was originally identified in the mid 1800's when these beetles were discovered to consume and destroy stored hides and meats (Weichbrod 1987). Dermestid beetles were initially viewed as destructive pests, but their scavenging tendencies were soon found to be advantageous by curators and preparators in natural history museums and other laboratories; if contained and properly maintained, the beetles could be used to easily deflesh remains with minimal human labor involvement. The first documented controlled use of dermestid beetles for defleshing skeletons began in the early 1900's in the laboratory of Charles Dean Bunker at the University of Kansas Museum of Natural History (Coleman and Zbijewska

1968; Hall and Russell 1933; Tiemeier 1939; Weichbrod 1987). Bunker found that due to an accidental infestation of *Attagenus piceus*, a smaller dermestid beetle, a collection of delicate bird skulls had been perfectly cleaned, yet left intact, with no human labor involved. After this incident, Bunker constructed a special "bug room" to contain the beetles for the purpose of defleshing skeletal remains for the museum collection (Horr 1952; Tiemeier 1939). After experimentation with the dermestid technique, Hall and Russell (1933) published a description of Bunker's bug room and formalized methods for initiating and maintaining a dermestid colony for defleshing purposes. This publication spurred interest in the use of dermestids in the laboratory, as is demonstrated in a series of publications that followed in the 1930's and 1940's to provide refinements to Hall and Russell's 1933 method (Bond 1939; Borell 1938; Scheffer 1940; Tiemeier 1939; Vorhies 1948).

The dermestid method of defleshing remains is quite simple; it involves merely placing a carcass into a container with a colony of dermestid beetles and removing the bones once clean of the unwanted soft tissue. However, if the life cycle and biology of these beetles are understood and proper conditions maintained, the efficiency and effectiveness of the method can be maximized and a superior final product produced (Russell 1947). For example, it is important that the preparator understand that the beetle larvae are the true defleshers as they ingest much more soft tissue than the adults, but adults are required for reproduction purposes to perpetuate the colony (Hall and Russell 1933; Sommer and Anderson 1974). Additionally, the population size and feeding activity of the colony is affected by environmental factors such as temperature and humidity (Weichbrod 1987). Dermestids work best in warmth, but the temperature-controlled room described by Hall and Russell (1933) is not necessary as long as the colony is not subjected to temperature extremes (Vorhies 1948). Optimal defleshing activity is typically

cited as within the temperature range of 21-29°C (70-85°F) (Gennaro and Salb 1972; Hall and Russell 1933; Laurie and Hill 1951; Mairs et al. 2004; Multhaup 1975; Sommer and Anderson 1974; Valcarcel and Johnson 1981; Vorhies 1948; Weichbrod 1987).

Other requisites for maintaining a healthy colony of dermestids are to store them in a dark place, since they are negatively phototropic (Weichbrod 1987); provide a place, such as cotton, for the larvae to pupate (Russell 1947); and provide enough healthy fats for survival (Russell 1947). A slight amount of humidity is important as well, but too much may facilitate the growth of mold which can kill a confined colony (Gennaro and Salb 1972; Russell 1947). The actual dermestid enclosure, or dermestarium, need not be a specialized "bug room" as described by Hall and Russell (1933) and Russell (1947) to maintain a dermestid colony. A container for the colony need only be large enough to hold the size of carcass desired to be cleaned and prevent the escape of the beetles. A wide variety of dermestid enclosures have been documented. An enclosure can be as simple as a coffee can (Borell 1938) or a 20 gallon garbage can (Grayson and Maser 1978), or more elaborate, like a repurposed chest freezer that can be kept outdoors (Gennaro and Salb 1972). A glass aquarium is easily obtained and may be used as a container for a dermestid colony as long it is kept in the dark (Weichbrod 1987). In order to prevent infestation due to the escape of rogue dermestids hiding in the small crevices of cleaned bones removed from the dermestarium, Florian (1990) and Bemis et al. (2004) recommend placing the bones in a freezer for at least 24 hours to kill hiding beetles.

The use of dermestids for defleshing remains is a simple process once a colony of the beetles has been established. Many researchers, particularly museum curators, favor the use of dermestid beetles as they are viewed as not damaging to bone tissue and require less monitoring/tending time than methods involving heat and chemicals (Hooper 1950; Horr 1952;

Russell 1947). Another reason for the popularity of using dermestids for processing remains in museums is the fact that the beetles can be used for the purpose of achieving a complete, fully articulated skeleton, as cartilage will remain intact if the skeleton is removed before these tissues are ingested by the beetles. If the removal of the skeleton is properly timed, even the teeth will remain tightly in their sockets (Weichbrod 1987). Williams (1991; 1992; 1999) has repeatedly proclaimed that the use of dermestids is the safest defleshing method to use for long-term stability of research-quality skeletons. Accurate measurements of the ash weight and mineral content of bone can be determined on dermestid-cleaned bones since it has been shown that this method does not alter these variables (Hefti et al. 1980).

In contrast, others have claimed that the use of dermestids is unsatisfactory as it is a comparatively lengthy process (days versus hours), defleshing is uneven, and the beetle colony must be maintained (Hangay and Dingley 1985; Quigley 2001; Thompson and Robel 1968) and is, thus, impractical for use in forensic anthropology (Walsh-Haney et al. 2008). Macroscopic bone damage due to the dermestids ingesting the bone tissue has been observed on delicate bones if they are not removed in a timely manner since they are "eating machines" (Graves 2005, p.35). Bones, especially of large animals, cleaned by dermestids tend to remain greasy and require an additional degreasing step (Hamon 1964). The major disadvantage that dissuades most from using this method is that a proper enclosure, even a separate room, is required to prevent the beetles from escaping and infesting the laboratory, since they can be quite destructive to other collections (Hangay and Dingley 1985; Mori 1979; Searfoss 1995). The use of dermestids is also considered "inconvenient if only a few specimens require preparation at infrequent intervals and also if large specimens, such as a horse's skull, need attention" (Chapman and Chapman 1969, p.522).

1.5 STATEMENT OF THE PROBLEM

As can be ascertained by the preceding brief review of defleshing literature, there is no obvious "best" defleshing method and, while many methods of soft tissue removal have been documented anecdotally, empirical testing of the effects of defleshing methods on bone tissue is lacking. Most literature on defleshing remains is dedicated to the description of a single method. The method described is typically the author's favored method of defleshing based on his or her specific needs. Each method has its own perceived advantages and coinciding disadvantages, which may differ based on the desired final product or personal preference of the preparator. Some authors anecdotally compare their preferred method to other defleshing methods, but these comparisons are also highly subject to personal opinion. Thus, the conclusions of the majority of the defleshing literature are subjective and few are agreed upon and relatively little scientific research on defleshing methods exist.

The most disconcerting issue is the fact that, to date, the amount of damaging effects is unknown for each method, as there is a lack of empirical testing. A common occurrence in the literature is when one or more researchers claim that a particular defleshing method is damaging to bones, but others using the same method elsewhere have observed no damage to the bones and commend the effectiveness of the method. These disparate descriptions leave readers attempting to use the literature to decide on the most appropriate defleshing method more confused and hesitant than when they started the review of the literature.

1.5.1 **Defleshing issues in forensic fields**

The problems in defleshing research are even more significant in forensic fields, such as anthropology, pathology, and odontology, which regularly require the removal soft tissues from bones. No defleshing method has been developed that is specific to forensic fields, despite the fact that the choice of a defleshing method is particularly important for the forensic investigator. Soft tissue must be removed from the bone in forensic cases to allow for a direct examination of bony features which will aid in the construction of a basic biological profile for the unknown individual to provide to law enforcement. The bones are also assessed for signs of trauma which could indicate the circumstances surrounding the death of the individual. Thus, the choice of a defleshing method poses a unique challenge to forensic anthropologists, as they are confronted with the issues of using a nondestructive method in order to preserve identifying features and fine marks of trauma, while remaining within time constraints for the quick identification of the deceased. Time constraints are even more imperative in mass disasters, when identifications must be made as quickly as possible by the anthropologist (Jensen 1999).

Another concern is the fact that years may pass between the time the remains are defleshed and analyzed and the time when the case goes to trial and the forensic anthropologist is requested as an expert witness. Therefore, one must consider any possible long-term effects that a defleshing method may have on the bone tissue or on trauma marks; however, this is difficult to determine since there is also a lack of studies involving the long-term effects of defleshing methods.

Forensic anthropologists must also consider the possibility of DNA extraction post-processing. Though DNA samples should be taken prior to processing the remains, a situation may arise where additional DNA samples are requested after the remains have been processed.

DNA is degraded by hydrolysis and oxidation, mechanisms of action of many of the common defleshing agents (Arismendi et al. 2004). Therefore, the selection of defleshing method may have an adverse effect on any DNA analyses that may be conducted on samples collected post-processing.

1.6 RESEARCH GOALS AND DESIGN

The current study provides empirical research that assesses the efficiency and effectiveness of 6 common defleshing methods as well as the possible destructive effects of each method on bone tissue and fine marks of trauma. The ultimate goal was to construct a protocol for soft tissue removal of fleshed remains that is quick and easy to implement, while safe enough for use on bones exhibiting marks of trauma. Though a variety of fields require removal of soft tissues from bone, this study focused on defleshing methods and concerns specific to forensic anthropology. In order to reach this goal, the following 4 research objectives were pursued:

1) Assess the **efficiency** of each defleshing method by calculating the net time investment and final monetary cost of each method.

2) Assess the **effectiveness** of each defleshing method by assessing differential effects on various tissue types and the final amount of soft tissue adhering to the bones by assessing the final bone condition.

3) Assess the **destructiveness** of each defleshing method to bone tissue by examining the surface of the bone macroscopically after processing, as well as pre- and post-defleshing mechanical testing of the structural integrity of the bone tissue.

4) Assess the **destructiveness** of each defleshing method to trauma marks on the bone by examining the marks of sharp force trauma (saw marks and cut marks) prior to and after each method via stereo-microscope.

5) Determine whether different concentrations (low, medium, high) of the tested enzyme and chemicals vary on the variables of efficiency, effectiveness, and destructiveness.

Each variable was assessed with the basic null hypothesis that there is no difference among the different defleshing treatments tested.

1.7 SIGNIFICANCE OF THE STUDY

The results of this research will impact the field of forensic anthropology on various levels. On a descriptive level, the proposed research will remedy the deficiency within the literature on soft tissue removal methods. Empirical research in this area is negligible and, therefore, the exact effects of the various defleshing methods on bone tissue are unknown. This study provides a detailed description of the overall efficiency and effectiveness of each of the tested methods as well as the effect on the bone tissue and the appearance of trauma marks with supportive quantitative results.

Secondly, this study will contribute to the methodology of soft tissue removal. Standardized methods for removing soft tissue from bone is lacking in many fields. Preparators use different methods as well as different concentrations and combinations of defleshing methods. This study utilizes distal deer hind limbs which consist of multiple types of soft tissue (muscle, tendons, ligaments, hyaline cartilage, and periosteum) to allow the assessment of

differential effects of various defleshing methods on these tissues. Therefore, the results of this study will aid in isolating an optimal defleshing method most effective for specific situations/elements.

Lastly, the results of this study will benefit any field that requires defleshing remains (human or non-human) for the accumulation of osteological collections or for research. However, the greatest impact will be in the field of forensic anthropology, where the selection criteria for a defleshing method are more critical. Defleshing methods used in this field must be quick, efficient, and nondestructive to the bone tissue or to fine trauma marks. Ineffective methods require tedious manual removal of soft tissue, which involves the use of scalpels and other sharp or abrasive utensils, potentially exposing the bone to further damage during processing. The method must be quick to remain within the time constraints of a medicolegal investigation, particularly in the case of a mass disaster. However, the method must not be too aggressive, as the integrity of the bone tissue or the appearance of trauma marks must be preserved for evidence. This study assesses the most common defleshing methods on variables important to forensic anthropology. It also considers trauma marks inflicted by weapons commonly used in crimes and the effect of the defleshing methods on their appearance. The results of this study will, thus, add to the knowledge of soft tissue removal for better practice in forensic anthropology.

2.0 MATERIALS AND METHODS

2.1 THE STUDY SAMPLE

White-tailed deer (*Odocoileus virginianus*) remains (n = 60) were used as an animal proxy for this study due to their similarity to human bones in size and composition and also due to the difficulty of obtaining a substantial sample of human remains. Distal hind limb segments were collected from a licensed deer processor in southwestern Pennsylvania during deer hunting season. A salvage permit to collect these remains was obtained from the Pennsylvania Game Commission. Each of the collected limb segments were individually bagged and placed in a deep freezer for storage before processing. Both doe and buck were in season during the time of sample collection, so the sex of the remains is unknown for this sample.

Additionally, the exact age-at-death of the deer was unknown at the time of collection. However, Pennsylvania instills antler point restrictions (APR) in order to protect younger bucks during hunting season (PA Game Commission 2011). At the time of sample collection, there was a 4 point-to-antler restriction in the region of collection, so it was assumed that most of the deer collected would be adult in age (over 1 year old). However, this assumption may not be applicable to the does in the sample as the regulation only pertains to bucks and it is difficult for hunters to age does in the field. Nevertheless, age may be an important confounding variable, as the bones of young individuals are more susceptible to destruction by various defleshing

techniques (Davis and Payne 1992). In order to control for this confounding variable, a method to estimate age-at-death using epiphyseal fusion of the bones comprising the distal limb samples was employed after each defleshing method had been conducted.

The distal hind limb segments (bones and soft tissues from the ankle to the toe) were used to test the effects of each defleshing method on adhering muscles, ligaments, tendons, periosteum, and hyaline cartilage, as well as on fine marks of trauma on the metatarsal. Figure 2.1 depicts the bones of a deer hind limb. The bones in the limb segments used in this study included the bones from the distal row of tarsals to the distal phalanges within the hoof. These elements were selected for this study since they are discarded by deer processors after butchering and are, therefore, readily available. Although there is a limited amount of muscle tissue in this area of the deer, the distal segments of the limbs are an appropriate choice for this research since there is a high concentration of tendons and ligaments. Muscle tissue is easily degraded during most processing methods, but these collagen-dense tissues are particularly difficult to remove and typically require manual removal using sharp instruments that may scar bones. Thus, this study will aid in ascertaining the most effective defleshing method(s) for removing the most stubborn tissues from bone.

2.2 RESEARCH LABORATORY AND SAFETY PRECAUTIONS

All processing of the remains was conducted in the osteology laboratory of the Department of Anthropology, University of Pittsburgh. This specific laboratory has been approved for the use of biological tissues by the University Environmental Health and Safety (EHS) office. This laboratory is equipped with a deep freezer for sample storage and a fume hood to safely conduct

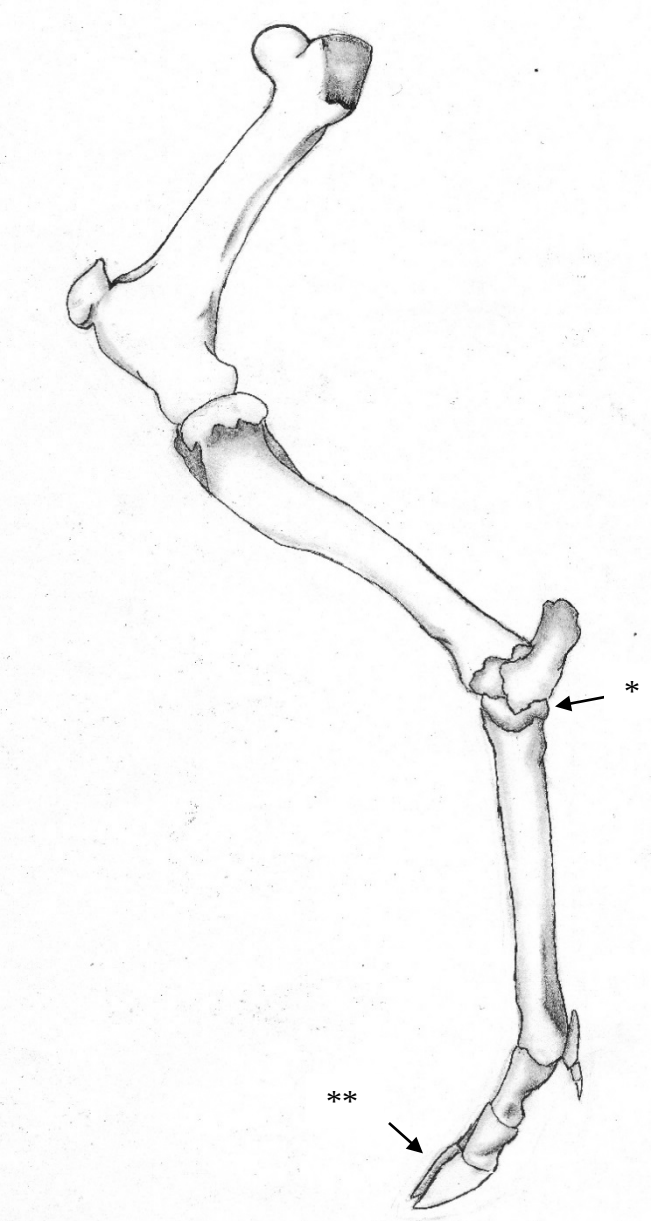

Figure 2.1: Deer hind limb anatomy.

The samples for this study included the limb bones from the distal row of tarsals* to the distal phalanges within the hoof**.

the various defleshing methods under study. There is also a faucet and sink located under the fume hood, which provided a ventilated area to pour the liquid from the maceration jars and cooking pots. The drain in this sink was fitted with a strainer so that small bones would not be lost and pieces of soft tissue would not clog the drain.

A table was positioned near the fume hood to be used as a work station. A stainless steel tray was placed on the table to lay the remains on at the check points when loose soft tissues would be lightly pulled from the remains. All soft tissues removed from the deer samples, including skin and fur, were deposited in zip-lock bags and stored in the deep freezer for later incineration, according to the University of Pittsburgh biohazardous waste disposal policy.

Several health and safety precautions were employed throughout the research process. Nitrile gloves were worn when handling the deer remains and various chemicals. Goggles and a plastic apron were worn when pouring liquid from the maceration jars or cooking pots into the drain, as splashing did occur. Goggles and a basic dust mask were worn when sawing and drilling the bones. Pots, utensils, and trays were cleaned with detergent between samples to impede mixing of the chemicals and/or enzymes used for processing. After a sample had been completed, the table and counter surfaces were disinfected with household bleach.

2.3 **PRE-PROCESSING TREATMENTS**

2.3.1 **Preparation of sample for defleshing**

A deer limb was unsystematically selected from the storage freezer and given an identification number which would stay with the limb segment and any bone samples taken from it throughout

the research process. The deer limb was then laid out to thaw on a stainless steel tray for 24 hours before the defleshing process would begin. After this thawing period, several standardized methods were completed before applying a defleshing treatment to the sample. The limb segments had been partially skinned by the deer processor during the butchering process prior to their collection for this study, so that 1/3 to 2/3 of the metatarsal bone was exposed. Digital photographs, both with and without a scale, were taken of each sample before preparation in order to document the amount of prior skinning, size of the specimen, and any variation in the condition of the remains prior to processing (see Figure 2.2).

Once photographed, the remains were carefully skinned using a scalpel. As much skin, fur, metatarsal gland, and foot pad was removed as possible without nicking the bone with the scalpel. The hoof and all periosteum, tendons, and ligaments were left intact so that the bones remained in articulation. The samples were photographed again once skinned to document any variation in the bones prior to trauma infliction and processing that may have been previously obscured by skin and fur (see Figure 2.3). This skinning process was implemented since it standardized all of the deer limbs before the various defleshing treatments were applied. It also simulated the typical methods employed in laboratories where animal or human remains are defleshed, as skinning (and eviscerating in complete remains) are common steps which precede the defleshing process in all but the smallest of animals in order to speed the process (Brown and Twigg 1967; Egerton 1968; Jakway et al. 1970; Nagorsen and Peterson 1980; Nawrocki 1997; Walsh-Haney et al. 2008).

