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Kamila KEMPA, Mirosława KULAWIK, Bartłomiej J. BARTYZEL[1], Mikołaj JAKUBOWSKI[2], Jacek SKUBIS[2], Piotr KOCZOŃ[3]

CHARACTERIZATION OF SELECTED TECHNIQUES OF MACERATION BONES OF *GALLUS GALLUS DOMESTICUS*

CHARAKTERYSTYKA WYBRANYCH TECHNIK MACERACJI KOŚCI *GALLUS GALLUS DOMESTICUS*

Department of Animal Anatomy, Poznań University of Life Sciences, Poland
[1]Department of Morphological Sciences, Warsaw University of Life Sciences, Warsaw, Poland
[2]Department of Game Management and Forest Protection, Poznań University of Life Sciences, Poland
[3]Department of Organic and Food Chemistry, Warsaw University of Life Sciences, Warsaw, Poland

Streszczenie. Pięć tuszek kur domowych *Gallus gallus domesticus* stanowiło materiał do badań. Trzy tuszki poddano obróbce termicznej w celu uzyskania elementów szkieletu. Następnie jeden komplet kości umieszczono w roztworze wodnym proszku do prania Persil®, drugi zanurzono w 5-procentowym roztworze nadtlenku wodoru, a trzeci wysuszono w temperaturze pokojowej. Czwarty macerowany był enzymatycznie w roztworze proszku piorącego Persil® w temperaturze 50°C. Kolejną tuszkę poddano maceracji chemicznej z użyciem 3-procentowego roztworu wodorotlenku sodu. W pracy oceniono przebieg zastosowanych metod i ich efekty końcowe. Badania wykazały, że najmniej czasochłonną techniką przygotowania kości było gotowanie, a najdłużej trwała maceracja enzymatyczna. Jasny materiał uzyskano po gotowaniu i bieleniu w 5-procentowym roztworze nadtlenku wodoru oraz po maceracji enzymatycznej. Po maceracji chemicznej elementy szkieletu były brunatne, jednakże połączenia między kośćmi zostały zachowane. Maceracji enzymatycznej towarzyszył nieprzyjemny zapach. Wybór odpowiedniej techniki zależy od przeznaczenia materiału oraz możliwości technicznych.

Key words: domestic hen, bones, preparation.
Słowa kluczowe: kura domowa, kości, preparacja.

INTRODUCTION

Single bones, their sets and entire skeletons serves as didactic equipment in schools and Universities. They are also presented as exhibits in museums, hunting trophies and scientific research material.

Excavated bones are important for science development (Geer et al. 2006). They serve for animals identification followed by assigning to proper taxonomic group (Boyle 2010). Morphological and morphometrical description of bones present widely in literature (Hidaka et al. 1998) can only be done after proper bones preparation.

Corresponding author – Adres do korespndencji: Mirosława Kulawik, Department of Animal Anatomy, Poznań University of Life Sciences, Wojska Polskiego 71 C, 60-625 Poznań, Poland, e-mail: kulawik@up.poznan.pl

There are several techniques to prepare bones from carcasses. The one probably oldest method to clean skeleton is burring. In this method the soft tissue decay is uncontrolled however. Furthermore, unable to remove discolorations of bones may occur due to action of different chemicals present in soil. The rate of soft tissue decay depends on type of soil and animal size (Hendry 1999).

In biological techniques use to prepare bones different bugs e.g. beetles from *Dermestes* group are used. They are placed together with material in bug room under specific conditions (Borell 1938; Sullivan and Romney, www.natsca.org/sites/default/files/publications/books/ /Vertebrates.pdf). After cleaning bones are frozen in –18°C to remove remaining bugs (Dumitru et al. 2013). This method is usually applied in museums in the case of middle and small size animals. The advantage is economy and very careful cleaning without damage of smallest bone's parts and elements (Sommer and Anderson 1974).

