Peculiarities of Preservative Materials Action on Wild Animal Row Skin Tissue

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The goal of the research was to develop and propose a successful and very easy applicable method for skin short-preservation in outdoor conditions. The obtained results have shown that problem of short term skin preservation method choice is enough complicated. The skins of wild animals a very different in their properties. The use of 15% of sodium chloride allows preservation of both hare and wild pig skin for 28 days. The bacteriostatic or bactericidal additives to sodium chloride are preferable but not essential if storage time is no longer than 28 days.

Keywords: preservation, skin, hare, wild-pig

The conversion of animal hides and skins (fur) into useful artifacts might be man's oldest technology. *Kaunas Tadas Ivanauskas Museum of Zoology* is one of the oldest museums in the Lithuania and the only Zoological Museum in the Baltic States. The whole history of the museum is closely related to the evolution of natural sciences in Lithuania. This museum has more than 250000 exponents in Museum's collection: invertebrates, insects, fishes, amphibian and reptile, birds, mammalian. The exhibits of museum collection are from expeditions, trips, and private persons. The collection is constantly supplemented. Sometimes, by poachers shoot animals become as exhibits. Therefore, it is very important to preserve upcoming exhibits effectively and fast.

The changes in equilibrium state in the collagen-water system and the secondary process, like, e.g., autolysis, bacterial growth and changes due to aging are related to the hide curing process [1]. The secondary processes start from the flaying moment; their intensity depends on the external conditions and they are insofar dangerous, as they may cause a quick and irreversible deterioration of skin. Taxidermy begins in the field, as soon as animal is harvested. Preservation (conservation, curing) of the skin, flayed off a slaughtered animal should prevent it against deterioration and, first of all, to prevent bacterial growth. Populations of microorganisms grow on raw hides firstly because of their ability to hydrolyze the proteins, with proteolysis begins degradation of the hide substance. Ideally, any treatment applied to skin should be reversible, without altering the properties of the skin [2]. It is known that the bacteria on hides can double in numbers in less than 4 hours at 25°C. Some incipient damage is often caused, whatever preservation system is used, and the process can only halt bacterial action and damage caused after flaying remains. A preservation of hide/skin stops the growing of microorganisms.

The following conditions favor putrefaction: *p*H 6-10, preferably *p*H 7-9; presence of nutrients; moisture content >25%; temperature >20°C [2]. Altering the *p*H of the skin could be a perfect solution for preservation. However, acidification could lead to acid swelling if there is no electrolyte present. Alkali treatment is limited, because at *p*H 10 and above there is a risk of immunizing the hair and adversely hair removal.

Basic condition for the preservation process is a change in the water content in the skin. An additional change is the use of bacteriostatic (disinfectants) and bactericides. Salt is most common method for preservation. Sodium chloride is used 30-40% of total flesh weight. Salt is not a real preservative, because it works as dehydration material. The decreasing of moisture in hides at the same time decreases bacteria action. To prevent skin and fur from the bacterial attack, bactericide could be added. This biggest method disadvantages are: large quantities needed and long rehydrate of so preserved skin. Alternatives to salt curing are: curing by potassium chloride [3], acids [4], drying [5], chilling [6] or even preservation by irradiation [7]. Also, could be used preservative for skins as phenolic-type, sulphates, sodium fluorosilicate [5] or boric acid [8]. Recently, Murugan et al. have developed desorption isothermal model for controlled drying depending on the relationship between equilibrium moisture content and water activity [9]. Researchers has proposed to use the alternative preservation technique using natural plant extracts as *Semecarpus anacardium* nut [10], *S. portulacastrum* [11] or thyme [12], eucalyptus, lavender essential oils [13]. These methods totally avoid or decrease amount of used sodium chloride.

Most of described and most promising methods of skin preservation are adapted to leather tanning manufactories. The methods require special materials or equipment and are not enough convenient in field conditions. Therefore, the simple method, which is easily and cheaply adaptable in outdoor conditions is necessary.

The goal of the presented research was to develop and propose a successful and very easy applicable method for skin short-preservation in outdoor conditions.

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Experimental part

Materials

The raw hare (*Lepus europaeus*) and wild pig (*Sus scrofa*) skins were used for experiments. Skins were cut into pieces 10 x 10 cm and series of samples were formed. Preservation materials or their mixtures were top-dressed on samples. The samples after treatment were stored in clean polyethylene bags at 28±2°C temperature 28 days. Skin preservation methods are presented in table 1.