Figure 2.2: Sample 10.060 prior to skinning (thawed).

Figure 2.3**:** Sample 10.060 after skinning.

2.3.2 **Trauma infliction**

Two types of sharp-force trauma were assessed in the current study: saw marks and cut marks. This trauma was inflicted after the skinned remains had been photographed. Once both types of trauma were inflicted, a stereo microscope connected to a digital camera was used to take close-up photographs of the marks. These photographs were later used to compare to the appearance of the marks pre- and post-processing to determine if any alteration to the marks had occurred during the processing method.

2.3.2.1 Saw marks

In order to prepare the sample for sawing, both ends of the metatarsal were thinly wrapped in paper towels. The limb segment was then laid on its medial surface on a 2"x 4" block of wood clamped to the counter. The paper towel-covered sections of bone were clamped to this wood/counter using Irwin Quick-Grip clamps with non-marring pads. The paper towels aided the clamp in gripping the slippery, rounded surface of the bone, while the soft, non-marring pads ensured that the bone would not be marked by the clamps during sawing. The metatarsal of each sample was then sawed 1/3 of the way down the shaft from the proximal end on the lateral surface using a Dewalt 18volt variable speed reciprocating saw. A Blu-Mol 6" bi-metal blade with 14 teeth per inch (TPI) was used with the reciprocating saw. The metatarsal was sawed completely through, resulting in two separate bone segments: the proximal 1/3 of the metatarsal, attached to the tarsals, and the distal 2/3 of the metatarsal, attached to the phalanges. Only the proximal kerf wall (i.e. the saw marks on the surface of the proximal segment of the bone) was observed in this study. The saw blade was changed after 30 samples (half-way through data collection) to ensure a sharp blade on all of the samples.

2.3.2.2 Cut marks

After removal of the sample from the clamps, a knife cut wound was created on the proximal metatarsal. The proximal segment of the metatarsal was cut on the lateral side, approximately 1/2" above the saw mark, with a stainless steel kitchen knife with an 8" non-serrated blade. The cut was made with the researcher's dominant (right) hand slicing the blade from forward to backward (towards the body), and the nondominant (left) hand holding the proximal end of the bone. Thus, right metatarsals were cut from anterior to posterior and left bones were cut from posterior to anterior.

2.3.3 **Bone core samples**

A core sample of the metatarsal cortical bone was taken prior to and following processing to be used in a mechanical test of the structural integrity of the bone tissue. The proximal segment was clamped into a Skil 10" bench top drill press. A cylindrical core of bone was drilled from the lateral side of the metatarsal prior to processing and from the medial side following processing using a 5/8" diamond encrusted tile hole saw bit. This core was taken approximately in the center (anterior to posterior) of the metatarsal, where the bone begins to flatten, so that the core sample was as flat as possible (see Figure 2.4).

After drilling through the bone, the core sample would remain inside the drill bit. Removal of the core was accomplished by taking the bit out of the drill press and gently tapping the bone with the blunt end of a bamboo skewer from the opposite end of the drill bit over a soft surface (such as a styrofoam plate).

The pre-processing bone core samples were cleaned of any excess soft tissue, such as bone marrow and periosteum, using gloved fingers and/or a bamboo skewer before being

prepared for storage in a chest freezer. Both pre- and post-processing core samples were marked with a colored permanent marker on the external surface of the cortical bone to reduce any possible confusion between the internal and external surfaces during later research. Storage preparation of the core samples involved individually wrapping each sample in gauze and dipping it into phosphate buffered saline (PBS) solution. The damp sample was then placed in a small labeled 2 mil. plastic resealable bag and placed in a freezer for storage until mechanical testing was performed.

Figure 2.4: Drilling of a bone core sample for mechanical analysis.

2.3.4 **Start weights**

Once the deer limb segments were skinned, traumatized, and drilled, the weight of what remained of each limb sample (see Figure 2.5) was recorded using a digital scale (Escali L600 High Precision digital scale; 0.1 gram resolution). This initial weight (in grams) was used to compare all of the samples prior to processing. These data were assessed using statistical analysis to determine if the remains significantly differed from each other in weight prior to processing since the sex of each sample was unknown and could not be controlled for.

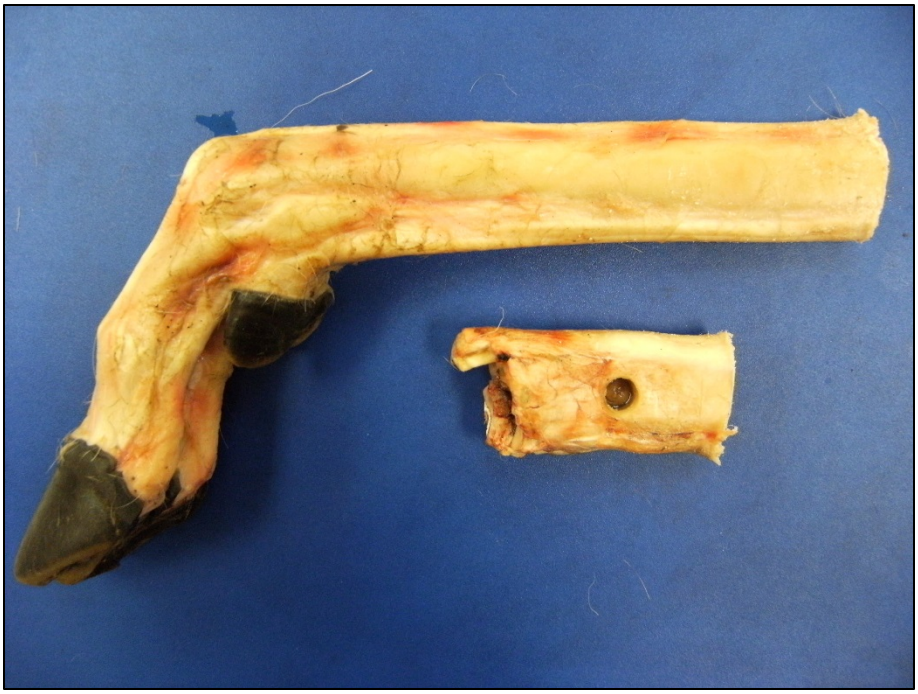

Figure 2.5: Sample 10.060 after trauma infliction and drilling.

Condition of the samples prior to defleshing method implementation.

2.4 METHODS OF SOFT TISSUE REMOVAL

The remains were processed using 5 general defleshing method types: 1) dermestid beetles, 2) plain water maceration, 3) heated plain water, 4) heated chemical solution, and 5) heated enzymatic solution. Two chemical, household bleach and sodium perborate, and one enzymatic method, enzymatic laundry detergent, were tested. Each of these chemical and enzymatic defleshing methods were varied at three concentration levels: low, medium, and high. The medium concentration was established using previously published methods. The high concentration was twice that of the medium, while the low concentration was half of the medium concentration. The low and high concentration variations allowed for the testing of the previously published methods at concentrations that were "too low" and "too high" to determine if these methods were still effective at a lower concentration and if a higher concentration would be destructive to the bone tissue or trauma marks. Table 2.1 provides a summary of the 12 defleshing method variations evaluated in this study.

Each method variation was implemented on 5 deer limb samples in order to determine if the defleshing methods were consistent and to strengthen the reliability of the results, leading to a total sample of 60 limbs. The deer limb samples were unsystematically assigned to each method variation.

All but the dermestid method, in which the remains were placed in a glass aquarium with a thriving dermestid colony, used tap water as the primary agent or solvent in the processing method. A 2 gallon glass jar with a loose-fitting lid was used for the maceration samples, while a 12 quart stainless steel stockpot with a strainer insert was used for all of the processing methods which implemented a heat treatment. A stainless steel pot was preferred for this study over another variety, such as aluminum, in order to reduce the chance of a metal, like aluminum,

affecting the chemical reactions of the processing methods. The strainer insert was essential for easy removal of the remains from the pot and it kept the remains from touching the bottom of the pot, where they would be in direct contact with the heat source. Heat was generated using electric camping burners with low, medium, and high temperature settings. The exact temperature (to the nearest degree Celsius) was monitored using a digital cooking thermometer with a probe and an alarm that would sound if the temperature exceeded the set number.

Table 2.1: Variations of defleshing methods tested.

Defleshing Method	Variations of Method	Concentration Levels
Maceration	NA	NA
Heated Maceration	NA	NA
Chemicals	Household Bleach	1. Low 2. Medium 3. High
	Sodium Perborate	
Enzymes	Biz Detergent	1. Low 2. Medium 3. High
Dermestids	NA	NA

NA = not applicable to method

Each method variation was observed at a set "check point." At each check point the remains were digitally photographed and the condition of the remains recorded, noting differential effects of the methods on various tissue types. The heated samples (plain water,

chemical, and enzyme samples) were checked every hour. Maceration and dermestid samples, due to the slower processes, were checked less frequently. Maceration check points were every 3 days, while dermestid samples were checked once a day. Due to this fact, many of these defleshing methods were able to be conducted simultaneously. For example, one set of remains could be placed in the dermestid tank as several remains could be soaking in maceration jars and two burners could be running at once for the heated tests.

The check points of the liquid defleshing methods involved recording the temperature and pH of the solution, digitally photographing the remains once removed from the solution, removal of loosened soft tissues and separation of any loosened joints, and digitally photographing the remains after the removal of this tissue. The temperature of the solutions was monitored and recorded in degrees Celsius with a digital cooking thermometer with a resolution to the nearest degree. The pH of the solution was recorded at each check point using a Hanna pH tester with an accuracy of 0.01pH. Because this particular pH tester cannot be used in water higher than 65°C, a 10ml sample of the solution from the pot or jar was collected using a syringe, placed in a small glass container, and left to cool for 10 minutes. Manual removal of the soft tissues occurred during this 10 minute interval, which was monitored with a digital timer. Light force was applied using fingers and forceps to remove soft tissues during these check points. No sharp tools, such as scalpels or scissors, were used during this process to avoid marking or damaging the bones. The check points of the non-liquid defleshing method, i.e. the dermestid method, did not involve manual removal of soft tissues, so as to not disturb the work of the beetles. However, photos were taken and the condition of the remains was recorded at each check point.

The remains were deemed "complete" once all soft tissues were removed from the bone or when no changes in the soft tissues were noted after two consecutive check points, since not every method was able to remove all soft tissues. These differential effects were noted at the end of processing. Once the remains were deemed complete, they were rinsed in warm, slow running tap water in a sink to remove any remaining loosely adhering soft tissues and any chemicals or enzymes used in processing. A strainer was placed over the sink drain to catch any small bones that may fall during the rinsing process. This strainer also protected the drain from clogging with small pieces of soft tissue that may be freed from the bone while rinsing. After rinsing, the bones were once again photographed and then laid out on paper towels on a tray labeled with the respective identification number of the sample to air-dry for 3 days. Once dry, the remains were weighed and placed in a resealable 2 mil. plastic bag labeled with the respective identification number for storage.

The "time-to-completion" (TTC) was defined as the time from when the remains were added to the processing treatment (water, chemical solution, enzyme solution, or dermestid tank) until they were deemed "complete" by the researcher. Thus, the TTC variable did not include the skinning, drilling, traumatizing, and photographing steps prior to processing. Nor did it include the post-processing steps of the final rinsing, drilling, air-drying, and photography. If the chemical or enzyme tests required more than a day of processing, the remains were left to soak in the solution off of the heat. The remains were placed in a new solution and the heat treatment was resumed in the morning. This soaking process was noted and calculated into the total processing time.

2.4.1 **Dermestids**

A pre-existing colony of *Dermestes maculatus* at the Carnegie Museum of Natural History (CMNH) in Pittsburgh, PA was used for this study. The CMNH dermestid colony resides in a shelter located outside of the museum. The CMNH dermestid colony was thriving at the time of the study (July 2010) and was divided into 3 medium-sized glass aquariums. Each deer sample was placed in a separate aquarium in a shallow cardboard box atop of a layer of cotton to provide a place for the dermestids to pupate. Since the effectiveness of the dermestids in defleshing is temperature-dependent, the temperature, as reported by the weather station at the Pittsburgh International Airport, was recorded daily for later reference. Humidity within the shed was not regulated or measured.

Samples in the dermestid tanks were checked every 24 hours. At this check point, photographs of the remains were taken while they were still in the aquarium as to not disturb or set free the dermestids (see Figure 2.6). However, after the photographs, loosened joints were disarticulated to expose more surface area for the dermestids to work by reaching into the aquarium and gently pulling the bones apart when possible. Additionally, water was sprayed with a spray bottle on any soft tissues that had become desiccated in order to moisten them for the dermestids. The samples were not able to be observed or accessed over the weekend since the dermestid shed was locked and the dermestid curator was off-duty, so beef jerky was added to the tanks on Friday and left over the weekend. This process was necessary to ensure there would be enough food for the dermestids if they had removed all soft tissues from the deer samples in this time. This also deterred the beetles from eating the bone tissue, as they would find the beef jerky more palatable.

Once the bones were sufficiently cleaned by the beetles, the remains were removed from the aquarium after brushing off as many straggling beetles as possible from the bones with fingers or a soft-bristled brush over the aquarium. Dermestid beetles tend to hide in small crevices and foramina of bones, so to prevent laboratory contamination, dermestid-cleaned remains were placed in a resealable plastic bag and stored in a chest freezer for 24 hours to kill any remaining beetles. After removal from the freezer, the remains were set out to thaw for 24 hours, then rinsed under slow-running tap water over a strainer and brushed with a soft-bristled toothbrush to clean the bones of any uneaten dried tissue, dead beetles, and beetle frass. The post-processing bone core was drilled and the bones were then laid out for 3 days to air-dry prior to photographing the trauma marks.

Figure 2.6: Dermestids at work on sample 10.003 (day 2).

2.4.2 **Maceration**

The maceration tests involved filling a 2 gallon glass jar 3/4 full with room temperature (23°C) tap water. This amount of water was enough to cover the remains and compensate for evaporation between check points. Once the jar was filled to the proper amount, it was left uncovered for 30 minutes to allow for the evaporation of the chlorine from the water, or dechlorination, prior to the addition of the remains, as the tap water supplied to the laboratory is treated with chlorine (Pittsburgh Water and Sewer Authority 2010) and chlorine may hinder the bacterial activity anticipated during maceration processing. This dechlorination step was not described in the literature, but it was thought that it would aid the maceration process for this study. After dechlorination, the remains were added and the loose-fitting lid placed on the jar. The jar was then positioned under a ventilation hood to reduce the possibility of strong odors permeating the laboratory.

It has been recommended that maceration water be changed regularly because molds may begin to grow at the water surface, which can either discolor or damage bones that come in contact with it (Hamon 1964). Thus, the water in the maceration jar was changed every 3 days at the check point. The water at this time would be cloudy to black in color, depending on the stage of decomposition of the remains, with a white film floating on top (see Figure 2.7). Additionally, during the maceration check points, the temperature and pH of the water was recorded and the remains removed with stainless steel tongs and gently placed on a stainless steel tray. While wearing an apron and goggles, the water was slowly and carefully poured from the jar through a strainer into a sink under the ventilation hood. Tap water was used to gently rinse out any residues on the interior of the jar. The jar was then refilled with clean tap water and once again left uncovered to dechlorinate for 30 minutes before the remains were replaced. After the

remains were photographed, separated of any loose soft tissues, and photographed again, they were gently placed in the clean water-filled jar, covered, and placed back under the ventilation hood until the next check point. After the last cleaning, the bones were rinsed under slow-running tap water over a strainer and brushed with a soft-bristled toothbrush to clean the bones of any remaining soft tissues or grease. The post-processing bone core was drilled and the bones were then laid out for 3 days to air-dry prior to photographing the trauma marks.

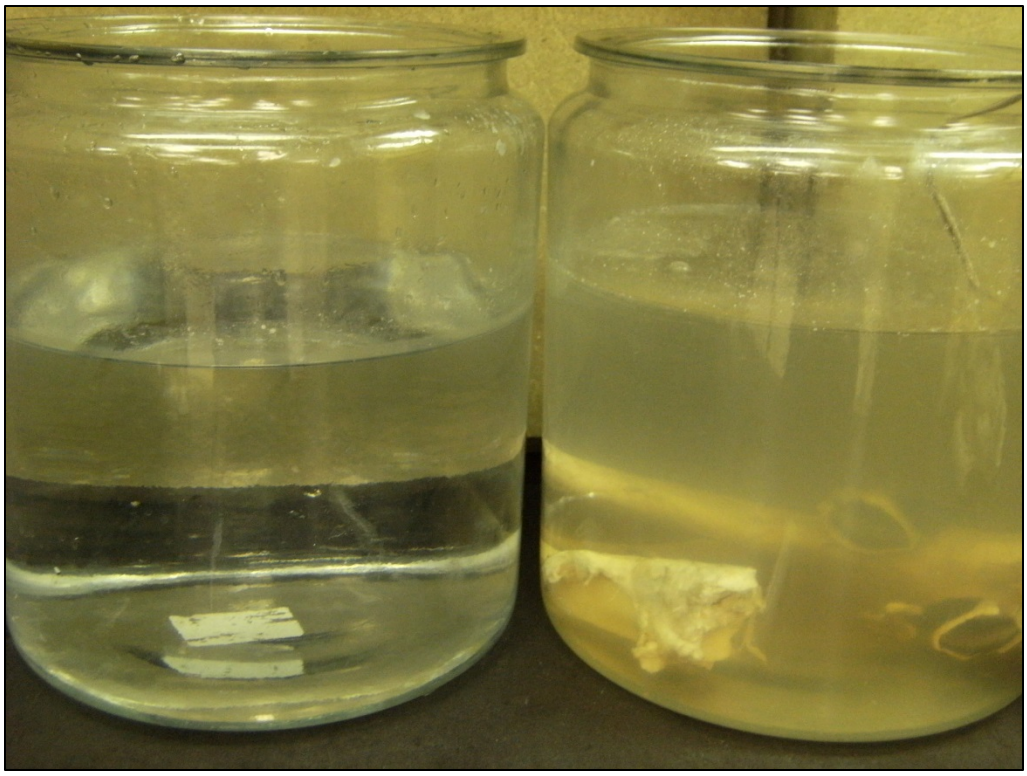

Figure 2.7: Maceration jars. The water was just changed in the jar on the left. The jar on the right shows the cloudiness of the water after 3 days of maceration; the remains have not been removed and the water has yet to be changed.

2.4.3 **Plain water boil**

In the plain water boil method, the remains were simply placed in a 12 quart stainless steel pot with 2 gallons of plain tap water and brought to a boil. This temperature (100ºC) was maintained until the soft tissues were easily removed with fingers and the bones were clean. After the last cleaning, the bones were rinsed under slow-running tap water over a strainer and brushed with a soft-bristled toothbrush to clean the bones of any remaining soft tissues and grease. The post-processing bone core was drilled and the bones were then laid out for 3 days to air-dry prior to photographing the trauma marks.

2.4.4 **Household bleach (Clorox®)**

The household bleach method used in this study was derived from Nawrocki (1997; 2008), who uses bleach to deflesh human remains from forensic cases in the University of Indianapolis Archeology and Forensics Laboratory. Nawrocki's recommended concentration of 1 cup of bleach to 1 gallon of water was used as the medium concentration in the current study. Although Nawrocki uses borax as a degreaser along with the bleach to deflesh remains, the current study used a bleach-water only solution in which the samples remained in, except during check points, until they were completely clean so as to assess the effects, if any, of bleach alone on the bones. Nawrocki also intimates that bleach should only be used in the first boil, and suggests that after a few hours in the bleach solution, the remains should be removed and rinsed and that the water in the pot should be changed for subsequent boiling of the remains. However, other researchers use bleach throughout the entire defleshing process (Gross and Gross 1966; Stephens 1979), therefore, the current study tested the effects of bleach when used for the entire boiling time.