Another method is immersing carcasses in water for certain time. This method requires continuous fill up with water that evaporates as preparing bones must be continuously immersed in liquid (Yamazaki 2010). Factors influencing time of this type of maceration are bath temperature, with the best around 32°C and size of animal (Sullivan and Romney, www.natsca.org/sites/default/files/publications/books/Vertebrates.pdf). Increasing temperature increases the rate of clearing hence boiling is also applied. The disadvantage in this case is bad odour that occurs. Therefore soft heating instead of boiling is recommended. Bad odour does not occur in this technique (Sullivan and Romney, www.natsca.org/sites/default/files/ /publications/books/Vertebrates.pdf). After temperature treatment completion, muscles are ease to remove.

Application of enzymes in the process of preparation of bones is relatively new technique. Although expensive and of intensive smell both trypsine and papin are commonly use. The alternative is application of any washing powder containing series of enzymes effectively removing stains and discolorations. Maceration time depends on total amount of proteases in the washing powder (Simonsen 2012). This method additionally requires pre-incubation in 37 to 50°C (Hendry 1999).

Chemical method base on immersing material in sodium or potassium hydroxide solution, respectively. Then system stays for some time in room temperature. This technique may cause partial or even complete damage of bones hence frequent control of process progress is required (Onwuama et al. 2010). It is also possible to boil in sodium hydroxide solution for 4–8 hours depend on animal size (Allouch 2014).

Literature data provides information on different methods of bones preparation, however there is a lot of discrepancies on process conditions required for desired effect e.g. time, solution concentration or temperature.

The aim of the investigation undertaken in this paper was to compare effectiveness of different methods to obtain bones for didactic puropses on the example of domestic hen.

MATERIAL AND METHODS

The material for this study was five carcasses of domestic hens (*Gallus gallus domesticus*), aging 21 weeks. The material comes from slaughterhouses. Following removal of feathers, skin, intestines and muscles the hen was divided into five parts i.e. head, back bone with rib and sternum, breast and pelvis limbs. Three methods of bones preparation were used i.e. boiling, enzymatic treatment and chemical treatment.

Three hens were boiled, one was enzymatically and one chemically treated. The parts of three hens were boiled in separate containers for 5 hours. After boiling bones were mechanically cleaned from remaining soft tissues and rinsed in warm water. Then skeleton elements of one hen was immersed in washing powder Persil® solution (200 grams of powder per 1 dm^3 of water) containing following chemicals: disodium carbonate (Na_2CO_3; 25%), hydrate of disodium carbonate with hydrogen peroxide ($Na_2CO_3 \cdot 1.5H_2O_2$; 13%) sodium salts of benzenesulfonic acid derivatives containing 10-13 carbon atoms in side chain (23%), sodium salt of silicic acid (general formula of silicic acid is $[SiO_x(OH)_{4-2x}]_n$ MR 7%), Ethoxylated alcohols containing 12–13 carbon atoms in the chain (2%), tetrasodium etidronate (1%). The second hen was immersed in 5% solution of hydrogen peroxide (H_2O_2), while third hen was left to dry in room conditions. After six days bones from two solutions were rinsed with regular water and dried in room temperature.

The fourth hen was macerated enzymatically. Parts obtained from this sample were put into water solution of washing powder (200 grams of powder per 1 dm^3 of water) and placed in dryer in temperature 50°C for 10 days. Then samples were mechanically separated from macerated muscles and put again into washing powder solution of the same concentration and placed in dryer again for 4 days (50°C). After that samples were rinsed with warm water to remove remaining soft tissues and deactivate enzymes and dried in room temperature.

The fifth hen sample was put into 3% sodium hydroxide (NaOH) solution for 4 days. Bones were then rinsed with water. Remaining of soft tissues were removed with surgery tools and bones were left to dry under room temperature.

RESULTS AND DISCUSSION

Investigation conducted proved absence of one universal and perfect method to prepare proper bones for both scientific, didactic and hobby purposes. All of tested methods have their specific advantages and disadvantages. The main aspects deciding on selection of bones preparation method are: laboratory and its equipment, financial capacity, animal size and use of bones. All methods tested allowed to remove soft tissues, however final bones differed. Specific features identifying those differences are gathered in Table 1.