Table 1
PRESERVATION METHOD OF SKIN (TOP DRESSING)

Nr.	Amount of used materials, % from hide mass
I	NaCl – 15.0%
II	NaCl – 15.0%; Na_2SiF_6 – 1.0%;
III	NaCl – 15.0%; $Na_2B_4O_7$ – 1.0%;
IV	NaCl – 15.0%; $Al_2(SO_4)_3$ – 1.0%.

Total bacterial count. On each consecutive time, the skin pieces average weight 5 g and the size of 2.5 cm x 2.5 cm were cut from samples and transferred into conical flask containing 100 mL sterile saline (0.85 percent). The flasks were then kept on rotary shaker (100 rpm) at temperature (28±2°C) for 3 h. The skin extract was then serially diluted. Selected dilutions were plated on standard plate count agar. The plates were incubated at 30°C and number of colonies were evaluated after 48 h. Total count of bacteria were determined in terms of colony forming units (cfu) per mL.

Chemical analysis methods. Determination of an amount of nitrogen extracted from skin was carried out in such way: the skin pieces (weight 5g) were cut from the samples and shacked with 25 mL of distilled water. The extract was prepared by shaking of skin pieces at 200 rpm during 30 min. The extract was hydrolyzed using 6 mol/L HCl and nitrogen content was determined by Kjeldahl method [14].For the determination of total nitrogen content in a skin, it was taken 0.2 g of absolutely dry skin and hydrolyzed using 6 mol/l HCl. After that, the nitrogen content was established using Kjeldahl procedure.

The amount of collagen proteins was estimated from the amount of hydroxyproline in the skin. The amount of hydroxyproline was determined using a photo colorimetric method [15].The *p*H was determined according to standard [16]. Shrinkage temperature [17], moisture [18] and matter soluble in dichloromethane contents [19] were determined according to standards.

Structure of skin was observed using a scanning electron microscope (SEM) Quanta 200 FEG (FEI, Netherlands).

Physical changes during preservation were estimated organoleptically. The preserved skin samples were observed for any physical change(s) with respect to three parameters as putrefaction symptoms: odor, exterior image, epidermis and hair loosening.

Results and discussions

Salting is the traditional method used for preservation of raw animal hide/skin wherein ~35 to 40% (w/w) salt is used immediately after flaying of the animal [5]. In our case very long preservation duration is not necessary; therefore amount of used NaCl was decreased till 15% (w/w). Also, it is known that salt penetration at room temperature if concentration is about 45% (w/w) takes approximately 48 h, if it decreases till ~15% (w/w) – 12 h [1]. As additional possibility to decrease microorganism's action to skin and avoid putrefaction is to use antimicrobial properties having materials as: sodium tetraborate, sodium fluorosilicate and aluminum sulphate. Dry top-dressing was carried out as mostly simple method for the carrying of the chemicals.

It is known that skins of different animals have different structure, density and hair covering. Also, therefore they have very different processing technologies. Raw material properties depend on age, species or status of health of animal. To estimate the following differences, for this study was used skins of two different animals: hare (*Lepus europaeus*) and wild pig (*Sus scrofa*). To visualize the differences of their skin structure was done SEM. The images are presented in figure 1. Wild pig skin as pigskin differs in structure from other animal skins in that there is no distinct grain layers [1, 2], the hair penetrates the full thickness of the skin. A hare hair coating is more evolved. Also, its skin structure is denser than wild pigs one and it is less fatty.

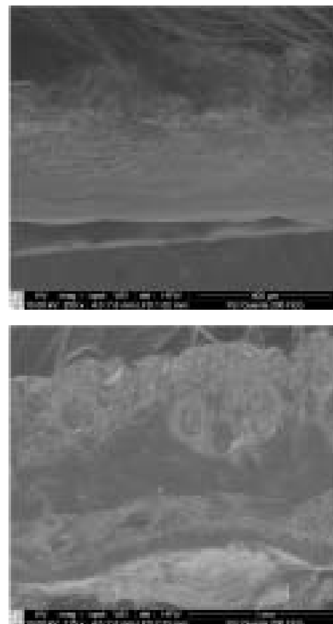

Fig. 1. SEM images of hare (a) and wild pig skin (b) cross-section

The others chemical and physical properties of hare and wild pig skin are presented in table 2.