Though many generic forms of household bleach (with an active ingredient of sodium hypochlorite) exist, the regular liquid formula of the name brand Clorox® was used in this study due to its notoriety and ready availability. This processing method involved adding a low (.5c/gal), medium (1c/gal), or high (2c/gal) concentration of Clorox® to 2 gallons of tap water in a 12 quart stainless steel stock pot. The remains were then added to the bleach solution and brought to a boil. This temperature (100ºC) was maintained until the soft tissues were easily removed by hand and the bones were clean. After the last cleaning, the bones were rinsed under slow-running tap water over a strainer and brushed with a soft-bristled toothbrush to clean the bones of any remaining soft tissues or chemicals. The post-processing bone core was drilled and the bones were then laid out for 3 days to air-dry prior to photographing the trauma marks.

2.4.5 **Sodium perborate (SPB)**

The sodium perborate (SPB) method utilized in this study was derived from several published studies as well as trial and error by the current researcher. The medium concentration for this study (70g/L) was chosen as it seemed to be a midpoint in the range of recommended concentrations (deWet et al. 1990; Jakway et al. 1970; McDonald and Vaughan 1999; Roche 1954). Though measurements in grams and liters are standard in science, the measurements for this concentration were converted to cups and gallons in order to simplify the method for utilization in American laboratories as it is speedier and more convenient to scoop a particular amount of the powdered chemical in a measuring cup than it is to weigh that same amount each time remains are to be defleshed. Accordingly, the 70g/L concentration was converted to 265g/gal, which, conveniently, equals 1.5 cups of sodium perborate per gallon of water.

Once the medium concentration was established, the methods suggested by Jakway *et al.* (1970) were used, with some alterations after trial and error. Jakway and colleagues first boil the water, then add the remains, and finally the sodium perborate. After the sodium perborate is added, the pot is removed from the heat source and left for several hours to return to room temperature. The pot is removed from the heat source after the sodium perborate is added to avoid the chemical reaction bubbling up over the pot. However, it was discovered early during experimentation that if the temperature dropped too low after the chemical was added, the sodium perborate would crystallize on the bones at the bottom of the pot, as cautioned by Chapman and Chapman (1969). Thus, informal trials were conducted using sodium perborate at various temperatures to find a temperature that would be low enough to keep the solution from bubbling over the pot, yet high enough to keep crystals from forming on the bones. A temperature of 80-85°C was found to work well during testing; both undesired effects were avoided at this temperature and the solution was effective at cleaning the bones.

The finalized methods utilized in the current study involved bringing two gallons of tap water to a boil in a 12 quart stainless steel stock pot. Once the water began to boil, a low (.75c/gal), medium (1.5c/gal), or high (3c/gal) concentration of sodium perborate was added. The remains were then placed in the solution and the temperature was decreased to the 'low' setting on the electric burner and the temperature was maintained at 80-85°C for the remainder of the processing method. After the last cleaning, the bones were rinsed under slow-running tap water over a strainer and brushed with a soft-bristled toothbrush to clean the bones of any remaining soft tissues or chemicals. The post-processing bone core was drilled and the bones were then laid out for 3 days to air-dry prior to photographing the trauma marks. Figures 2.8 –

2.13 show a deer limb sample after removal of loose soft tissues at each check point throughout the entire sodium perborate defleshing process (6 hours for this particular sample).

2.4.6 **Enzymatic laundry detergent (Biz®)**

These methods were derived from Mooney et al. (1982) who used a 10% v/v solution of a commercial enzyme active laundry detergent (Biz®) heated to 75-80°C to deflesh various skulls. Both the concentration and recommended heat treatment were used in the methods of the current study, however, the concentration was converted to cups and gallons such that the medium concentration was determined to be 1.5 cups of detergent to 1 gallon of water. This conversion was completed to simplify the measuring process for preparators in other laboratories.

In the current study this processing method involved adding a low (.75c/gal), medium (1.5c/gal), or high (3c/gal) concentration of Biz® powdered enzymatic laundry detergent to 2 gallons of tap water in a 12 quart stainless steel stock pot. The remains were then added to the enzyme solution and brought to a temperature of 75-80°C. This temperature was maintained until the soft tissues were easily removed with fingers and the bones were clean. After the last cleaning, the bones were rinsed under slow-running tap water over a strainer and brushed with a soft-bristled toothbrush to clean the bones of any remaining soft tissues or enzymes. The post-processing bone core was drilled and the bones were then laid out for 3 days to air-dry prior to photographing the trauma marks.

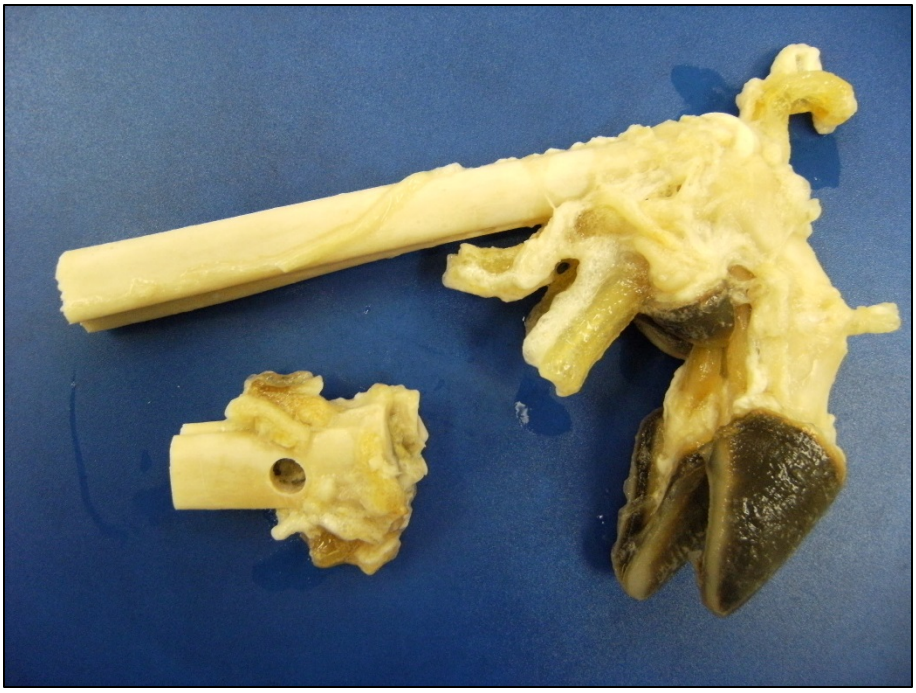

Figure 2.8: Sample 10.060 after 1 hour of SPB treatment.

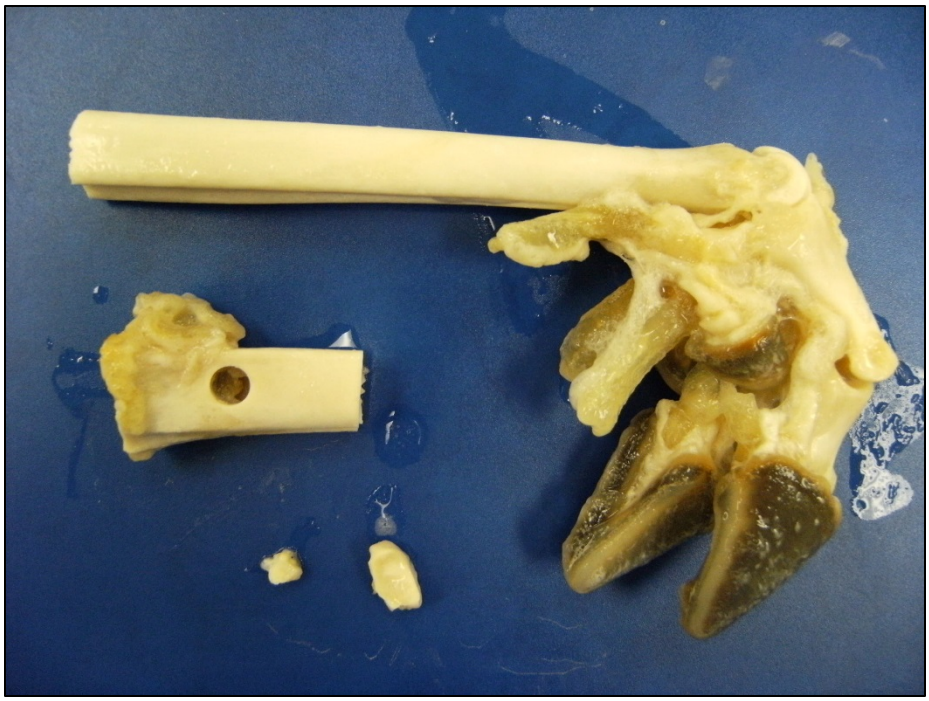

Figure 2.9: Sample 10.060 after 2 hours of SPB treatment.

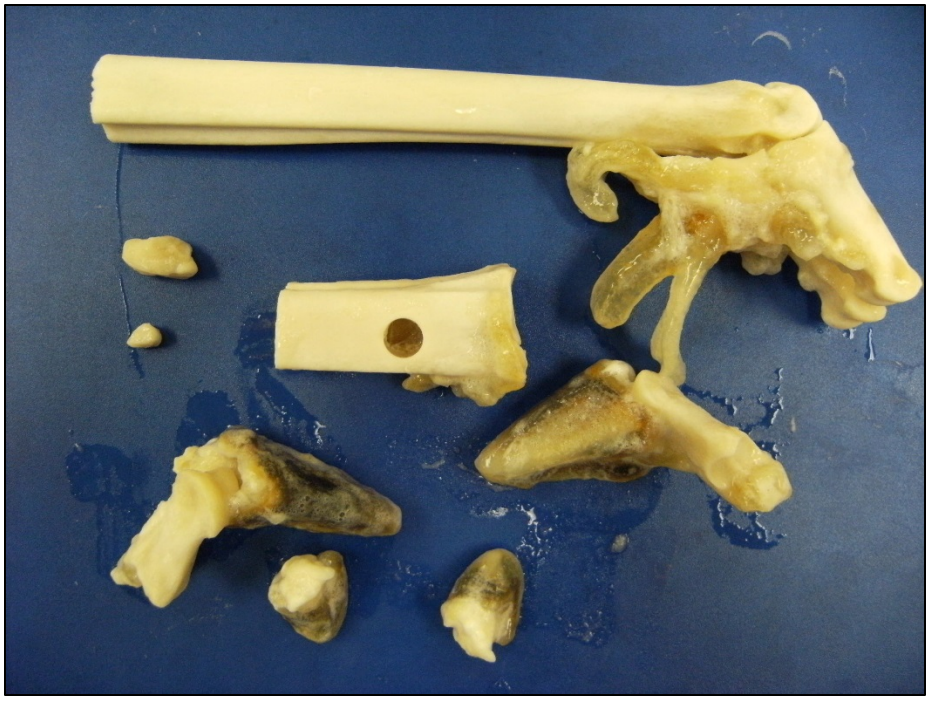

Figure 2.10: Sample 10.060 after 3 hours of SPB treatment.

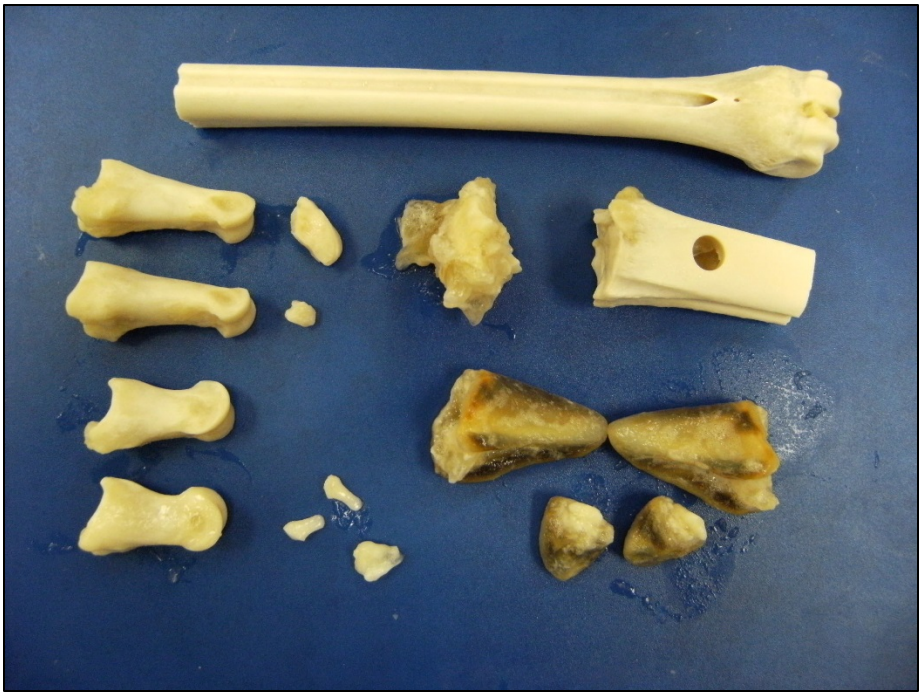

Figure 2.11: Sample 10.060 after 4 hours of SPB treatment.

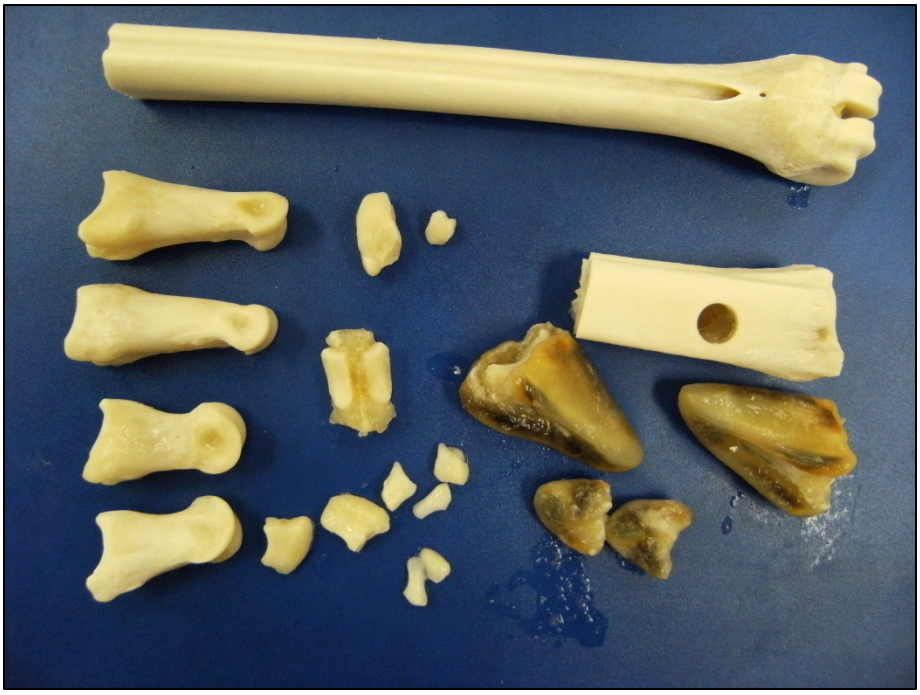

Figure 2.12: Sample 10.060 after 5 hours of SPB treatment.

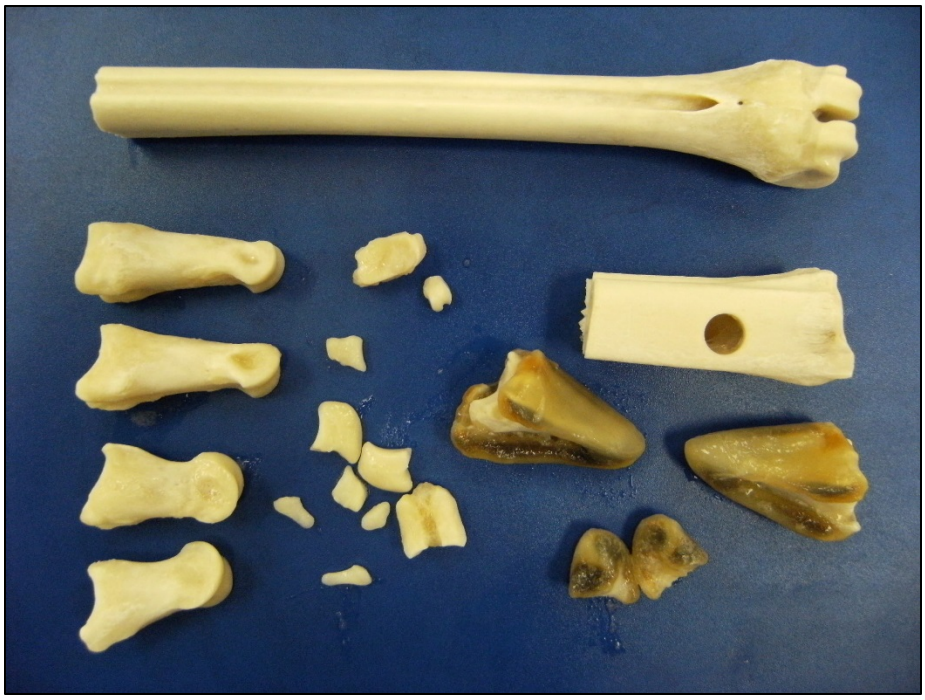

Figure 2.13: Sample 10.060 after 6 hours of SPB treatment.

Remains deemed "complete". Note that the distal phalanges were not able to be removed from the hooves and some of the sesamoid bones are still connected by ligaments.

2.5 **POST-PROCESSING METHODS**

2.5.1 **Dry weights**

The remains of each sample were weighed (in grams) with a digital scale (Escali L600 High Precision digital scale; 0.1 gram resolution) after air-drying for 3 days. These weights were used in statistical tests to determine if the samples differed in weight post-processing. If a significant difference in post-processing weight existed in the remains but not in pre-processing weight, it could be deduced that the difference was caused by the processing method. Differences in weight could be a result of differing grease content of the bones, damage to the bone tissue, or due to the amount of soft tissues remaining attached to the bones. Thus, the dry weights of the remains post-processing were used to discover differences in the efficiency or possible damaging effects of the tested processing methods.

2.5.2 **Estimation of age-at-death**

The various defleshing methods removed the soft tissues previously obscuring the epiphyseal plates such that the level of epiphyseal fusion of the distal metatarsal, first phalanx, and second phalanx could be used to estimate the age-at-death for each deer sample using methods outlined by Purdue (1983). Each of these bones was scored as unfused, fusing, or completely fused, then compared to Purdue's data to estimate the age of each deer sample. Based on the pattern of

epiphyseal fusion of these bones, the deer samples were classified into 3 age groups: less than 1 year old, 1-2 years old, and greater than 2 years old.

2.5.3 **Assessment of bone damage**

Bone damage was operationalized in this study as any observable alteration in the bone tissue or in the inflicted sharp-force trauma marks. Three methods were used to determine whether the bone tissue of the samples had been altered by the defleshing method. Each of the samples underwent a gross (macroscopic) inspection of the bone tissue, a comparison of close-up views of the cut and saw marks prior to and after defleshing treatment, as well as mechanical testing of the bone tissue prior to and after defleshing treatment.

2.5.3.1 Macroscopic assessment

Macroscopic bone damage was assessed via a visual and tactile inspection of the entire external surface of each of the bones of the limb samples after processing. The gross appearance of the bones were scored on an ordinal scale for bone quality developed by Steadman et al. (2006):

(1) Brittle, fragile, easily broken
(2) No cortical erosion but bone is lighter in weight and porous
(3) Softer, more pliable than normal bone but no cortical damage
(4) Cortex eroding and/or flaking but bone will not easily fracture
(5) Strong, normal bone texture and quality.

2.5.3.2 Assessment of trauma marks

Prior to and following defleshing treatment, the saw marks on the proximal kerf wall and the cut mark on the proximal metatarsal were photographed using 2 methods: a digital SLR camera fitted with a 10X macro lens and a Leica MZ12 stereo microscope connected to a digital camera.

The use of the stereo microscope allowed for a closer view of the trauma marks. However, a scale was not able to be included in the stereo microscope photos since the bone filled the frame, so the SLR photographs were taken to have pictures of the trauma marks with a scale. Figures 2.14 and 2.15 show the saw marks on a sample from the study pre- and post-processing using both methods of photography. Once photographed via each method, the pre-processing photos were compared to the post-processing photos and evaluated for visible changes in the striations comprising the respective trauma marks.