Table 1. The comparison of specific features of bones obtained with different methods.
Tabela 1. Porównanie efektów końcowych różnych metod przygotowania materiału kostnego

Features Cechy	Boiling Gotowanie	Boiling + + enzymes Gotowanie + + enzymy	Boiling + + H_2O_2 Gotowanie + + H_2O_2	Enzymatic maceration Maceracja enzymatyczna	Chemical maceration Maceracja chemiczna
Time Czas	5 h	5 h boiling + 6 days enzymes 5 h gotowanie + + 6 dni enzymy	5 h boiling + 6 days H_2O_2 5 h gotowanie + + 6 dni H_2O_2	14 days in dryer (50°C) 14 dni w cieplarce (50°C)	4 days NaOH 4 dni NaOH
Odor Zapach	–	–	–	very strong bardzo silny	–
Color Barwa	dark brown discolorations brunatne przebarwienia	creame brown kremowo-brązowy	uniformly white jednolicie biały	creamy kremowy	brown brązowy
Damages Uszkodzenia	–	–	–	–	small nieznaczne
Surface Powierzchnia	smooth gładka	smooth gładka	smooth gładka	smooth gładka	rough szorstka

Boiling was the fastest and enzymatic treatment with double solution exchange the slowest procedure. Boiling followed by use of washing powder solution to remove dark discolorations consumed the same amount of time as boiling with bleaching with hydrogen peroxide.

It is to be noticed that during enzymatic maceration applied as independent method unpleasant odor was produced. Good ventilation of laboratory room where the dryer was located was essential in this method.

The color of final material was the essential parameter. Most visually acceptable, white bones were obtained in boiling method followed by bleaching in 5% hydrogen peroxide solution. Enzymatic maceration with double exchange of solution produced neat, white bones as well. Merged. Skeleton elements after boiling alone were of many discolorations and those from chemical maceration were dark brown (Fig. 1–5).

Fig. 1. The skeleton of a bird obtained throught the boiling technique
Ryc. 1. Szkielet ptaka uzyskany dzięki technice gotowania

Fig. 2. Bird bones obtained after boiling and enzymatic maceration
Ryc. 2. Kości ptaka otrzymane po gotowaniu i maceracji enzymatycznej

Fig. 3. Uniformly white skeleton after boiling and using H_2O_2
Ryc. 3. Jednolicie biały szkielet po gotowaniu i użyciu H_2O_2

Fig. 4. Hen bones after enzymatic maceration
Ryc. 4. Kości kury po maceracji enzymatycznej

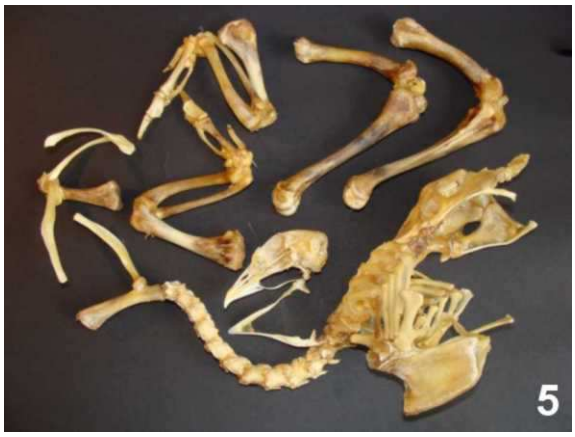

Fig. 5. The skeleton obtained through the chemical maceration
Ryc. 5. Szkielet uzyskany dzięki maceracji enzymatycznej

No method except chemical treatment damaged bones. Small defects occurred as soften of most sensitive parts of skeleton. Those damages do not eliminate bones from scientific, didactic or hobby purposes. Chemical maceration was found to be most destructive for bones surface. Bones obtained with this method were rough. However joint between bones were preserved which allows their detailed anatomical analysis. Using this method requires following strict laboratory safety rules due to corrosive properties of sodium hydroxide.

The process of bones preparation covers several stages including soft tissues removal, body defragmentation, maceration or boiling. Bones bleaching, degreasing and marking is also included. Selected processes application depends on further use of bones. The time of given stage performance depends on animal size (Boyle 2010). Removal of skin, organs and muscles must be performed with care, so that bones are not damaged. Big animals are recommended to be divided into smaller pieces.