A constitutions of skins very differ. The hare skin has relatively heavy (comparing with derma weight) and dry hair covering. Due to this the content of moisture is lower than in the skin of wild dick, which has very thick derma and relatively lightweight hair. The skins very differ in collagen content. It is know that amount of collagen in rabbit skin depends on rabbit age, and it varies from 3% till 15% from wet skin when age varies from 2 weeks till 9

Table 2
SKIN CHEMICAL AND PHYSICAL PROPERTIES

Indexes	Hare skin	Wild pig skin
Moisture, %	46.7	60.2
pH	6.2	6.6
Shrinkage temperature, °C	65.0	65.3
Amount of collagen, %	29.1	70.4
Total amount of nitrogen, %	7.6	13.3
Amount of matter soluble in dichloromethane, %	14.6	24.0

Note: amount of collagen, total nitrogen and matter soluble in dichloromethane were calculated from absolutely dry skin mass

month [20]. Collagen amount in pig skin is around 70% [1]. The indexes of *p*H and shrinkage temperature for both skins are close.

Fresh hare and wild pig skins immediately after flaying were treated according to methods presented in table 1. The preservation efficiency was estimated in conformity with the total bacterial count (TBC), amount of extracted nitrogen, *p*H and moisture content, which had been determined every 7 days.

The *p*H alterations of preserved hare skin samples are presented in figure 2. No significant alteration during preservation period was observed. The *p*H of samples after the treatment is ordained by properties of materials used together with sodium chloride. So, highest *p*H was of samples preserved with $NaCl+Na_2B_4O_7$ (III, fig. 2) and lowest preserved with $NaCl+Na_2SiF_6$ mixture (II, fig. 2).

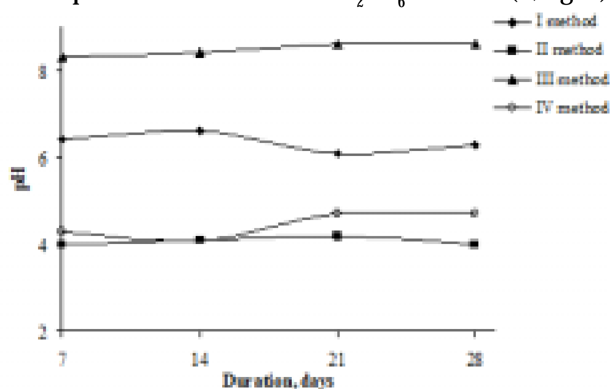

Fig. 2. Alteration of hare skin *p*H during storage

Every skin/hide as collagen based material has something amount of soluble in water proteins. During storage time and due to action of microorganisms a hydrolysis of nitrogen containing materials in skin occurs and ammonia forms. So, the amount of released and extracted from skin nitrogen content can serve as indicator showing quality of skin. The established initial amount of extracted from raw hare skin nitrogen was 1.7 g/kg. During one day of the preservation this amount slightly decreased. The explanation could be a protein denaturation. Denaturation process of proteins could run by application of compound such as a strong acid or base, a concentrated inorganic salt, an organic solvent (e.g., alcohol or trichlormethane), or heat [1]. Denatured proteins can exhibit a wide range of characteristics, from loss of solubility to communal aggregation [2]. In all preservation recipes were used materials which could impact in water soluble proteins denaturation, therefore after preservation the amount of in water soluble proteins decreased, and this reflected as the decrease of the extracted nitrogen content. Further, this index increases. The highest extracted nitrogen content after 28 days was in hare skin samples treated with NaCl and $Na_2B_4O_7$ (table 3, III). The faster increase of nitrogen content was observed for the samples treated using NaCl and Na_2SiF_6 (table 3, II) but later this index had stabilized. On the other hand, after 28 days of storage the values of nitrogen content were very close for all samples.

As was mentioned above, for preservation is preferable when moisture content is less than 25% [2]. The evaluation

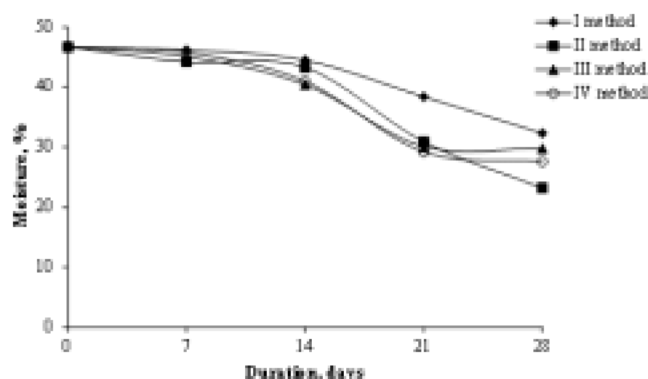

Fig. 3. Alteration of moisture content in hare skin during storage

of moisture content kinetics in hare skin shows that dehydration in this skin goes on slowly and during 28 days reaches 24-32% (fig. 3). The final value practically does not depend on the treatment method.