2.5.3.3 Mechanical testing

Unconfined compression tests on the pre- and post-processing bone core samples were conducted to determine whether the processing methods used altered the mechanical properties of the bone tissue. Mechanical testing took place at the Center for Craniofacial Regeneration in the School of Dental Medicine at the University of Pittsburgh. Prior to mechanical testing, the bone core samples were allowed to thaw and hydrate in phosphate buffered saline (PBS) for 1 hour. Once thawed, the specimen diameter and thickness was measured using digital calipers and recorded for use in determining stress and strain. Then the endosteal surface of each bone core was secured to a compression platen using cyanoacrylate so that the periosteal surface faced up and compression was applied to the external surface of the bone. The tank was filled with PBS, and heated to 37°C. Each core was loaded to 1790N or to 50% of the caliper thickness of the sample, whichever value was attained first. The load was applied along the transverse axis of the bone, or the thickness of the cylinder, at a strain rate of 0.1mm/min. (testing machine: Instron model 5564; 2kN load cell, Bluehill 2). The force and distance traveled was recorded and normalized to stress and strain. The extension was held for 20 minutes to allow for relaxation. After each mechanical test, the bone core and any pieces that may have fragmented from it were

again wrapped in gauze, dampened in PBS, and replaced in its respectively labeled bag to be refrozen for possible later testing.

The collected data were then used to generate stress-strain curves. The stress and strain values were recorded at the first peak, signifying when the sample first broke, and at 1790N, the maximum load. Some samples did not break before reaching the maximum load, so only show a peak at 1790N on the stress-strain curve; while some broke before reaching 1790N, and thus display more than 1 peak. Typical stress-strain curves are depicted in Figures 2.16 and 2.17. The tangent modulus was found by taking the slope of the linear region of the stress-strain loading curve for the last 2 percent of strain before the first peak.

Three biomechanics variables, strain at 1790N, stress at first peak, and tangent modulus, were selected for further assessment and statistical analysis. Since strain is the amount of deformation of the bone core relative to its original thickness, the strain at 1790N reflects the compressibility of the bone. The stress at first peak indicates the amount of stress required to initially fracture the sample and, thus, reflects the strength of the bone. The tangent modulus is the slope of the stress-strain curve and reflects the stiffness of the sample.

2.5.4 **Per-Sample Cost**

The final monetary cost of implementing each defleshing method was determined based on how much it cost (in dollars) to deflesh a single sample. The value calculated was a cost per sample and did not include start-up materials or equipment such as pots and burners.

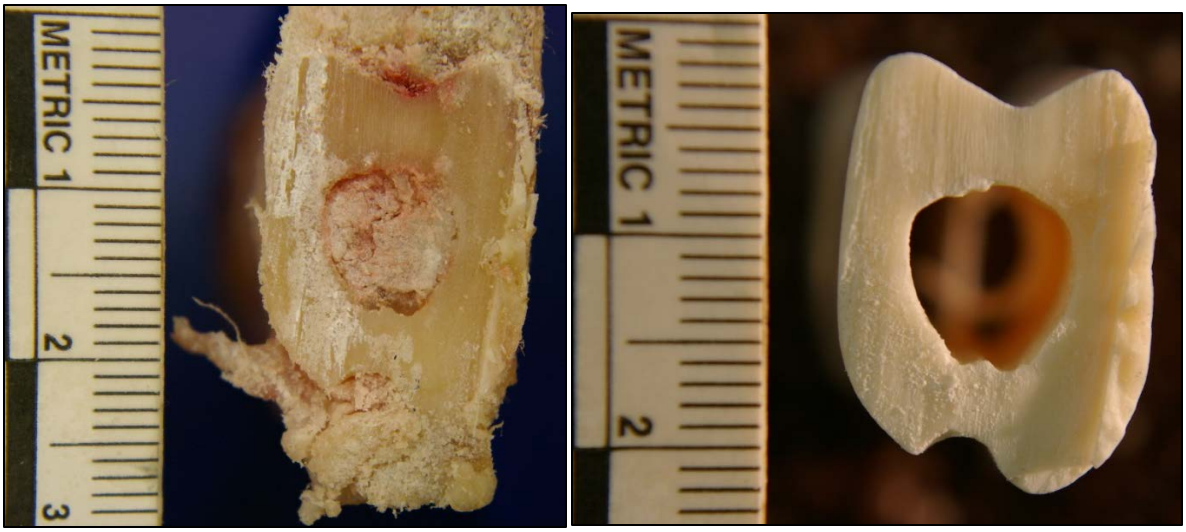

Figure 2.14: Sample 10.060 saw marks prior to (left) and following (right) defleshing treatment (SPB). Photos taken using a digital SLR camera with a 10X macro lens. The white on the kerf surface of the pre-processing sample is paint residue from the saw blade, which was removed during processing.

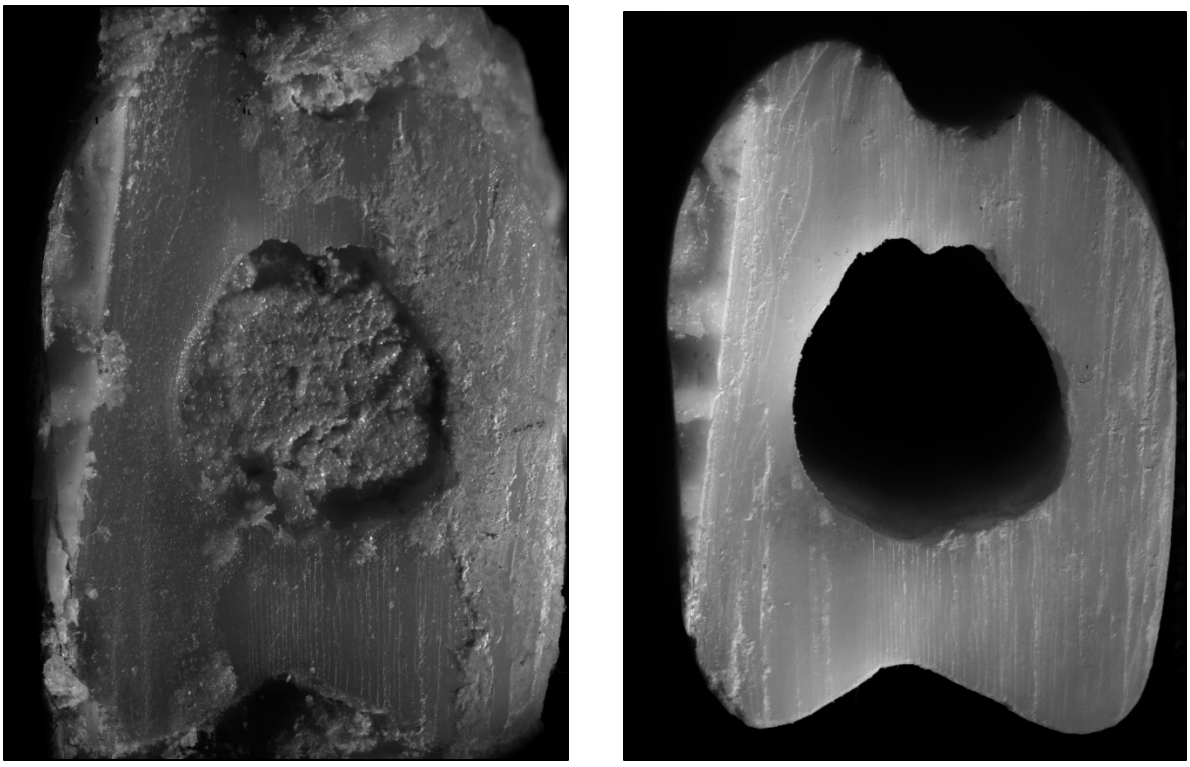

Figure 2.15: Sample 10.060 saw marks prior to (left) and following (right) defleshing treatment (SPB). Photos taken using a stereo microscope connected to a digital camera.

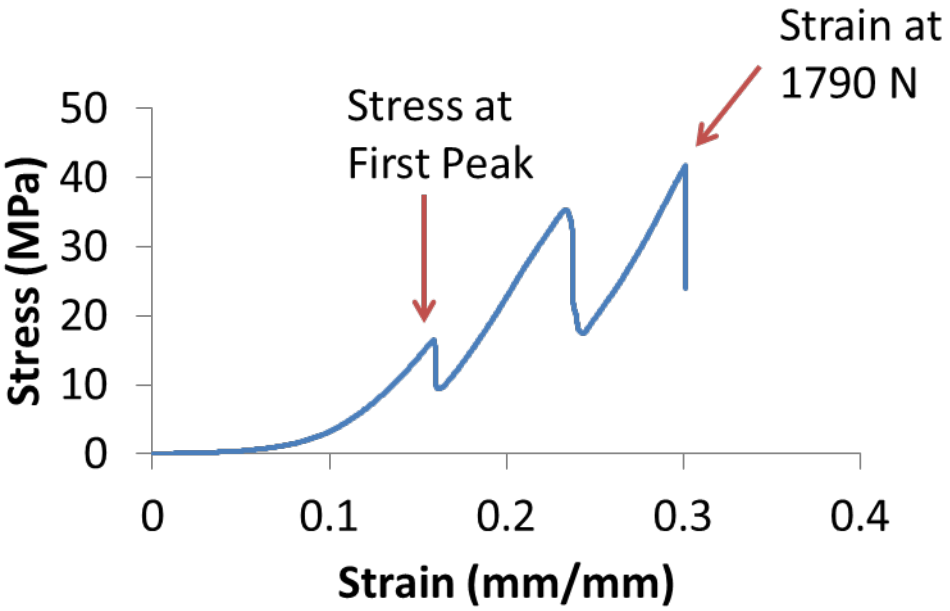

Figure 2.16: Typical stress-strain curve.

Stress at first peak and strain at 1790N occurred at different times in this instance and are labeled.

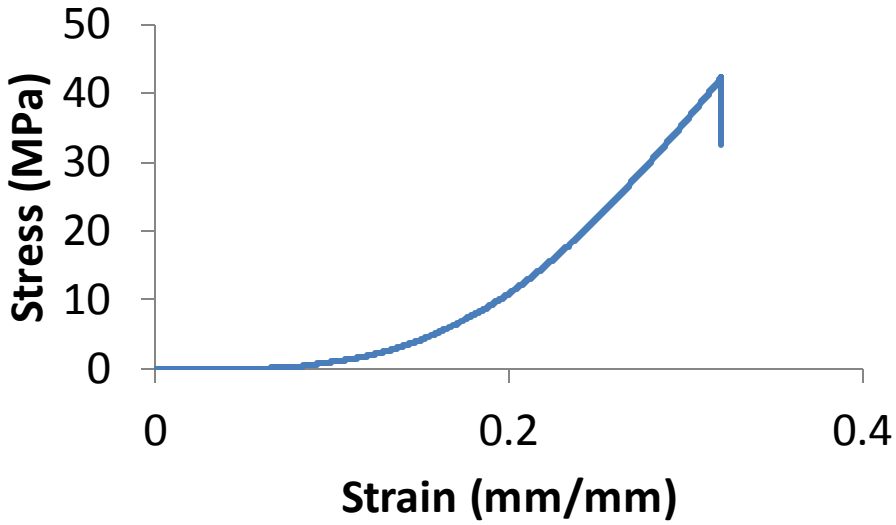

Figure 2.17: A stress-strain curve where the sample did not break before 1790N.

Stress at first peak and strain at 1790N are the same value in this instance.

2.6 **STATISTICAL ANALYSES**

Statistical analyses were conducted using the Statistical Package for the Social Sciences (SPSS) version 22.0. Descriptive statistics were compiled for all data. Continuous variables were assessed for normality using the Shapiro-Wilks test and homogeneity of variance using Levene's F test in order to test the assumptions of the various quantitative tests. If these assumptions were found to be violated, nonparametric alternatives with less rigorous assumptions were implemented. Results were considered significant at the $p < 0.05$ level (all two-tailed).

The composition and distribution of the sample was examined by assessing the frequency of limb sides (left or right), estimated age of the deer, and weight of each deer limb prior to defleshing (start weight). The local air temperatures were recorded for the 2 week period the dermestid colony was used and are reported with temperature averages for this time period. Additionally, descriptive statistics for the pH of the water solutions were calculated to determine whether some defleshing methods tended to become acidic or alkaline, or remained neutral. The remaining quantitative variables were first considered for each defleshing treatment separately in order to assess the efficiency and possible destructiveness of each method. The efficiency of the defleshing methods was examined by computing the average time-to-completion (TTC) of each treatment and bone destruction was assessed statistically by analysis of the biomechanic variables (strain at 1790N, stress at 1st peak, and tangent modulus) via paired t-tests.

The TTC results were then compared among all of the defleshing treatments using a Kruskal-Wallis test in order to assess defleshing efficiency in comparison to the rest of the methods. This nonparametric rank test was employed because the TTC data violated multiple analysis of variance (ANOVA) assumptions. The final multiple comparison test was a repeated

measures ANOVA to compare the start weight to the corresponding dry weight of the samples in each defleshing treatment.

3.0 **RESULTS**

This study investigated the efficiency, effectiveness, and possible damaging effects of 6 defleshing methods on the distal segment of deer hind limbs. The 3 methods involving chemical or enzyme solutions were tested in low, medium, and high concentrations, thus, there was a total of 12 defleshing treatments. The efficiency of the various methods was measured as time-to-completion (TTC) and the monetary cost to implement the method per sample. The effectiveness was assessed qualitatively as descriptions of tissues unable to be removed during processing or bones unable to be separated and descriptions of the final greasiness of the bone. Bone damage was assessed qualitatively by visual inspection of the bone and of the marks of trauma. Bone damage was also assessed quantitatively by 3 biomechanics variables (strain at 1790N, stress at first peak, and tangent modulus) which evaluated changes in the integrity of the bone tissue post-processing.

The results of these tests and evaluations are presented in the subsequent text after a description of the deer samples used in the study. The results are first presented for each of the 6 defleshing methods separately, followed by the results of the analyses comparing all of the methods. The results of the specific assumption tests of each of the statistical tests conducted are reported in Appendix B.

3.1 DESCRIPTIVE STATISTICS OF THE DEER SAMPLES

Frequency data and descriptive statistics for the side, age, and start weight, of the deer limb samples are provided. Chi square analyses were not able to be conducted on the frequency data for these variables due to the small sample sizes in each of the defleshing treatments.

3.1.1 Side of the deer samples

A total of 31 right and 29 left deer limbs were used in this study. The frequency of left and right limbs used in each defleshing treatment is displayed in Table 3.1.

Table 3.1: Frequency of limb sides by defleshing treatment.

Method	Concentration Variation	Right Limbs	Left Limbs	Total
Dermestids	NA	4	1	5
Maceration	NA	3	2	5
Boil	NA	4	1	5
Clorox®	Low	2	3	5
	Medium	2	3	5
	High	3	2	5
SPB	Low	3	2	5
	Medium	1	4	5
	High	2	3	5
Biz®	Low	2	3	5
	Medium	2	3	5
	High	3	2	5
Total		31	29	60

3.1.2 **Age-at-death of deer samples**

The frequency of deer samples in each of the 3 age categories (less than 1 year old, 1-2 years old, and greater than 2 years old) is displayed in Table 3.2. Only 4 (6.7%) of the deer were under a year old and, likely, in their first Fall. Of these fawns, defined as less than 1 year old, only 1 sample possessed unfused epiphyses on all of the observed bones. The remaining deer in the study (n = 56, 93.3%) were over a year old (in at least their second Fall), and considered adult. Table 3.3 presents the distribution of the age groups in each defleshing treatment.

Table 3.2: Frequency of deer age groups in sample.

Age Group	Frequency	Percent
< 1yr	4	6.7
1-2yr	21	35.0
> 2yr	35	58.3
Total	60	100.0

Table 3.3: Frequency of age groups in each defleshing treatment.

Method	Concentration Variation	< 1 Year	1-2 Years	> 2 Years	Total
Dermestids	NA	0	2	3	5
Maceration	NA	2	0	3	5
Boil	NA	2	1	2	5
Clorox®	Low	0	2	3	5
	Medium	0	3	2	5
	High	0	2	3	5
SPB	Low	0	3	2	5
	Medium	0	2	3	5
	High	0	1	4	5
Biz®	Low	0	2	3	5
	Medium	0	2	3	5
	High	0	1	4	5
Total		4	21	35	60

3.1.3 **Start weight of samples**

The descriptive statistics for the weight of the deer limb samples prior to defleshing treatment, or start weight, are displayed in Table 3.4. The start weights of 2 of the samples were inadvertently left out during the study, so the sample size for this variable is 58. The weights ranged from 181.00g to 473.00g (M = 313.293, SD = 77.607).

A one-way between-subjects analysis of variance (ANOVA) was performed on the start weight of the deer samples to assess whether the samples randomly assigned to the 12 defleshing treatments differed in weight prior to processing. The ANOVA showed no significant difference in the start weight of the deer samples across the 12 defleshing treatments, $F(11, 46) = 1.096$, $p = 0.386$.

Table 3.4: Descriptive statistics for the start weight of the deer limb samples.

Method	Concentration Variation	N	Mean Weight (grams)	SD	Std. Error
Dermestids	NA	5	322.800	105.063	46.986
Maceration	NA	5	315.280	107.687	48.159
Boil	NA	5	286.760	70.396	31.482
Clorox®	Low	4	288.425	88.784	44.392
	Medium	5	256.600	49.410	22.097
	High	4	384.225	91.830	45.915
SPB	Low	5	258.920	59.532	26.623
	Medium	5	326.940	50.866	22.748
	High	5	347.120	93.182	41.672
Biz®	Low	5	340.120	85.215	38.109
	Medium	5	338.180	37.602	16.816
	High	5	303.360	47.387	21.192
Total		58	313.293	77.607	10.190

3.2 RESULTS BY DEFLESHING METHOD

Descriptive statistics for time-to-completion (TTC) and the 3 biomechanics variables (strain at 1790N, stress at first peak, and tangent modulus) are reported below for each defleshing treatment, as well as the local air temperature data for the dermestid samples and the pH data for the samples involving submersion in water. The pH of the water solutions was taken at each check point, which was every 3 days for the maceration method and every hour for all of the methods which involved heated water solutions. Table 3.5 presents the descriptive statistics for TTC for all of the defleshing treatments.

Paired-samples t-tests were conducted on the 3 biomechanics variables to determine if there was a significant difference between the mean values of the pre-processing samples and the post-processing samples for each defleshing treatment. The results of these tests are reported for each defleshing method separately.

Table 3.5: Descriptive statistics for TTC in all 12 treatment groups.

Method	Concentration Variation	N	Mean (in hours)	SD	Minimum		Maximum	
					Hours	Days	Hours	Days
Dermestids	NA	5	134.40	46.785	72	3	168	7
Maceration	NA	5	1137.60	365.005	792	33	1656	69
Boil	NA	5	6.60	.548	6	.25	7	.29
Clorox®	Low	5	6.80	.837	6	.25	8	.33
	Medium	5	6.40	.548	6	.25	7	.29
	High	5	6.40	.548	6	.25	7	.29
SPB	Low	5	4.80	.837	4	.17	6	.25
	Medium	5	5.20	.447	5	.21	6	.25
	High	5	4.20	.837	3	.13	5	.21
Biz®	Low	5	15.40	6.768	8	.33	21	.88
	Medium	5	12.80	6.573	8	.33	20	.83
	High	5	10.00	5.612	7	.29	20	.83
Total		60	112.55	327.983	3	.13	1656	69

3.2.1 **Dermestids**

3.2.1.1 Temperature

During the 2 July weeks that the dermestid colony was utilized, the average high temperature in Pittsburgh was 30.2°C and did not exceed 34°C. The average low temperature during this period was 20.8°C and did not drop below 19°C. The high and low temperatures for the specific dates of dermestid colony use are listed in Table 3.6.