Current study shows that all considered methods are efficient. Different procedures require different time of performance and result different final effects. Boiling is popular method to prepare bones. It is cheap and does not require specific equipment. Time of boiling depends on total size of animal or size of animal parts. The smaller parts the shortest time and the lower temperature (Hendry 1999). The overrunning procedure may cause osteological damages. Following Dumitru et al. (2013) data damages resulting from this method are significant. The clear advantage of this method is short time. However final bones contain dark discolorations that according to current results can be removed by

bleaching in hydrogen peroxide or washing powder solution. Sękowski (1995) claims that exposing to sunlight produces same bleaching effect. Bleaching with hydrogen peroxide is easy and cheap. It is to notice that increase in hydrogen peroxide concentration and time of bath bones undergo makes them soften (Hussain et al. 2007). In current investigation however, application of 5% hydrogen peroxide solution for six days did not produce any bones damage.

Onwuama et al. (2012) comparing water maceration, boiling and chemical maceration with use of 3% and 5% NaOH solution for preparation of bones from *Cricetomys gambianus* concluded chemical maceration to be the best. Clear advantage was short time of maceration, i.e. 6 or 7 hours for 5% and 3% solutions respectively and absence of unpleasant odor. Authors applied lower concentrations of NaOH solution which is claimed to cause damages, followed by frequent control of process, every half an hour.

According to Onwuama et al. (2012) chemical bleaching of hen's bones with hydrogen peroxide is very effective. This procedure allows to preserve joints between bones. With this method didactic sets of limb's bones, axial skeleton and even whole animal skeletons can be prepared. Bones can be further bleached and stored in formalin.

In current study enzymatic maceration produced good bones of cream color without any damages. Disadvantage of this method is unpleasant smell of decaying soft tissues. This conclusion is in accordance with Onwuama et al. (2012) results. The highest temperature applied can be 50°C, as enzymes undergo deactivation in higher temperatures hence rate of the process decreases significantly. Repeating change of enzymatic solution improves color of final bones due to removal of dark brown solution and acting of enzymes at constant level. For this method adequate laboratory is required for safety reasons.

The selection of bones preparation method depends solely on purpose of final material. In science and didactic single bones or their sets are required to present and discus shape and function hence dark color is acceptable. For hobby and hunting purposes mainly skulls of perfectly white color without any discolorations are required (Sękowski 1995; Sullivan and Romney, www.natsca.org/sites/default/files/publications/books/Vertebrates.pdf).

For exhibition purposes e.g. museum beetles from *Dermestes* group are used for cleaning bones usually due to presence of single sample animals only. To avoid the risk of loss of any skeleton element determines selection of this method (Sommer and Anderson 1974).

CONCULSIONS

1. The shortest method to clean bones is boiling.
2. Uniformly white bones can be obtained merging boiling with 5% H_2O_2 solution bath bleaching.
3. Enzymatic maceration enables obtaining elegant creamy bones, but process is associated with occurrence of unpleasant smell.
4. Boiling followed by bathing in washing powder solution enables obtaining light bones and avoid unpleasant smell.
5. Chemical maceration with 3% NaOH solution enables preservation of joins between bones however rough surface is formed, and most sensitive elements can be damaged.

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Abstract. Five carcasses of domestic hens *Gallus gallus domesticus* were starting material for this study. Three carcasses were thermally treated to obtain all parts of skeleton. One set of initial material was placed in water solution of washing powder i.e. Persil®, second one was placed in 5% hydrogen peroxide water solution, the third one was dried in room temperature. Fourth was enzymatic macerated in washing powder solution Persil® of the temperature 50°C. The last one was macerated chemically with use of 3% sodium hydroxide solution. The conductance and effects of procedures applied were evaluated. Results showed boiling to be the shortest and enzymatic process to be the longest procedure to prepare bones of desired quality. Bright material was obtained after both boiling and whitening in 5% hydrogen peroxide and enzymatic process. Chemical maceration produced brown elements of skeleton, however joints remained undamaged. Yet, enzymatic maceration was accompanied with unpleasant odour. The selection of procedure to obtain skeleton depends mainly on desired use of final product as well as the technical capabilities.