TBC determination results are presented in table 4. Overall, the situation with microorganisms amount change in the hare skin during its storage is sufficiently complicated. During first two weeks goes intense multiplication of bacteria. This process practically does not depends on the method of skin treatment. Despite of this fact, no weakening of hair bond with derma, no bad odor, no other symptoms of skin deterioration were observed. Presumably, the reproduction of bacteria goes on in fine hair covering in higher level than in skin tissue which has the absorbed preservative materials. Since hair is more resistant for an action of bacteria than derma, the high general TBC does not mean the putrefaction of skin tissue. The positive decrease of the bacteria content was observed during 4th week of storage, and this effect might be resulted by the reduction of moisture content [10].

If compare treatment methods, lowest TCB was observed after hare skin treatment with NaCl and $Na_2B_4O_7$ mixture (table 4, III), but lowest content of bacteria after 28 days storage had samples treated NaCl and $Al_2(SO_4)_3$ (table 4, IV).

The *p*H variation of wild pig samples during storage was similar like in hare case (fig. 4). The disparity between *p*H values of hare and wild pig skins samples treated in same way could be explained by different skin thickness, content of moisture and different structure.

The evaluation of the released from wild pig skin nitrogen has shown that best result was obtained using $NaCl+Na_2SiF_6$ and $NaCl+Na_2B_4O_7$ mixtures. The treatment using NaCl only leads to observable increase of the nitrogen in skin, especially, during fourth week of storing (table 5, I).

Kinetics of moisture content change in wild pig skin differs from change in hare one. The most significant decrease of moisture occurs over first week of storage. Afterward, the moisture level continues to lower very slowly, and after 28 days it is 37-43% (fig. 5). The lowest content of moisture was established in samples treated using NaCl and $Al_2(SO_4)_3$ (fig. 5, IV).

If to estimate a treatment method on the ground of TCB results, the best results were achieved using mixture of NaCl and Na_2SiF_6 (table 6, II). Also relatively good preservation was observed after treatment with NaCl and

Table 3
THE HARE SKIN DEGRADATION PROFILE IN TERMS OF EXTRACTED NITROGEN CONTENT

Preservation method	Preservation duration, days				
	1	7	14	21	28
	Amount of nitrogen extracted from skin, g from kg skin				
I	1.6	1.7	1.7	2.2	2.3
II	1.6	2.1	2.1	2.2	2.2
III	1.6	1.6	1.7	2.3	2.4
IV	1.6	1.8	2.1	2.2	2.2

Preservation method	Preservation duration, days				
	1	7	14	21	28
	Total bacterial count, cfu/ml				
I	$1.35x10^5$	$2.3x10^7$	$4.3x10^7$	$6.3x10^7$	$2.1x10^7$
II	$1.1x10^4$	$2.8x10^7$	$3.5x10^7$	$5.5x10^7$	$1.8x10^7$
III	$0.92x10^4$	$1.1x10^4$	$1.8x10^6$	$8.0x10^7$	$1.5x10^5$
IV	$0.13x10^4$	$3.8x10^6$	$2.3x10^7$	$8.0x10^7$	$2.5x10^4$

Note: the total bacterial count in raw hare skin was $4.7x10^1$ cfu/ml.