Table 3.6: Local temperatures for the time of dermestid colony use.

	High		**Low**	
Date	**°C**	**°F**	**°C**	**°F**
7/12/2010	28	82	20	68
7/13/2010	27	80	21	69
7/14/2010	30	86	20	68
7/15/2010	31	88	19	67
7/16/2010	31	87	22	71
7/17/2010	31	88	19	67
7/18/2010	32	89	22	72
7/19/2010	29	85	21	69
7/20/2010	28	82	20	68
7/21/2010	30	86	21	70
7/22/2010	31	87	20	68
7/23/2010	34	93	25	77
Average	30.17	86.08	20.83	69.50

3.2.1.2 TTC

The dermestid samples had a TTC of 3 to 7 days (M = 5.6, SD = 1.949). Three samples took 7 days to complete (Monday to Monday). The CMNH dermestid shelter was locked over the weekends, so these samples were not able to be observed or accessed on Saturday or Sunday. The remaining 2 samples took less than a week to complete and did not have to be left over the

weekend. Of the 2 samples that took less than a week to complete, 1 sample took 4 days to complete (Monday to Friday) and the other sample took 3 days to complete (Monday-Thursday).

3.2.1.3 Biomechanics

The samples defleshed via dermestids showed a significant difference between pre- and post-processing samples in tangent modulus. The tangent modulus significantly decreased after processing. The paired-samples t-tests found no significant difference between pre- and post-processing samples in strain at 1790N or in stress at first peak. Table 3.7 displays the descriptive statistics for these variables and the results of the paired-samples t-tests.

Table 3.7: Descriptive statistics and paired-samples t-test values for the biomechanics tests on the dermestid samples.

Variable	Pre or Post	N	Mean	SD	*t*	df	*p*
Strain at 1790N	Pre-processing	5	.242	.094	-1.621	4	.180
	Post-processing	5	.274	.067			
Stress at 1st Peak	Pre-processing	5	35.122	7.760	1.566	4	.192
	Post-processing	5	20.589	14.149			
Tangent Modulus	Pre-processing	5	386.624	117.786	3.454	4	.026*
	Post-processing	5	183.792	84.148			

* = statistical significance

3.2.1.4 Per-sample cost of method

There was no per-sample cost involved in the dermestid method. A pre-existing colony was utilized and no purchases were required to deflesh each sample for this study.

3.2.1.5 Qualitative results

Most all of the soft tissues were digested by the dermestids; however, some ligament remnants remained attached to the proximal end of the metatarsal, distal phalanges of digits 2 and 5, and

sesamoid bones of 1 of the samples. On the other 4 samples, the distal phalanges of digits 2 and 5 were not separated from their hooves, and on 1 of these samples all 4 sesamoid bones remained attached. When complete, the bones were visibly greasy (dark yellow/orange in color) and had a strong greasy odor. Damage was observed on 2 samples on the proximal surface of the metatarsal (see Figure 3.1). On these bones, holes were eaten through the thin bone between the articular facets. No damage to the trauma marks was observed.

Figure 3.1: Holes eaten through the proximal surface of the metatarsal of a dermestid-cleaned sample.

3.2.2 **Maceration**

3.2.2.1 pH

The pH of the maceration water was recorded at the check point every 3 days. Values ranged from 5.46 to 9.24 (M = 7.08, SD = 0.839). Descriptive statistics for the pH at each check point are provided in Table 3.8.

3.2.2.2 TTC

The maceration samples had a time-to-completion (TTC) of 33 to 69 days (M = 47.40, SD = 15.209). Thus, all samples took over 1 month to complete.

3.2.2.3 Biomechanics

The samples defleshed via maceration showed a significant difference between pre- and post-processing samples in strain at 1790N, which increased after processing. The paired-samples t-tests found no significant difference between pre- and post-processing samples in stress at first peak or tangent modulus. Table 3.9 displays the descriptive statistics for these variables and the results of the paired-samples t-tests.

3.2.2.4 Per-sample cost of method

Once the glass jars are obtained for maceration, there are no further costs per sample since only tap water is required for this method.

3.2.2.5 Qualitative results

Adipocere was formed on all maceration samples. The earliest adipocere was observed was by day 27 of maceration. All bones were separated and all soft tissues removed during processing. Once complete, the bones had a bright white color and a strong offensive odor. This odor dissipated during the air-drying period, but was still present when sniffed at a close distance. Adipocere remained in the bone crevices of 3 samples. No damage was observed on the bones or the trauma marks of the maceration samples.

Table 3.8: Descriptive statistics for the pH at each check point of the maceration samples.

Check	N	Min.	Max.	Mean	SD
Day 3	5	5.76	7.64	6.54	.929
Day 6	5	5.47	7.39	6.28	.928
Day 9	5	5.56	7.33	6.35	.852
Day 12	5	5.76	7.14	6.42	.627
Day 15	5	5.46	7.48	6.42	.877
Day 18	5	6.51	8.54	7.12	.862
Day 21	5	7.36	7.43	7.39	.031
Day 24	5	7.02	8.86	7.58	.754
Day 27	5	7.17	9.04	7.67	.783
Day 30	5	7.31	7.35	7.33	.014
Day 33	5	7.42	7.97	7.70	.194
Day 36	3	6.91	7.22	7.09	.159
Day 39	3	7.09	7.09	7.09	.000
Day 42	3	7.17	7.47	7.36	.163
Day 45	3	7.36	7.36	7.36	.000
Day 48	3	7.44	7.59	7.51	.076
Day 51	2	7.33	7.55	7.44	.156
Day 54	2	7.76	8.29	8.03	.375
Day 57	1	9.24	9.24	9.24	-
Day 60	1	7.11	7.11	7.11	-
Day 63	1	7.39	7.39	7.39	-
Day 66	1	7.62	7.62	7.62	-
Day 69	1	7.48	7.48	7.48	-

Table 3.9: Descriptive statistics and paired-samples t-test values for the biomechanics tests on the maceration samples.

Variable	Pre or Post	N	Mean	SD	*t*	df	*p*
Strain at 1790N	Pre-processing	5	.283	.055	-3.046	4	.038*
	Post-processing	5	.373	.050			
Stress at 1st Peak	Pre-processing	5	25.015	12.156	2.634	4	.058
	Post-processing	5	9.049	5.431			
Tangent Modulus	Pre-processing	5	276.968	152.901	1.815	4	.144
	Post-processing	5	134.095	100.759			

* = statistical significance

3.2.3 **Plain water boil**

3.2.3.1 pH

The pH of the water for the plain water boil samples was recorded at the check point every hour. Values ranged from 5.50 to 9.86 (M = 8.51, SD = 1.115). Descriptive statistics for each check point are provided in Table 3.10.

Table 3.10: Descriptive statistics for the pH at each check point of the plain water boil samples.

Check	N	Min.	Max.	Mean	SD
1hr	5	5.50	7.25	6.44	.652
2hr	5	7.59	8.23	7.89	.280
3hr	5	8.30	9.21	8.62	.365
4hr	5	8.68	9.64	9.02	.436
5hr	5	8.90	9.75	9.28	.367
6hr	5	8.84	9.77	9.31	.397
7hr	2	9.68	9.86	9.77	.127

3.2.3.2 TTC

The plain water boil samples had a TTC of 6 to 7 hours (M = 6.60, SD = 0.548).

3.2.3.3 Biomechanics

The paired-samples t-tests for the plain water boiling samples found no significant difference between pre- and post-processing samples in strain at 1790N, stress at first peak, or tangent modulus. Table 3.11 displays the descriptive statistics for these variables and the results of the paired-samples t-tests.

Table 3.11: Descriptive statistics and paired-samples t-test values for the biomechanics tests on the plain water boil samples.

Variable	Pre or Post	N	Mean	SD	*t*	df	*p*
Strain at 1790N	Pre-processing	5	.296	.063	-.161	4	.880
	Post-processing	5	.302	.035			
Stress at 1st Peak	Pre-processing	5	31.173	14.358	-.477	4	.659
	Post-processing	5	31.980	13.828			
Tangent Modulus	Pre-processing	5	225.379	109.130	-.875	4	.431
	Post-processing	5	274.176	106.913			

3.2.3.4 Per-sample cost of method

Once the burners and pots for boiling are obtained, there are no further costs per sample since only tap water is required for this method.

3.2.3.5 Qualitative results

All of the bones were separated and all soft tissues removed during processing by plain water boiling. Once complete, the bones had a normal bone color and a slight greasy odor. Damage occurred to the medial cuneiform of 1 sample. The thin bone became soggy during processing and a hole formed in this bone. No other damage was observed on the bone or the trauma marks.

3.2.4 **Clorox®**

3.2.4.1 pH

The pH of the water for the Clorox® samples was recorded at the check point every hour. Values ranged from 7.31 to 10.05 (M = 8.93, SD = 0.684). Descriptive statistics for each check point are provided for the low concentration (Table 3.12), medium concentration (Table 3.13), and the high concentration samples (Table 3.14).

Table 3.12: Descriptive statistics for the pH at each check point of the low concentration Clorox® samples.

Check	N	Min.	Max.	Mean	SD
1hr	5	7.31	8.85	7.89	.641
2hr	5	7.67	9.27	8.39	.602
3hr	5	8.45	9.68	8.98	.452
4hr	5	8.74	9.64	9.17	.333
5hr	5	8.87	9.85	9.30	.353
6hr	5	9.03	9.92	9.36	.336
7hr	3	9.22	9.36	9.27	.078
8hr	1	9.39	9.39	9.39	-

Table 3.13: Descriptive statistics for the pH at each check point of the medium concentration Clorox® samples.

Check	N	Min.	Max.	Mean	SD
1hr	5	7.48	8.81	8.29	.580
2hr	5	7.68	9.49	8.52	.650
3hr	5	7.45	9.64	8.68	.823
4hr	5	7.73	9.65	8.86	.795
5hr	5	8.17	9.87	9.24	.674
6hr	5	8.30	9.87	9.31	.757
7hr	2	8.33	9.90	9.12	1.110

Table 3.14: Descriptive statistics for the pH at each check point of the high concentration Clorox® samples.

Check	N	Min.	Max.	Mean	SD
1hr	5	7.55	8.43	7.97	.336
2hr	5	8.21	9.27	8.82	.426
3hr	5	8.44	9.65	9.02	.462
4hr	5	9.03	9.68	9.36	.284
5hr	5	9.16	10.05	9.52	.367
6hr	5	8.95	9.97	9.41	.452
7hr	2	9.26	9.55	9.41	.205

3.2.4.2 TTC

The Clorox® samples took 6 to 8 hours to complete (M = 6.53, SD = 0.640). The low concentrations had a mean TTC of 6.80 hours (SD = 0.837), while both the medium and high concentrations had a mean TTC of 6.40 hours (SD = 0.548).

3.2.4.3 Biomechanics

The paired-samples t-tests for the Clorox® samples found a significant difference between pre- and post-processing samples in tangent modulus for the high concentration samples. The tangent modulus significantly decreased in these samples. There was no significant difference between pre- and post-processing samples in strain at 1790N or stress at first peak. Tables 3.15, 3.16, and 3.17 display the descriptive statistics for these variables and the results of the paired-samples t-tests.

3.2.4.4 Per-sample cost of method

Once the start-up equipment is obtained, the only ensuing cost to implement the method is the purchase of a sodium hypochlorite solution, such as Clorox®. The Clorox® used in this study was purchased in a 96oz. jug at a local department store for $3.19. Thus, it cost $0.26 per fluid cup (8 ounces) of Clorox®. Table 3.18 displays the cost per sample for low, medium, and high Clorox® concentrations.

3.2.4.5 Qualitative results

All of the bones were separated and all soft tissues removed during processing by Clorox®. Once complete, the bones had a normal bone color and a slight greasy odor. Damage in the form of cortical bone exfoliation was observed on the third day of air-drying on one of the high

Table 3.15: Descriptive statistics and paired-samples t-test values for the biomechanics tests on the low concentration Clorox® samples.

Variable	Pre or Post	N	Mean	SD	*t*	df	*p*
Strain at 1790N	Pre-processing	5	.277	.041	-1.448	4	.221
	Post-processing	5	.311	.033			
Stress at 1st Peak	Pre-processing	5	27.678	9.036	.401	4	.709
	Post-processing	5	25.181	8.992			
Tangent Modulus	Pre-processing	5	266.130	58.121	.868	4	.434
	Post-processing	5	219.470	84.147			

Table 3.16: Descriptive statistics and paired-samples t-test values for the biomechanics tests on the medium concentration Clorox® samples.

Variable	Pre or Post	N	Mean	SD	*t*	df	*p*
Strain at 1790N	Pre-processing	5	.402	.070	.958	4	.392
	Post-processing	5	.358	.093			
Stress at 1st Peak	Pre-processing	5	27.157	13.481	.435	4	.686
	Post-processing	5	23.601	15.967			
Tangent Modulus	Pre-processing	5	224.424	89.143	1.099	4	.333
	Post-processing	5	164.868	88.956			

Table 3.17: Descriptive statistics and paired-samples t-test values for the biomechanics tests on the high concentration Clorox® samples.

Variable	Pre or Post	N	Mean	SD	*t*	df	*p*
Strain at 1790N	Pre-processing	5	.272	.085	-.396	4	.712
	Post-processing	5	.283	.072			
Stress at 1st Peak	Pre-processing	5	34.900	11.362	1.489	4	.211
	Post-processing	5	23.844	18.365			
Tangent Modulus	Pre-processing	5	342.574	115.404	3.224	4	.032*
	Post-processing	5	199.115	137.368			

* = statistical significance

concentration samples. This particular sample was younger (placed in the 1-2 year age category) and exhibited juvenile bone tissue. No other damage was observed on the bone or the trauma marks.

Table 3.18: Cost of Clorox® method per-sample.

Concentration		Price per 1gal Solution	Price per 2gal Solution
Low	.5c/gal	$0.13	$0.26
Medium	1c/gal	$0.26	$0.53
High	2c/gal	$0.53	$1.06

3.2.5 **Sodium perborate (SPB)**

3.2.5.1 pH

The pH of the water for the SPB samples was recorded at the check point every hour. Values ranged from 9.94 to 10.61 (M = 10.15, SD = 0.132). Descriptive statistics for each check point are provided for the low concentration (Table 3.19), medium concentration (Table 3.20), and the high concentration samples (Table 3.21).

3.2.5.2 TTC

The sodium perborate (SPB) samples took 3 to 6 hours to complete (M = 4.73, SD = 0.799). The low concentrations had a mean TTC of 4.80 hours (SD = 0.837), while the medium concentrations had a mean TTC of 5.20 hours (SD = 0.447) and high concentrations had a mean of 4.20 hours (SD = 0.837).

Table 3.19: Descriptive statistics for the pH at each check point of the low concentration SPB samples.

Check	N	Min.	Max.	Mean	SD
1hr	5	10.12	10.16	10.15	.016
2hr	5	10.04	10.18	10.13	.054
3hr	5	10.00	10.14	10.09	.055
4hr	5	9.94	10.16	10.09	.085
5hr	2	10.09	10.14	10.12	.035
6hr	1	10.15	10.15	10.15	-

Table 3.20: Descriptive statistics for the pH at each check point of the medium concentration SPB samples.

Check	N	Min.	Max.	Mean	SD
1hr	5	10.12	10.14	10.13	.009
2hr	5	10.04	10.10	10.07	.024
3hr	5	9.99	10.07	10.03	.029
4hr	5	9.98	10.04	10.01	.024
5hr	5	10.00	10.07	10.03	.029
6hr	1	9.94	9.94	9.94	-
7hr	1	9.98	9.98	9.98	-

Table 3.21: Descriptive statistics for the pH at each check point of the high concentration SPB samples.

Check	N	Min.	Max.	Mean	SD
1hr	5	10.28	10.61	10.39	.129
2hr	5	10.26	10.38	10.31	.050
3hr	5	10.27	10.38	10.31	.050
4hr	4	10.26	10.34	10.29	.036
5hr	2	10.30	10.32	10.31	.014

3.2.5.3 Biomechanics

During the process of drilling the bone core samples, 2 medium concentration SPB post-processing cores were destroyed and unable to be tested. Thus, only 3 post-processing core samples were tested for the medium concentration of SPB, while there were 5 core samples tested for each of the other variations.

The paired-samples t-tests for the SPB samples found no significant difference between pre- and post-processing samples in strain at 1790N, stress at first peak, or tangent modulus. Tables 3.22, 3.23, and 3.24 display the descriptive statistics for these variables and the results of the paired-samples t-tests.

3.2.5.4 Per-sample cost of method

Once the start-up equipment is obtained, the only ensuing cost to implement the method is the purchase of the powdered SPB. The sodium perborate tetrahydrate used in this study was purchased online from a soap making supplies store (www.thechemistrystore.com). Multiple quantities of the chemical were available for purchase on this site. A 6lb pail was initially purchased, but did not contain enough chemical for the study, so a 33lb pail was also purchased.

The price per weight of the SPB was determined by the quantity purchased since the price varied based on the size of the pail. The 6lb pail cost $25.67, which equates to $4.28 per pound (or $0.009 per gram) of SPB. Whereas, the 33lb pail cost $75.79, which equates to only $2.30 per pound (or $0.005 per gram) of SPB. Both pail prices include shipping and handling charges. Table 25 displays the cost per sample for low, medium, and high SPB concentrations.

Table 3.22: Descriptive statistics and paired-samples t-test values for the biomechanics tests on the low concentration SPB samples.

Variable	Pre or Post	N	Mean	SD	*t*	df	*p*
Strain at 1790N	Pre-processing	5	.309	.103	-.029	4	.978
	Post-processing	5	.310	.046			
Stress at 1st Peak	Pre-processing	5	18.964	13.719	2.212	4	.091
	Post-processing	5	14.750	14.981			
Tangent Modulus	Pre-processing	5	203.361	151.116	1.849	4	.138
	Post-processing	5	138.327	82.900			

Table 3.23: Descriptive statistics and paired-samples t-test values for the biomechanics tests on the medium concentration SPB samples.

Variable	Pre or Post	N	Mean	SD	*t*	df	*p*
Strain at 1790N	Pre-processing	3	.215	.015	-3.568	2	.070
	Post-processing	3	.277	.029			
Stress at 1st Peak	Pre-processing	3	31.304	9.059	2.453	2	.134
	Post-processing	3	12.571	6.921			
Tangent Modulus	Pre-processing	3	333.420	26.937	3.782	2	.063
	Post-processing	3	169.133	48.677			

Table 3.24: Descriptive statistics and paired-samples t-test values for the biomechanics tests on the high concentration SPB samples.

Variable	Pre or Post	N	Mean	SD	*t*	df	*p*
Strain at 1790N	Pre-processing	5	.236	.027	-2.303	4	.083
	Post-processing	5	.264	.020			
Stress at 1st Peak	Pre-processing	5	27.377	13.266	2.525	4	.065
	Post-processing	5	15.555	15.034			
Tangent Modulus	Pre-processing	5	237.954	52.576	1.707	4	.163
	Post-processing	5	179.737	88.908			

Table 3.25: Cost of sodium perborate method per sample.

Pail of SPB	Concentration		Price per 1 gal Solution	Price per 2 gal Solution
6lb Pail	Low	.75c/gal	$1.25	$2.49
	Medium	1.5c/gal	$2.49	$4.98
	High	3c/gal	$4.98	$9.96
33lb Pail	Low	.75c/gal	$0.66	$1.33
	Medium	1.5c/gal	$1.33	$2.65
	High	3c/gal	$2.65	$5.30

3.2.5.5 Qualitative results

All of the bones were separated and all soft tissues removed during processing by SPB with a few exceptions. The distal phalanges of digits 2 and 5 were not able to be removed from their hooves in 1 low concentration sample. Additionally, a small amount of remnant ligaments remained on some of the sesamoid bones of 2 low concentration samples. Once complete, the bones were light in color and had a slight greasy odor. All hooves were bleached and lighter in color. No damage was observed on the bone or the trauma marks.

3.2.6 **Biz®**

3.2.6.1 pH

The pH of the water for the Biz® samples was recorded at the check point every hour. Samples which were not complete by 8 hours were left in the solution to soak for 12 hours, and the processing and check points resumed the following day at hour 20. Values ranged from 10.20 to 11.62 (M = 10.94, SD = 0.206). Descriptive statistics for each check point are provided for the low concentration (Table 3.26), medium concentration (Table 3.27), and the high concentration samples (Table 3.28).

Table 3.26: Descriptive statistics for the pH at each check point of the low concentration Biz® samples.

Check	N	Min.	Max.	Mean	SD
1hr	5	10.99	11.07	11.04	.034
2hr	5	10.94	10.99	10.97	.020
3hr	5	10.85	10.98	10.93	.054
4hr	5	10.79	10.89	10.85	.039
5hr	5	10.78	10.83	10.81	.021
6hr	5	10.73	10.81	10.78	.037
7hr	5	10.69	10.78	10.74	.037
8hr	5	10.68	10.80	10.73	.045
20hr	3	10.84	10.90	10.87	.031
21hr	1	10.95	10.95	10.95	-

Table 3.27: Descriptive statistics for the pH at each check point of the medium concentration Biz® samples.

Check	N	Min.	Max.	Mean	SD
1hr	5	10.20	11.12	10.74	.365
2hr	5	10.86	11.05	10.92	.078
3hr	5	10.85	11.25	11.04	.183
4hr	5	10.85	11.48	11.09	.286
5hr	5	10.82	11.56	11.10	.344
6hr	5	10.81	11.61	11.10	.381
7hr	5	10.80	11.62	11.09	.388
8hr	5	10.79	11.61	11.08	.397
20hr	2	10.73	10.93	10.83	.141

Table 3.28: Descriptive statistics for the pH at each check point of the high concentration Biz® samples.

Check	N	Min.	Max.	Mean	SD
1hr	5	11.04	11.15	11.10	.042
2hr	5	10.98	11.01	11.01	.027
3hr	5	10.94	10.97	10.97	.046
4hr	5	10.91	10.94	10.94	.038
5hr	5	10.89	10.93	10.91	.015
6hr	5	10.85	10.87	10.86	.010
7hr	5	10.83	10.86	10.84	.014
8hr	5	10.81	10.83	10.82	.012
20hr	3	11.03	11.03	11.03	-

3.2.6.2 TTC

The Biz® samples took 8 to 21 hours to complete (M = 12.73, SD = 6.296). The low concentrations had a mean TTC of 15.40 hours (SD = 6.768), while the medium concentrations had a mean of 12.80 hours (SD = 6.573) and high concentrations a mean of 10.00 hours (SD = 5.612). Three samples were not complete within one 8 hour work day and were left to soak for 12 hours overnight. Processing resumed on the preceding morning at hour 20.

3.2.6.3 Biomechanics

The paired-samples t-tests for the Biz® samples found no significant difference between pre- and post-processing samples in strain at 1790N, stress at first peak, or tangent modulus. Tables 3.29, 3.30, and 3.31 display the descriptive statistics for these variables and the results of the paired-samples t-tests.

3.2.6.4 Per-sample cost of method

Once the start-up equipment is obtained, the only ensuing cost to implement the method is the purchase of the laundry detergent. The powdered form of Biz® used in this study was purchased in a 5lb (80oz) box at a local department store for $5.99, which worked out to be a cost of $0.51 per cup of the detergent. Table 3.32 displays the cost per sample for low, medium, and high Biz® concentrations.

3.2.6.5 Qualitative results

Most of the bones were separated during processing by Biz®. Some distal phalanges were not able to be removed from their hooves, but no other ligaments remained. Once complete, the

Table 3.29: Descriptive statistics and paired-samples t-test values for the biomechanics tests on the low concentration Biz® samples.

Variable	Pre or Post	N	Mean	SD	*t*	df	*p*
Strain at 1790N	Pre-processing	5	.260	.067	-.839	4	.449
	Post-processing	5	.283	.084			
Stress at 1st Peak	Pre-processing	5	33.468	11.757	2.522	4	.065
	Post-processing	5	23.517	8.683			
Tangent Modulus	Pre-processing	5	328.650	102.672	1.385	4	.238
	Post-processing	5	274.548	108.808			

Table 3.30: Descriptive statistics and paired-samples t-test values for the biomechanics tests on the medium concentration Biz® samples.

Variable	Pre or Post	N	Mean	SD	*t*	df	*p*
Strain at 1790N	Pre-processing	5	.289	.037	.347	4	.746
	Post-processing	5	.279	.063			
Stress at 1st Peak	Pre-processing	5	18.424	2.927	-1.307	4	.261
	Post-processing	5	24.570	10.531			
Tangent Modulus	Pre-processing	5	201.028	30.032	-1.235	4	.284
	Post-processing	5	271.860	139.215			

Table 3.31: Descriptive statistics and paired-samples t-test values for the biomechanics tests on the high concentration Biz® samples.

Variable	Pre or Post	N	Mean	SD	*t*	df	*p*
Strain at 1790N	Pre-processing	5	.288	.046	-.517	4	.633
	Post-processing	5	.306	.069			
Stress at 1st Peak	Pre-processing	5	33.773	13.796	1.283	4	.269
	Post-processing	5	21.708	12.060			
Tangent Modulus*	Pre-processing	5	284.358	102.386	1.368	4	.243
	Post-processing	5	204.724	52.280			

Table 3.32: Cost of Biz® method per sample.

Concentration		Price per 1 gal Solution	Price per 2 gal Solution
Low	.75c/gal	$0.38	$0.76
Medium	1.5c/gal	$0.76	$1.53
High	3c/gal	$1.53	$3.05

bones had a normal bone color and a slight greasy odor. No damage was observed on the bone or the trauma marks.

3.3 COMPARISON OF DEFLESHING METHODS

3.3.1 Time-to-completion (TTC)

Time-to-completion (TTC) was defined as the time from when the remains were added to the processing treatment (water, chemical solution, enzyme solution, or dermestid tank) until they were deemed "complete" by the researcher and was measured in hours and days. Maceration and dermestid methods took days or months to complete, while the other methods took less than 24 hours to complete. The complete descriptive statistics for TTC were previously listed in Table 3.5.

A one-way between-subjects analysis of variance was not able to be applied to the TTC data to compare the defleshing treatments, as the data violated many of the essential assumptions of the test (see Appendix B). Transformations were not able to normalize the data since the distributions for the 12 defleshing treatments were oppositely skewed.

Due to the fact that the TTC data violated multiple assumptions of analysis of variance, a Kruskal-Wallis H test was chosen as a non-parametric alternative. Distributions of TTC data

were not similar for all groups, as assessed by visual inspection of a boxplot. Thus, the distribution of mean ranks were compared rather than medians. A statistically significant difference in the mean ranks of TTC among the 12 defleshing treatments was found (χ^2 (11) = 54.187, $p < 0.001$).

Because there was such a large difference in TTC of the dermestid and maceration methods, which took days to months to complete, while the rest of the methods took less than 24 hours to complete, the dermestid and maceration samples were treated as outliers and removed from the analysis to tease out any differences among the quicker acting heated treatments. A second Kruskal-Wallis H test was completed on the TTC of just the plain water boil, 3 Biz® concentrations, 3 Clorox® concentrations, and 3 sodium perborate concentrations. A statistically significant difference in the mean ranks of TTC among these 10 defleshing treatments was found (χ^2 (9) = 42.077, $p < 0.001$). Table 3.33 provides the mean ranks in TTC for these defleshing treatments.

Pairwise comparisons were performed with a Bonferroni correction for multiple comparisons. This post hoc analysis revealed statistically significant differences in the mean ranks in TTC between most of the SPB and Biz® samples. These defleshing methods consisted of the shortest and longest times to completion, respectively, when dermestid and maceration samples are removed. Table 3.34 displays the comparisons with statistically significant differences and their respective *p*-values.

3.3.2 **Dry weight**

The descriptive statistics for the dry weight of the deer limb samples after defleshing treatment and 3 days of air-drying are displayed in Table 3.35. The dry weight of all the deer limb samples

Table 3.33: Mean ranks in TTC with dermestid and maceration samples removed.

Method	Concentration Variation	N	Mean Rank
Boil	NA	5	26.40
Clorox®	Low	5	28.30
	Medium	5	24.10
	High	5	24.10
SPB	Low	5	9.10
	Medium	5	11.50
	High	5	5.40
Biz®	Low	5	45.00
	Medium	5	43.10
	High	5	38.00
Total		60	

Table 3.34: Defleshing treatments with statistically significant differences in TTC mean ranks.

Comparison		*p*
Low SPB	Low Biz®	.003
	Medium Biz®	.008
Medium SPB	Low Biz®	.010
	Medium Biz®	.022
High SPB	Low Biz®	.001
	Medium Biz®	.001
	High Biz®	.014

were taken, so the sample size for this variable is 60. The dry weights ranged from 86.50g to 253.00g (M = 166.602, SD = 43.094).

A one-way between-subjects analysis of variance was performed on the end dry weight of the deer samples to assess whether the samples differed in weight among the 12 defleshing treatments once processing was complete and the samples had air-dried for 3 days. The analysis of variance showed no significant difference in the dry weight of the deer samples among the 12 defleshing treatments, $F(11, 48) = .885$, $p = 0.561$.

Due to the fact that no significant difference was found in start weight or in dry weight among the defleshing treatments, further analysis, such as a repeated measures ANOVA, was not conducted.

Table 3.35: Descriptive statistics for the dry weight of the deer limb samples.

Method	Concentration Variation	N	Mean Weight (grams)	Std. Dev.	Std. Error
Dermestids	NA	5	163.020	55.412	24.781
Maceration	NA	5	163.620	62.834	28.100
Boil	NA	5	151.180	37.347	16.702
Clorox®	Low	4	166.040	49.569	22.168
	Medium	5	137.720	27.135	12.135
	High	4	178.980	59.905	26.791
SPB	Low	5	134.200	31.849	14.243
	Medium	5	172.180	29.299	13.103
	High	5	181.340	46.811	20.935
Biz®	Low	5	190.020	44.889	20.075
	Medium	5	190.380	23.730	10.612
	High	5	169.540	30.617	13.692
Total		60	166.602	43.094	5.563

4.0 DISCUSSION

4.1 SUMMARY AND INTERPRETATION OF RESULTS

4.1.1 Goals and Hypotheses

This study evaluated the effectiveness, efficiency, and destructiveness of 6 common defleshing methods on the distal segment of white-tailed deer (*Odocoileus virgineanus*) hind limbs. Qualitative and quantitative assessments were conducted in order to test the basic null hypothesis that the 6 different defleshing treatments do not significantly differ among the variables considered. This section provides a summary of the results for each variable examined and compares these results among the defleshing treatments. The results of this study are then compared to findings and statements made within the defleshing literature to recognize how the current results differ from or confirm previous findings.

4.1.2 Sample Composition

The deer limbs used in this study were collected from a single deer processor over 2 days, during a period when both doe and buck were in season. The age-at-death and sex of the deer were not able to be controlled for during sample collection as the leg segments had already been severed from the rest of the body by the processor. However, the age of the deer was estimated by

assessing the epiphyseal fusion of the metatarsal and phalanges and size was accounted for by recording the weight of each sample prior to processing.

Several variables were considered in order to determine if the deer samples significantly differed from one another prior to processing. The anatomical side of the limb, estimated age-at-death, and start weight of the remains were compared among the defleshing treatments. A nearly equal amount of right and left deer limbs were used in this study and the majority (93.3%) of the deer were estimated to be adult (> 1 year old). There were 4 sub-adult remains in the sample, which, unfortunately by chance, were placed in 2 defleshing treatments (maceration and plain boil) instead of being more evenly distributed within the sample.

The weight of the deer limb samples prior to processing did not differ significantly among the defleshing treatments. Thus, even though there was a mixture of ages and sexes that were unable to be accounted for prior to defleshing, the limb samples were distributed evenly among the defleshing treatments such that they did not statistically differ in weight.

4.1.3 **Efficiency**

4.1.3.1 Time-to-completion

The efficiency of each defleshing method was assessed by computing the time-to-completion (TTC) for each sample. The TTC results were then compared using a Kruskal-Wallis test. Maceration was the longest method, requiring over a month (33-69 days) to completely clean the bones. Dermestids required 3-7 days to complete, and the remaining methods, which used a heated water treatment, were completely cleaned in less than 24 hours. All of the heated methods were able to be completed within an 8 hour work day, except for 6 of the Biz® samples (3 low, 2 medium, and 1 high concentration). These 6 Biz® samples required an overnight soak

in the solution after 8 hours of processing. Five of these samples were complete after the 8-hour overnight soak, but 1 low concentration sample required an additional hour of heat treatment after the soaking process.

A post hoc analysis comparing the heated defleshing treatments revealed statistically significant differences in the mean ranks in TTC between most of the SPB and Biz® samples. These defleshing methods consisted of the shortest and longest times to completion, respectively, when dermestid and maceration samples were removed from the analysis.

4.1.3.2 Per-sample cost of methods

The efficiency of each defleshing method was also assessed by considering the monetary cost to deflesh a single sample (the per-sample cost of the method). Costs involved with procuring the equipment required to implement each method, such as burners and boiling pots, were not considered in this study. Thus, defleshing methods that have no costs to implement once the respective start-up equipment has been obtained were considered to have no per-sample cost. For example, the dermestid, maceration, and plain water boiling methods had no per-sample cost to defleshing. The dermestid method utilized an existing dermestid colony at the Carnegie Museum of Natural History (CMNH) and no additional equipment was required for the beetles to deflesh the deer samples. Similarly, once the maceration jars, pots, and burners were obtained for the maceration and plain water boil methods, only tap water was required to deflesh each sample.

The only cost involved in implementing the remaining defleshing methods tested was the chemicals or enzymes added to the heated water. The cost of each of these methods is directly proportional to the concentration of the chemical or enzyme solution used and varies based on

the quantity of the chemical or enzyme purchased. For example, purchasing a bulk quantity of sodium perborate reduced the per-sample cost to implement the method by nearly half.

Of the defleshing methods with per-sample costs, Clorox® was the cheapest to implement. The Biz® cost nearly 3 times as much as Clorox® per-sample and the most expensive method, sodium perborate, cost approximately 5 times as much as Clorox® when purchased in bulk. Sodium perborate cost nearly 10 times as much as Clorox® to implement per-sample when smaller quantities of the chemical were purchased.

4.1.4 **Effectiveness**

4.1.4.1 Soft tissue remnants

The effectiveness of the methods was measured qualitatively by the amount of tissues remaining on the bones and the condition (color, odor, and greasiness) of the bones after processing. Most all of the defleshing treatments separated all of the bones of the distal hind limb and removed all soft tissues. When soft tissues were not able to be removed or digested, they tended to be the ligaments attaching the distal phalanges to their respective hooves, especially of the 2nd and 5th digits and/or the thick flexor tendons attached to the sesamoid bones.

Though in very limited quantities in the limb segments used, muscle tissue was the most easily removed soft tissue and was able to be removed from all bones in the study. Muscle tissue is easily degraded by bacterial activity in maceration and by heat, oxidation, and enzymatic hydrolysis in the heated solutions. Muscle tissue is also easily and readily ingested by the dermestids. The beetles prefer this protein source to the tougher collagen-dense tissues, which they tend to ingest only after the muscle tissue has been consumed.

Tendons and ligaments, which are comprised of dense regular connective tissue, were more difficult to degrade or ingest, and thus, took longer to remove. In some samples, remnants of tendons or ligaments remained on the bones. There were remnants of tendons and ligaments adhering on some bones of all of the dermestid samples. Two samples cleaned by a low concentration of sodium perborate had adhering remnant soft tissues by the end of processing, as did 4 samples cleaned by Biz® (2 low, 1 medium, and 1 high concentration samples).

4.1.4.2 Bone condition

The condition of the processed bones was subjectively assessed in this study by the greasiness, color, and smell of the cleaned bones of each sample. Each of these variables were dependent on the amount of grease remaining within the bones after processing. The dermestid method yielded the greasiest bones since no solvent was used to leach out the grease. So, once clean, these bones were dark yellow to orange in color from the grease and emitted a strong greasy odor. All of the other defleshing treatments degreased the bones to some degree and had no odor unless sniffed at a close distance.

Maceration produced the whitest bones, but an offensive odor was evident at a close distance and adipocere remained within various crevices in the bones. Defleshing via plain water boil, Clorox®, and Biz® produced bones with a normal bone color and a slight greasy odor, while SPB produced lighter colored bones with only a very slight greasy odor.

4.1.5 **Destructiveness**

4.1.5.1 Macroscopic Bone Damage

The destructiveness of the methods was assessed by examining the bones for macroscopic damage after processing. Steadman and colleagues' (2006) ordinal scale of macroscopic bone damage was to be implemented in this study to compare bone quality post-processing, but all samples except 1 were scored as 5 (strong, normal bone texture and quality), so the results of this comparison were not assessed any further.

Macroscopic bone damage was observed on 2 dermestid-cleaned samples, 1 plain water boiled sample, and on 1 Clorox® sample. The 2 dermestid-cleaned samples each exhibited holes on the proximal surface of the metatarsal through the thinnest cortical bone. These holes were likely due to the beetles eating through the bone to gain access to the marrow in the medullary cavity.

A small hole was also discovered on the medial cuneiform of a sample cleaned by plain water boiling. This hole was also located through the thinnest area of cortical bone, likely due to a softening of the bone during the boiling process. It is unknown whether this hole was formed while boiling or if it was caused by the methods used to remove the soft tissues during the hourly check points. Damage in the form of cortical bone exfoliation was observed on 1 sample cleaned by a high concentration of Clorox® after the third day of air-drying. This damage may indicate that the chemicals comprising Clorox®, such as the sodium hypochlorite or the sodium hydroxide, may continue to affect the bone tissue after processing and rinsing.

4.1.5.2 Erosion of Trauma Marks

The destructiveness of the methods was also assessed by examining the inflicted cut and saw marks prior to and following processing to determine if the fine marks had been altered due to the various defleshing treatments. No erosion was evident on any of the trauma marks in this study using the stereomicroscope and close-up photography. These comparisons, however, were rudimentary. Methods using photo superimposition or scanning electron microscopy (SEM) may be more likely to discern minor alterations in fine trauma marks which could affect a trauma analysis of remains from a human forensic case. Mairs and colleagues (2004) used environmental SEM (ESEM) and found no erosion of trauma marks on 2 human dismemberment cases after processing with the enzymatic laundry detergent, Persil®, but other defleshing treatments have not been similarly tested.

4.1.5.3 Mechanical Properties

Mechanical testing of bone core samples taken prior to and following defleshing treatment provided an additional assessment of the possible destructiveness of the defleshing methods by quantifying changes in the structural integrity of the bone tissue. A cylindrical bone core sample was extracted from the lateral side of the proximal metatarsal prior to processing and from the medial side following processing using a drill press. Two medium concentration sodium perborate post-processing core samples were destroyed during extraction and were unable to be used in the mechanical tests.

The bone core samples were compressed to the maximum capacity of the Instron (1790N) or to 50% of the caliper thickness of the bone core at a strain rate of 0.1 mm/min. Only 2 samples (both pre-processing medium concentration Clorox®) reached 50% of the sample thickness. The rest of the samples were tested to 1790N. The force and deformation were

recorded and normalized to stress and strain. Three mechanical variables, strain at 1790N, stress at first peak, and tangent modulus, were considered for each sample and then pre- and post-processing values were compared via paired samples t-tests.

Strain at 1790N

Since strain is the amount of deformation of the bone core relative to its original thickness, the strain at 1790N reflects the compressibility of the sample. The larger the strain value, the more the sample was compressed.