Table 4
THE ALTERATION OF TOTAL COUNT OF BACTERIA'S ON THE HARE SKIN DURING PRESERVATION

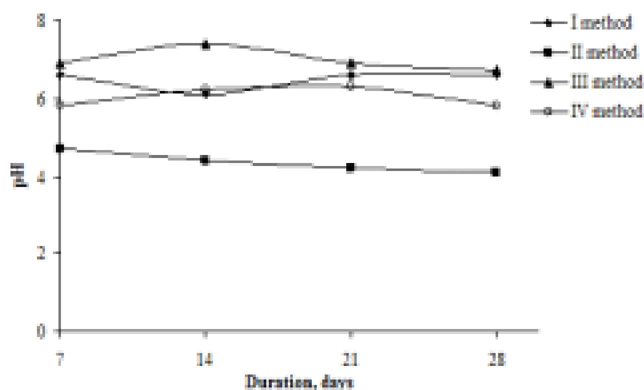

Fig. 4. Alteration of *p*H of wild pig skin during storage

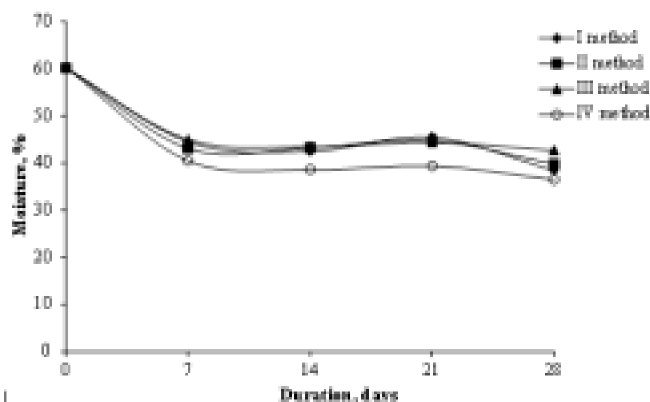

Fig. 5. Alteration of moisture content in wild pig skin during storage

Preservation method	Preservation duration, days				
	1	7	14	21	28
	Amount of nitrogen extracted from skin, g from kg skin				
I	1.5	1.5	1.6	1.8	2.3
II	1.4	1.4	1.4	1.5	1.6
III	1.4	1.4	1.5	1.7	1.7
IV	1.5	1.6	1.8	1.8	1.9

Note: initial amount extracted from wild pig skin nitrogen was 1.4 g/kg of skin

Table 5
THE WILD PIG SKIN DEGRADATION PROFILE IN TERMS OF RELEASED NITROGEN

Preservation method	Preservation duration, days				
	1	7	14	21	28
	Total amount of bacteria's, cfu/ml				
I	$3.8x10^5$	$1.6x10^7$	$1.4x10^8$	$4.4x10^8$	$7.5x10^8$
II	0	100	190	40	60
III	$1.7x10^4$	$1.8x10^4$	$6.4x10^4$	$3x10^5$	$10x10^7$
IV	0	$2.3x10^4$	$5.1x10^7$	$3.6x10^7$	$0.5x10^4$

Note: the total amount of bacteria's on raw wild pig skin before preservation was $1.0x 10^5$ cfu/ml.

Table 6
THE ALTERATION OF TOTAL COUNT OF BACTERIA'S ON THE WILD PIG SKIN DURING PRESERVATION

$Na_2B_4O_7$ (table 6, III). On the other way, as in the case with hare skin no skin decay indications had been observed in the treated wild pig skin during all storage period independently on treatment method.

Since a degradation of proteins due to possible putrefaction leads to destabilization of collagen, and in turn, of skin structure. One of most important criterion which allows estimation of this process progress is skin shrinkage temperature decrease. Shrinkage temperature shows the hydrothermal stability of the skin. The evaluation results of the shrinkage temperature of the skins during all observation period are presented in figure 6. The established values lead as to conclusion that during preservation period do not occur any structural changes and any damages, which can be reflected as alteration of shrinkage temperature.

As was mentioned above, the organoleptic evaluation haven't shown any symptoms of skin decay: no putrefactive odor, epidermis or hair loosening.

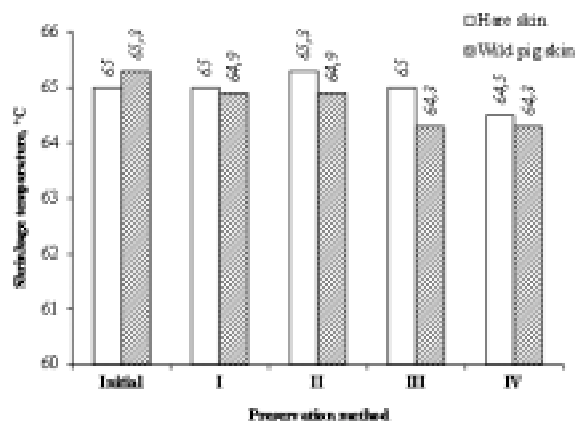

Fig. 6. Shrinkage temperature of hare and wild pig skins after 28 days

Conclusions

The obtained results have shown that problem of short term skin preservation method choice is enough complicated. The skins a very different in their properties: both chemical and physical. Also, very different hair covering also takes place in bacteria growing processes. The use of 15% of sodium chloride allows preservation of both hare and wild pig skin for 28 days. The decrease of moisture content has different kinetics and it depends on initial moisture amount in skin. When initial amount of moisture is less, the further remove of moisture goes slower. The action of sodium chloride on bacteria is slower as well.

The bacteriostatic or bactericidal additives to sodium chloride are preferable but not essential if storage time is no longer than 28 days. Sodium fluorosilicate has approved as most suitable additive is such case, and the best skin preservation is obtained using 15% sodium chloride and 1% sodium fluorosilicate mixture. This preservation method cam be recommended as enough simple, cheap and secure for possible exhibits preservation.

Acknowledgement: The research was carried out according to cooperative agreement with Kaunas Tadas Ivanauskas Museum of Zoology.

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Manuscript received: 6.05.2014