In this study, the strain at 1790N was higher in the post-processing samples than in the pre-processing samples for all of the defleshing treatments except for the medium concentrations of Clorox® and Biz®, where the results were opposite. The differences between pre- and post-processing samples were only statistically significant for the maceration method, though. The post-maceration samples had a statistically higher strain (M=0.378, SD=0.049) than the pre-maceration samples (M=0.289, SD=0.059) at 1790N. Thus, the post-maceration bone cores were compressed more during mechanical testing, indicating that the maceration process significantly weakened the bone tissue. This alteration in structural integrity is likely due to the fact that these bones were submerged in water for over a month.

Stress at First Peak

The stress at the first peak on the stress-strain curve reflects the strength of the sample. A peak on the stress-strain curve depicts either when a bone core fractured before the sample reached the maximum load of 1790N or if the sample did not fracture before reaching maximum load. Thus, the stress at first peak indicates the amount of stress required to initially fracture the sample, so the larger the stress value at this point, the stronger the sample. In this study, the

stress at first peak tended to be lower in the post-processing samples than in the pre-processing samples. This indicates that there was a trend of decreased strength in the post-processing samples, though there was a slight increase in stress at first peak in the post-processed plain water boil samples and medium concentration of Biz® samples. However, none of the defleshing methods exhibited a statistically significant difference between pre- and post-processing bone core samples in stress at first peak.

Tangent Modulus

The tangent modulus is the slope of the compression stress-strain curve and reflects the stiffness of the sample. The larger the modulus value, the stiffer the sample. In all of the defleshing treatments, except for the plain water boil and medium concentration of Biz® treatments, the tangent modulus was lower in the post-processing samples than in the pre-processing samples. This indicates a trend of a post-processing decrease in bone stiffness, though only samples defleshed via dermestids and a high concentration of Clorox® had a statistically significant difference between pre- and post-processing bone core samples in tangent modulus. In both cases the tangent modulus significantly decreased the stiffness of the bone after processing.

4.1.6 **pH of Water Solutions**

The pH of all of the defleshing methods utilizing water was recorded at each check point in order to make assumptions of possible effects on bone tissue. The assumptions were that if a solution became acidic, damage could occur to the inorganic components of the bone tissue and if a solution became alkaline, damage could occur to the organic components of the bone tissue.

These assumptions are only approximate since the muscle tissue itself after death is slightly acidic due to the anaerobic breakdown of muscle glycogen, which produces lactic acid. Lactic acid build-up is even greater in animals struggling at the time of death, which is possible of hunted deer that comprised the samples in this study (Romans et al. 1994).

The water in the maceration samples remained approximately neutral, which were the lowest pH values and mean pH. Biz® solutions were the most alkaline solutions with the highest pH values and mean pH.

4.2 COMMENTS ON DEFLESHING METHODS

Table 4.1 presents a summary of the results for the 6 defleshing methods so that easy comparisons can be made.

4.2.1 Dermestids

4.2.1.1 Efficiency

Since the efficiency of the dermestid beetle method is largely temperature-dependent, the ambient temperature was recorded during the study. The temperatures recorded during the times the CMNH dermestid colony was in use for this study were optimal for defleshing purposes, with an average high of 30.2°C (86.1°F) and an average low of 20.8°C (69.5°F). All of the dermestid samples were completed within a week (3-7 days). The dermestid method involved the least amount of manual labor, as the only labor involved in this method was the gentle separation of the loosened joints at the check points to expose more soft tissues for the beetles.

Table 4.1: Comparison of results for all variables.

		Dermestids	Maceration	Plain Boil	Clorox	SPB	Biz
Efficiency	**Cost**	0	0	0	$ ($0.53)	$$$ ($2.65)	$$ ($1.53)
	TTC	3-7 days M=5.6 SD=1.95	33-69 days M=47.4 SD=15.21	6-7 hours M=6.53 SD=0.55	6-8 hours M=6.53 SD=0.64	3-6 hours M=4.73 SD=0.80	8-21 hours M=12.73 SD=6.30
Effectiveness		5	0	0	0	3 (low)	4 (2 low, 1 med, 1 high)
Destructiveness	**Macroscopic**	2	0	1	1 (high)	0	0
	Mechanics	↓ Tangent Modulus (↓ stiffness)	↑ Strain @ 1790 (↑ compressibility)	No significant change	↓ Tangent Modulus (↓ stiffness) *High concentration only	No significant change	No significant change

Cost = cost to implement the method per sample (0 = $0, $ = least expensive, $$ = intermediate expense, $$$ = most expensive).
TTC = time-to-completion, measured in days or hours.
Effectiveness = the number of samples with soft tissues remaining on the bones after processing (specific concentrations are provided in parentheses).
Macroscopic = the number of samples exhibiting a form of macroscopic bone damage (specific concentrations are provided in parentheses).
Mechanics = statistically significant change in a bone mechanics variable post-processing.

The per-sample cost of this method was considered to be $0 since only the established beetle colony was used in defleshing these samples. There are costs to start-up and maintain a dermestid colony, however these costs tend to be quite low. Dermestid beetles can be collected for free from carcasses in nature, or can be purchased from online distributers (Graves 2005; Knudsen 1966). Miscellaneous items used in maintaining a dermestid colony are common products that can be obtained many times for free. For example, Stephen Rogers of the CMNH uses shallow cardboard boxes obtained from products purchased for his home to place remains in the dermestid aquarium. Cotton is required to provide a place for pupation. This cotton may be purchased cheaply, or obtained for free from old mattresses or jewelry boxes.

Additionally, a steady supply of food is required which provides enough nutritional value to properly sustain and propagate the colony. If there is time between remains that need to be defleshed, the dermestids can be fed raw beef or dog/cat food (Weichbrod 1987).

4.2.1.2 Effectiveness

The dermestids ingested all of the skeletal muscle tissue from the samples, but remnants of tendons and ligaments remained on some of the bones of every sample. The dermestids did not ingest the ligaments which attach the distal phalanges of digits 2 and 5. It is possible that these small hooves could have been removed if they remained in the aquarium longer, but more damage to thin cortical bone would likely occur on other bones from the dermestids searching for more food. A remedy to reduce this risk is to remove the cleaned bones from the colony and to move the bones with remaining soft tissues to a smaller container with fewer beetles. Warmed bacon or beef fat, cod-liver, or vegetable oil may be brushed onto the remaining soft tissues,

particularly if they are very dry by this point, to make them more appetizing to the beetles (Hooper 1956; Laurie and Hill 1951).

The dermestid method yielded the greasiest bones since no solvent was used to leach out the grease. These bones, when complete, were dark yellow to orange in color from the grease and emitted a strong greasy odor. Many preparators who use this method of defleshing employ a post-processing degreasing and whitening step using ammonia (Bemis et al. 2004; Borell 1938; Grayson and Maser 1978; Hall and Russell 1933; Hooper 1950; Sommer and Anderson 1974; Tiemeier 1939; Weichbrod 1987), trichloroethylene (Bemis et al. 2004; Sommer and Anderson 1974), or hydrogen peroxide (Coleman and Zbijewska 1968; Graves 2005). These additional treatments could have detrimental effects on the bone tissue, but, as in the defleshing literature, little is published on this topic.

4.2.1.3 Destructiveness

Macroscopic damage was observed on the proximal articular surface of the metatarsal of 2 samples. Both of these bones exhibited a small hole through the thinnest area of cortical bone and were likely caused by the beetles eating through the thin bone to get to the marrow inside. No alterations were observed in the trauma marks and there was no significant change in strain at 1790N or in stress at first peak between pre- and post-processing samples.

However, the tangent modulus significantly decreased from pre- to post-dermestid processing, indicating that the bone had become less stiff post-processing with dermestids. The exact reason for this significant decrease in the stiffness of the bone is unknown. One possible explanation is that dermestids may secrete digestive enzymes in their waste or saliva that could affect bone tissue. Conversely, though, Caldeira et al. (2007) explained that most of the dermestid lava's digestive enzymes are reabsorbed by the gut before being excreted. Another

possible explanation is that the second freezing process required in the dermestid method to kill the remaining beetles hiding in the bones may cause additional microfractures due to the expansion of the freezing fluids within the bone, which may then reduce the stiffness of the bone tissue. Some researchers have found that freezing can alter the mechanical properties of bone (Cowin 2001; Kang and Kim 1995), but most agree that very little to no changes occur in the mechanical properties of bone due to freezing at typical freezing temperatures, i.e. 0°C to -20°C (Cowin 2001; Pelker et al. 1984). Even multiple freeze-thaw cycles have been shown to not significantly affect the stiffness of bone tissue (Kang et al. 1997). Therefore, it is unclear why the dermestid samples within this study demonstrated a decreased stiffness after processing. A future study could include extracting the post-dermestid processing bone core sample prior to the extra freezing step and comparing subsequent mechanical testing results to the results of this study in order to determine whether the cause of the decreased bone stiffness was the beetles themselves or the additional freezing step to de-bug the remains.

4.2.1.4 Pros, Cons, and Comments

The dermestid method is popular with museum curators of vertebrate collections due to the little amount of manual labor involved, low cost, effectiveness, and the ability to produce ligamentary skeletons if desired (Causey and Trimble 2005). Another major advantage is that the method itself does not require the bones to be exposed to water or harsh chemicals (Williams 1992), but the resulting dermestid-cleaned bones typically require additional preparation steps before the remains are able to be displayed or added to a research collection.

A “de-bugging” step is necessary to prevent infestation of the laboratory from beetles hiding within the bones after removal from the colony. The de-bugging process could involve subjecting the bones to temperature extremes (heat or freezing) or dipping them in ethanol, but

these processes are desiccating and may degrade lipids and proteins, altering the structure of the bone (Williams 1991). Williams (1991) recommends the bones be placed in an air-tight container, such as a glass vial with a rubber stopper, for a 3 week quarantine period. He claims that this time period is based on the life cycle of *Dermestes* and is sufficient to kill larvae hatching from eggs laid on the bones. Though advantageous because the bones are not subjected to possibly damaging temperature extremes or chemicals, this method is only easily implemented on small remains and not feasible on large remains, such as an adult human. The additional degreasing step that many preparators employ due to the greasiness of the cleaned bones, may also cause alterations to the bone tissue that could affect the future research value of the remains.

Furthermore, the preparator must keep careful watch over the colony while they are defleshing remains as dermestids are "eating machines" and will eat through bone once the soft tissues are gone (Graves 2005, p.35). This type of damage was observed on the metatarsals of 2 of the 5 dermestid samples.

4.2.2 **Maceration**

4.2.2.1 Efficiency

The mean pH of the maceration water remained neutral (M = 7.08, SD = 0.839), which is optimal for most microorganisms to thrive (Romans et al. 1994) . However, all maceration samples required over a month to complete, making maceration the slowest defleshing method assessed in this study. The per-sample cost of this method was considered to be $0 since simply tap water was used in defleshing these samples once the glass maceration jars were purchased. The microorganisms already on the remains before placing them in the water act to degrade the soft tissues. Placing the remains in the water reduces the decomposition odor, especially if the

maceration container has a lid and is placed under a fume hood, and facilitates the action of anaerobic bacteria.

4.2.2.2 Effectiveness

All bones were separated and all soft tissues removed during processing. Maceration produced the whitest bones. The macerated bones were not greasy, as most of the grease leached out during the lengthy maceration process and, though strong offensive odors were produced during processing, these smells dissipated from the bones of this study during the 3 day air-drying period, as asserted by Hamon (1964).

Saponification of the lipids occurred during processing of all of the maceration samples. The adipocere produced remained within crevices of the bones post-processing. Adipocere is difficult to remove from small crevices and can obscure small features in the bone that could hold potential evidentiary value.

4.2.2.3 Destructiveness

There was no macroscopic damage or erosion of the trauma marks observed on any of the maceration-cleaned samples. However, the structural integrity of the bone was altered after maceration. There was as significant increase in the strain at 1790N, so the bone samples compressed more after maceration. This increase in compressibility is conceivably due to the lengthy soak in water. No other significant change in mechanical properties was observed.

4.2.2.4 Pros, Cons, and Comments

The maceration method was effective at cleaning the bones and required little manual labor. It did not require a full day of tending as did the defleshing methods involving heated

water solutions. The resulting bones were white, degreased, and did not preserve the strong, foul odor that is famously produced during this method. Although this smell is not retained in the dry bones and does not permeate the laboratory if placed under a fume hood in a container with a lid, the preparator must handle the pungent odor every 2-4 days for at least a month when the water is to be poured off and changed, making this a very undesirable task.

Another disadvantage to this method is the saponification of the soft tissues that occurs during maceration. The adipocere that collects in the small crevices of the bone is difficult to remove and can obscure details of the bone necessary for analysis. Adding a post-processing step using a cleaning solution with lipases could aid in removing the adipocere. There are also additional health risks when employing this method since maceration facilitates the growth of anaerobic microorganisms and no heat or other disinfecting agents are implented. Therefore, health precautions should be taken when handling the maceration water and the remains. For example, a face shield, gloves, and protective clothing such as a vinyl apron should be used when pouring the maceration water from the jar and handling the remains.

Though no harsh chemicals are utilized in this method, the long soak in water can be considered a potentially damaging agent. This study found that maceration significantly increased the compressibility of the bone samples. So, although no macroscopic damage was observed on the bones or trauma marks, mechanical testing reveals that processing via maceration alters the mechanical properties of bone tissue.

The time requirement involved in maceration is a major concern in a laboratory where remains must be processed quickly. Maceration is also not optimal when there is a steady supply of large remains, such as humans, to deflesh.

4.2.3 **Plain Water Boil**

4.2.3.1 Efficiency

All of the samples boiled in plain water were complete in 6-7 hours, which did not statistically differ from the TTC of the other defleshing methods involving heat. The per-sample cost of this method was considered to be $0 since only tap water was used in defleshing these samples once the start-up equipment, such as pots and burners, were purchased. The boiling temperature alone acted to degrade the proteins of the soft tissues, allowing them to be picked off by hand.

4.2.3.2 Effectiveness

All of the bones were separated and all of the soft tissues were removed during processing. The resulting bones were similar in color and smell to the bones cleaned via the Clorox® and Biz® methods.

4.2.3.3 Destructiveness

There was no observable erosion of the trauma marks or significant change in the mechanical properties of the bone detected after boiling. However, there was a macroscopic hole observed on the medial cuneiform of one of the samples. This hole formed on the thinnest area of cortical bone and was likely due to the softening of the bone during the boiling process.

4.2.3.4 Pros, Cons, and Comments

Boiling in plain water was found to be an effective and efficient method, as it produced clean bones relatively quickly; the remains were complete within hours versus days or months.

No harsh chemicals were used, but the method involves the use of a high temperature (100°C) to degrade the soft tissue proteins. Due to this high temperature, the remains need to be tended frequently during processing to keep the water from boiling over and create a mess or boil off and burn the remains. If the remains sit directly on the bottom of the pot, the bones could scorch from the heating element. Additionally, the boiling water could be a damaging agent by making the bones, especially thin subchondral bone, soggy and easily broken (Mann and Berryman 2012). This type of damage was evident on the medial cuneiform of 1 sample, but there was no significant change in mechanical properties of the bone after boiling.

4.2.4 **Clorox®**

4.2.4.1 Efficiency

All of the Clorox® samples were complete in 6-8 hours, with a mean of 6.53 hours. There was no significant difference in TTC among the 3 concentrations of Clorox®, that is, increasing the concentration of Clorox® did not significantly decrease the time required to clean the remains. Additionally, boiling the remains in a Clorox® solution did not statistically differ in time-to-completion from the other heated treatments. These results suggest that the addition of Clorox® to the boiling water does not significantly reduce the time required to clean the remains over the plain water boil method.

The per-sample cost of using Clorox® was low, at only $0.26 per fluid cup (8oz). This cost could be lowered even further if a generic brand of household bleach containing sodium hypochlorite is used.

4.2.4.2 Effectiveness

All of the bones were separated and all of the soft tissues were removed during processing with all concentrations of Clorox®. The resulting bones were similar in color and smell to the bones cleaned via the plain water boil and Biz® methods. Surprisingly, the oxidation effects of the Clorox® did not whiten the bones any more than boiling in plain water and not as much as the maceration method.

4.2.4.3 Destructiveness

Samples defleshed using low and medium concentrations of Clorox® exhibited no observable macroscopic damage or erosion of the trauma marks; and there was no significant change in the mechanical properties of the bone detected after processing of these samples. Macroscopic damage in the form of bone exfoliation was observed on a high concentration sample by the third day of air-drying. Additionally, the tangent modulus of these samples decreased significantly after processing, indicating a decrease in the stiffness of the bone after using a high concentration of Clorox®. These results imply that Clorox® may be damaging to bone tissue at high concentrations.

4.2.4.4 Pros, Cons, and Comments

The Clorox® method, even in a high concentration, did not significantly differ from the plain water boil method in effectiveness or efficiency. The only significant differences detected between these methods was the cortical bone exfoliation observed on a high concentration sample and the decrease in bone stiffness after processing with a high concentration of Clorox®. Based on the variables assessed in this study, Clorox® does not seem to offer any advantages over using plain boiling tap water and can be damaging to bone at high concentrations.

Household bleach is also known to lower the quality of DNA retrieval (Lee et al. 2010). Therefore, unless it is found that bleach can sterilize biohazards that boiling alone cannot, it should be avoided in forensic cases as it has the potential to damage bones if not used properly.

4.2.5 **Sodium Perborate**

4.2.5.1 Efficiency

All of the SPB samples were complete in 3-6 hours, with a mean TTC of 4.73 hours, making SPB the quickest defleshing method used. There was no statistically significant difference in mean rank of TTC among the 3 concentrations of SPB. However, all concentrations of SPB were significantly faster than the Biz® method. The low and medium concentrations of SPB were significantly faster than both the low and medium concentrations of Biz®, and the high concentration of SPB was significantly faster than all 3 concentrations of Biz®. Though many of the SPB times were lower than the other methods as well, they did not statistically differ from the other heated treatments.

SPB was the most expensive method assessed in this study. The per-sample cost was dependent on the quantity of the chemical purchased, since purchasing in bulk decreased the cost by nearly half. Purchasing a 6 pound pail of SPB had a cost of $4.28 per pound, whereas purchasing a 33 pound pail had a cost of $2.30 per pound.

4.2.5.2 Effectiveness

All bones were separated and all soft tissues removed during processing except in 1 low concentration sample where the proximal phalanx of digits 2 and 5 remained within their respective hooves. The resulting bones were bleached whiter than normal bone-color, but not as

white as the maceration-cleaned bones. The bones were not greasy and had only a very slight greasy odor.

4.2.5.3 Destructiveness

There was no observable macroscopic damage or erosion of the trauma marks on any of the SPB-cleaned samples. Moreover, there was no significant change in the mechanical properties of the bone detected after processing. However, 2 of the 5 medium concentration SPB post-processing core samples broke during extraction and were unable to be used in the compression tests, which may have affected the statistical comparisons.

4.2.5.4 Pros, Cons, and Comments

Though there was not a statistically significant difference in TTC, the method was faster and involved less manual labor than the other heated methods. Therefore, SPB may be worth the extra money if time and labor involvement are concerns within a particular laboratory. Sodium perborate is a compound commonly used as a bleaching agent in household laundry detergents. If a laundry detergent containing SPB is used for defleshing rather than the pure chemical, costs may be lower, but the ingredients of commercially produced detergents are unknown to the public. The additional ingredients of these detergents could be potentially damaging to bone tissue, so specific research should be conducted on these detergents and compared to the results of using pure SPB.

4.2.6 **Biz®**

4.2.6.1 Efficiency

All of the Biz® samples were complete within 8-21 hours, with a mean of 12.73 hours. This TTC includes the overnight soaking times for 6 samples. Due to this required soak time in addition to the processing time, low and medium concentrations of Biz® took significantly longer than all 3 concentrations of SPB and the high concentration of Biz® took significantly longer than the high concentration of SPB. However, the Biz® solutions did not statistically differ in the mean rank of TTC among the 3 concentrations or among the other heated methods.

The per-sample cost of using Biz®, at $0.51 per cup, was intermediate between Clorox® and SPB. It is difficult to compare the results of Biz® to generic enzyme-active detergents since the types of enzymes and additional ingredients will vary, and the exact components of these detergents are unknown to the public due to proprietary knowledge.

4.2.6.2 Effectiveness

All bones were separated and all soft tissues removed during processing except in 1 low and 1 medium concentration samples. In both cases, the proximal phalanx of digits 2 and 5 remained within their respective hooves. In the low concentration sample, the proximal phalanx of digit 3 also was not able to be removed from the hoof. The resulting bones were similar in color and smell to the bones cleaned via the plain water boil and Clorox® methods. Thus, the various chemicals within Biz® did not whiten the bones any more than boiling in plain water and not as much as the maceration method.

Though the resulting bones did not have an odor, during processing the Biz® produced laundry-fresh smells which made it the defleshing method with the most pleasant smells. The

detergent, which included lipases, also acted to degrease the pots during processing, making clean-up easier for the preparator.

4.2.6.3 Destructiveness

There was no observable macroscopic damage or erosion of the trauma marks on any of the Biz®-cleaned samples. Furthermore, there was no significant change in the mechanical properties of the bone detected after processing.

4.2.6.4 Pros, Cons, and Comments

An advantage of the Biz® method is that temperatures below boiling (80-85°C) are used with enzymatic laundry detergent for optimal action of the enzymes, so the potentially harmful effects of high temperatures on bone are reduced. But, the Biz® method was found to be less efficient and effective than boiling in plain water. This method was also the only heated method that required an overnight soak in the solution in order to completely clean the remains.

Unfortunately the specific ingredients of enzymatic detergents are unknown to the public due to proprietary knowledge, and the company may change the formula over time without warning. This is likely the reason for the difference in results between Mooney et al. (1982) and the current study. Mooney and colleagues found Biz® to be a quick and effective method of defleshing remains, however, Biz® was the slowest of the heated defleshing methods assessed in the current study, and was not as effective as boiling with plain water.

5.0 CONCLUSIONS

5.1 SELECTING A DEFLESHING METHOD

Due to the fact that there is a lack of research on defleshing methods and no established protocol for producing skeletal remains in any field, many preparators are left asking the question, "What is the 'best' method for defleshing remains?". Unfortunately, there is not an easy answer to this question, as evidenced by the conflicting opinions within the literature and the results of the current study. The decision is typically based upon the ease of the method, the resources available to the laboratory, and the experience of the preaparator. Selection of a defleshing method, however, should not be made hastily or thought of as a menial task. The defleshing process may have long-lasting effects on the resulting bones that could obstruct future research or even damage bones meant for collection or display purposes. Therefore, the decision of a defleshing method should not be based solely on these superficial concerns and the desired finished product (level of articulation, acceptable amount of greasiness, whiteness, etc.). The primary consideration in defleshing method selection should be the effects of the method on the bone tissue, especially if the material to be processed are human remains from a medicolegal investigation and exhibit signs of perimortem trauma in the bone.

Every defleshing method has its own advantages and disadvantages and can be potentially damaging to bone if not carefully implemented. These potentially damaging effects

should be considered and weighed against the advantages prior to defleshing. Based on this study, bone can be damaged macroscopically and altered microscopically by the defleshing method used. These alterations in the bone tissue can then affect future research on the bones, such as chemical, histological, mechanical, and genetic analyses (Williams 1992; Williams 1999). The long-term research value can further be diminished if chemicals or enzymes are not properly washed off so that they can continue to act on and degrade the bone, as was suspected in the Ossian osteological collection (Shelton and Buckley 1990).

An excellent example of the need to understand the effects of a defleshing method before utilizing it for skeletal processing is demonstrated by the comparison of results of this study for plain water boiling and boiling with Clorox®. This study surprisingly found that the addition of Clorox® has no obvious advantage over boiling the remains in plain water. The bones cleaned by both methods were completely cleared of all soft tissues and the bones were all similar in color and greasiness, so Clorox® did not increase the effectiveness of plain water. The Clorox® did not even whiten the bones more than boiling in plain water as one would expect. Additionally, Clorox® did not clean the samples any faster than the plain water, as there was no significant difference in time-to-completion between the methods, meaning Clorox® did not increase the efficiency any further than plain water either. The primary difference in results between these 2 methods was found in the assessment of destructiveness. Though a small hole was found on one of the plain water boil bones, no cortical bone exfoliation occurred and there was no significant change in the mechanical properties of the bones post-processing. Conversely, cortical bone exfoliation was observed on a sample cleaned by a high concentration of Clorox® and the high concentration was found to significantly decrease the stiffness of the bone. Therefore, Clorox®, which is commonly used in defleshing, was found to have no benefit

over boiling in plain water and to have potentially damaging effects if used in high concentrations, which could diminish the long-term research value of the remains.

5.2 LIMITATIONS OF THIS STUDY

5.2.1 Sample Limitations

The results of this study are limited due to the choice of the particular remains used as samples. The distal segment of the hind limb of white-tailed deer was chosen as a proxy for human remains. Though it has been explained that a basic animal model of bone can be utilized and generalized to human bone due to the similarities in chemical composition and physiology between humans and other mammals (Mooney and Siegel 2005), there are some differences in microstructure between human and deer bone that could affect the results of this study. Human Haversian systems tend to be more circular and regularly-shaped than any other mammal (Crescimanno and Stout 2012; Martiniakova et al. 2006), which may or may not affect the mechanical testing results. Also, young deer bone is comprised of a combination of Haversian bone and plexiform bone, which does not typically occur in humans (Hillier and Bell 2007; Martiniakova et al. 2006).

Additionally, the deer bones comprising the distal hind limb have thicker cortical bone than most human bones. None of the bones within the samples had the thin cortical bone and high density of trabecular bone as human tarsals or vertebral bodies, nor were they as fragile as the thin bones of the cranium. Bones such as these could be more susceptible to damage during

the processing methods employed than the thick deer limb bones. Furthermore, teeth were not included in this study, so assumptions cannot be drawn from these results on the effects of the defleshing treatments on dental tissues. Teeth are notoriously more vulnerable to cracking under temperature extremes and drying too quickly than are bones (Nawrocki 1997; Neves et al. 1995; Williams 1991).

Since the deer limbs were collected after the deer processor had removed and discarded them, the specific age-at-death and sex of the deer comprising the study sample were unknown. Thus, these 2 important co-variables could not be controlled for accurately within the analyses. To remedy this obstacle in future research, collection of the limbs could occur while the deer processor is removing them from the animal. The sex could then be easily determined and the age-at-death could be more accurately estimated from the teeth and antlers, if present.

5.2.2 **Methods Limitations**

The equipment used to implement the selected defleshing methods in this study was not high-grade scientific equipment. Instead, the equipment was relatively inexpensive and easily obtained from local department stores. Though this is the type of equipment more likely to be utilized in an anthropology laboratory, the results produced may not be as reliable as those produced with higher quality scientific equipment.

There was a small sample size (n = 5) within each treatment group of this study, which led to difficulties in the ensuing statistical analyses.

Since the metatarsals in this study were sawed transversely through the shaft so that the saw marks could be assessed and had 2 bone cores removed for mechanical testing, none of the metatarsals were processed complete with an unaltered medullary cavity. Therefore, processing

using the methods described in this research with unaltered long bones could produce greasier final products than described here since the marrow would not be as exposed to the processing agents.

5.3 RECOMMENDATIONS FOR FUTURE RESEARCH

These results provide insight into the performance of 6 defleshing techniques and their effects on bone tissue. However, additional assessments of bone alteration could be conducted to enhance our understanding even further. For example, the use of histological analysis could provide a more detailed microscopic assessment of bone damage, allowing views of the bone tissue at the osteon level. Similarly, scanning electron microscopy (SEM) would offer a more detailed view of fine trauma marks in bone and likely display erosion of these marks more clearly. An ash weight and chemical analysis of the remains would provide a more comprehensive assessment of any alterations that may occur to the mineral component of bone tissue due to the processing methods.

Additional mechanical tests could be implemented to offer more knowledge on alterations to the mechanical properties of bone. This study only tested the mechanical properties of cortical bone in compression along a transverse axis. Samples could also be tested in compression along a longitudinal axis, in tension, or in torsion; or an indention test could be executed.

Only a small region of a large mammal with limited amounts of muscle tissue was defleshed in this study. A study should be conducted using larger body regions or entire human bodies so that the effects of the defleshing methods can be assessed on large muscle masses and

on human bone tissues. The time-to-completion for each method would likely be greatly increased if an entire large mammal body were to be defleshed rather than a small region of a limb. Thus, the bones would be subjected to the defleshing agents for longer periods of time, which could have more adverse effects on the bone tissue than revealed in the current study. Further research should also replicate these methods using juvenile bone. Since juvenile bone is denser in organic matrix than adult bone, they may be more susceptible to certain defleshing methods and produce different results than observed for the adult bones used in this study.

The bones of each sample from this study are currently stored at room temperature in individually labeled resealable plastic bags in cardboard boxes. Since the defleshing treatments used on each sample was recorded in detail, these samples are primed for a study of the potential long-term effects of these defleshing methods on bone tissue and trauma marks.

After mechanical testing, the bone core samples were rehydrated in PBS-soaked gauze and stored in a freezer for future DNA analysis. These samples will allow for a future study comparing the ability to retrieve and amplify DNA prior to and following the different defleshing treatments.

5.4 SIGNIFICANCE OF THIS RESEARCH

Although, more research should be conducted to paint a more complete picture of the effects of defleshing treatments on bone, the current study provides empirical research that assesses the efficiency, effectiveness, and destructiveness of 6 common methods of soft tissue removal from bone. Descriptions of these methods and their effects on bone tissue are provided, as are descriptions of how differing concentrations of Clorox®, sodium perborate, and Biz® affect the

efficiency, effectiveness, and destructiveness of these methods. These comparisons and descriptions offer an improved understanding of the methodology of soft tissue removal and the potential hazardous effects of these techniques in the hope of directing fields employing defleshing techniques towards standardized procedures and utilizing practices safer for bone tissue.

APPENDIX A

DEER HIND LIMB ANATOMY

All of the limbs were dismembered by the deer processor distal to the astragalus (talus), so no sample in this study included the calcaneus or astragalus from the proximal row of tarsals. Tarsals in the distal hind limb segments used in the current study include the naviculocuboid (NC) from the proximal row and the medial cuneiform (MC) and intermediate/lateral cuneiform (ILC) from the distal row of tarsals. Figure A.1 depicts the medial view of the tarsals of the left limb. The metatarsal (MT) or "metapodial" in the deer is a single bone that is comprised of a fused MT3 and MT4. Digits 2 and 5 are greatly reduced in deer and the first digit is completely missing.

There are 4 small half-moon-shaped sesamoid bones that form within the flexor tendons located on the distal posterior surface of the metatarsal. Three phalanges, a proximal, middle, and distal, form each of the 4 digits (digits 2-5). Each distal phalanx of digit 2-5 is encased in a keratinized hoof. Digits 3 and 4 together form the cloven hoof that carries the weight of the deer. Digits 2 and 5 are more proximally located and form the small dew claws of the deer.

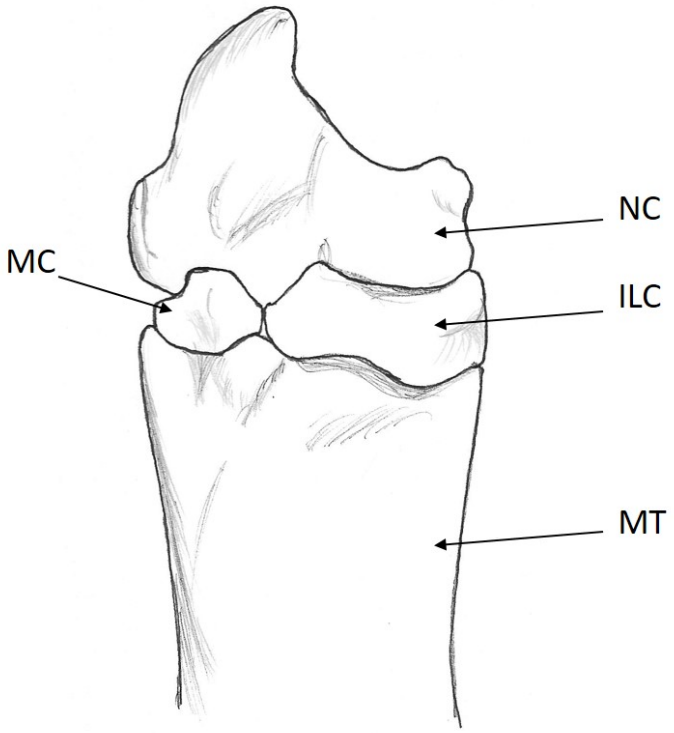

Figure A.1: Medial view of tarsals and metatarsal of the left limb.

APPENDIX B

ASSUMPTION TESTS FOR STATISTICAL ANALYSES

Start Weight of Samples

A one-way between-subjects ANOVA was performed on the start weight of the deer samples to assess whether the samples randomly assigned to the 12 defleshing treatments differed in weight prior to processing. The assumption of homogeneity of variance was met, Levene $F(11, 46) = 1.442$, $p = .187$. The assumption of normality was met for all defleshing groups except for the low concentration of sodium perborate (Table B1). All other assumptions for this test were met.

Time-to-Completion (TTC)

A one-way between-subjects ANOVA was not able to be applied to the TTC data as the assumption of homogeneity of variance was not met, Levene $F(11, 48) = 9.906$, $p < .001$. Additionally, the assumption of normality was not met for all of the defleshing groups (Table B2) and the distributions for the 12 defleshing groups were oppositely skewed.

Table B1: Shapiro-Wilk test of normality for start weight.

Statistical significance means that the assumption of normality was not met.

Method	Concentration Variation	df	Statistic	*p*
Dermestids	NA	5	0.975	0.905
Maceration	NA	5	0.871	0.271
Boil	NA	5	0.862	0.235
Clorox®	Low	4	0.862	0.267
	Medium	5	0.902	0.419
	High	4	0.947	0.697
SPB	Low	5	0.721	0.016*
	Medium	5	0.904	0.431
	High	5	0.923	0.552
Biz®	Low	5	0.957	0.786
	Medium	5	0.929	0.593
	High	5	0.959	0.801

*Statistical significance

Table B2: Shapiro-Wilk test of normality for TTC.

Statistical significance means that the assumption of normality was not met.

Method	Concentration Variation	df	Statistic	*p*
Dermestids	NA	5	0.753	.032*
Maceration	NA	5	0.909	.460
Boil	NA	5	0.684	.006*
Clorox®	Low	5	0.881	.314
	Medium	5	0.684	.006*
	High	5	0.684	.006*
SPB	Low	5	0.881	.314
	Medium	5	0.552	.000*
	High	5	0.881	.314
Biz®	Low	5	0.726	.018*
	Medium	5	0.684	.006*
	High	5	0.626	.001*

*Statistical significance

Biomechanics Variables

Paired samples t-tests were conducted on the 3 biomechanics variables. Normality of these data was tested using the Shapiro-Wilk test of normality. For the variable of strain at 1790N, all of the pre-processing samples and all but the low concentration Clorox® post-processing samples met the assumption of normality (Table B3). Three pre-processing groups (high concentration of Biz®, low concentration of Clorox®, and medium concentration of sodium perborate) violated the assumption of normality for the variable of stress at first peak, but all post-processing samples were normally distributed for this variable (Table B4). All of samples met the assumption of normality for the tangent modulus (Table B5). All other assumptions were met for these tests.

Dry Weight of Sample

A one-way between-subjects ANOVA was performed on the end dry weight of the deer samples. The assumption of homogeneity of variance was met, Levene $F(11, 48) = 1.895$, $p = .064$. The assumption of normality was met for all defleshing groups and all other assumptions for this test were met (Table B6).

Table B3: Shapiro-Wilk test of normality for strain at 1790N.

Statistical significance means that the assumption of normality was not met.

Method	**Concentration Variation**	**Sample**	**Statistic**	**df**	***p***
Dermestids	NA	Pre	0.82	5	0.116
		Post	0.979	5	0.927
Maceration	NA	Pre	0.935	5	0.631
		Post	0.951	5	0.747
Boil	NA	Pre	0.956	5	0.779
		Post	0.91	5	0.466
Clorox®	Low	Pre	0.933	5	0.616
		Post	0.743	5	0.026*
	Medium	Pre	0.994	5	0.993
		Post	0.95	5	0.735
	High	Pre	0.934	5	0.621
		Post	0.938	5	0.654
SPB	Low	Pre	0.962	5	0.825
		Post	0.953	5	0.755
	Medium	Pre	0.856	3	0.258
		Post	0.897	3	0.376
	High	Pre	0.936	5	0.637
		Post	0.964	5	0.836
Biz®	Low	Pre	0.996	5	0.996
		Post	0.898	5	0.400
	Medium	Pre	0.983	5	0.950
		Post	0.97	5	0.878
	High	Pre	0.949	5	0.729
		Post	0.91	5	0.469

*Statistical significance

Table B4: Shapiro-Wilk test of normality for stress at first peak. Statistical significance means that the assumption of normality was not met.

Method	**Concentration Variation**	**Sample**	**Statistic**	**df**	***p***
Dermestids	NA	Pre	0.867	5	0.253
		Post	0.915	5	0.501
Maceration	NA	Pre	0.834	5	0.149
		Post	0.904	5	0.430
Boil	NA	Pre	0.833	5	0.148
		Post	0.98	5	0.937
Clorox®	Low	Pre	0.904	5	0.434
		Post	0.845	5	0.180
	Medium	Pre	0.801	5	0.082
		Post	0.986	5	0.965
	High	Pre	0.644	5	0.002*
		Post	0.82	5	0.117
SPB	Low	Pre	0.892	5	0.368
		Post	0.782	5	0.057
	Medium	Pre	0.758	3	0.019*
		Post	0.822	3	0.169
	High	Pre	0.871	5	0.270
		Post	0.981	5	0.938
Biz®	Low	Pre	0.783	5	0.059
		Post	0.961	5	0.812
	Medium	Pre	0.91	5	0.466
		Post	0.84	5	0.166
	High	Pre	0.691	5	0.008*
		Post	0.901	5	0.413

*Statistical significance

Table B5: Shapiro-Wilk test of normality for tangent modulus. Statistical significance means that the assumption of normality was not met.

Method	**Concentration Variation**	**Sample**	**Statistic**	**df**	***p***
Dermestids	NA	Pre	0.883	5	0.323
		Post	0.898	5	0.400
Maceration	NA	Pre	0.831	5	0.141
		Post	0.86	5	0.227
Boil	NA	Pre	0.98	5	0.933
		Post	0.925	5	0.560
Clorox®	Low	Pre	0.878	5	0.298
		Post	0.968	5	0.865
	Medium	Pre	0.847	5	0.186
		Post	0.892	5	0.365
	High	Pre	0.943	5	0.691
		Post	0.958	5	0.793
SPB	Low	Pre	0.84	5	0.164
		Post	0.959	5	0.804
	Medium	Pre	0.863	3	0.276
		Post	0.802	3	0.120
	High	Pre	0.988	5	0.972
		Post	0.92	5	0.528
Biz®	Low	Pre	0.824	5	0.126
		Post	0.823	5	0.123
	Medium	Pre	0.919	5	0.521
		Post	0.84	5	0.165
	High	Pre	0.974	5	0.903
		Post	0.934	5	0.624

Table B6: Shapiro-Wilk test of normality for dry weight.

Statistical significance means that the assumption of normality was not met.

Method	Concentration Variation	df	Statistic	*p*
Dermestids	NA	5	0.979	0.930
Maceration	NA	5	0.853	0.587
Boil	NA	5	0.929	0.587
Clorox®	Low	5	0.845	0.181
	Medium	5	0.933	0.617
	High	5	0.850	0.195
SPB	Low	5	0.846	0.183
	Medium	5	0.952	0.749
	High	5	0.942	0.677
Biz®	Low	5	0.950	0.735
	Medium	5	0.913	0.487
	High	5	0.982	0.945

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