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Impact of aging and sweat action on properties of parchment as a biopolymer of animal origin of aging

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Abstract. The processes of accelerated artificial aging and sweat action of leather parchment manufactured in different methods from sheep's skin were investigated. By means of standard physical and mechanical methods of analysis and method of gel electrophoresis, the effect of high temperature and sweat was analysed on the behaviour of this biopolymer over time. The multidirectional of these influences on the most important physical and mechanical properties and the structure of parchment have been founds, which can be explained by the different degree of separation of the structural elements during the liming and the presence of plasticizing components that can prevent the influence of external factors.

1. Introduction

Parchment as a biopolymer of animal origin is one of the oldest types of natural leather, whose uniqueness lies in the production specifics (the tanning process is excluded from the technological cycle), as well as in significant strength and durability due to the peculiarities of structure and properties of collagen which is its main component. This allows being to parchment one of the best materials for writing, restoration of historical manuscripts and book editions, manufacturing of decorative arts and crafts [1-2]. However, as well as many other materials, the disadvantage of parchment is instability to various types of influence (mechanical, thermal, chemical and biologically active environment, etc.) that being to change its properties, direct to the deterioration and even destruction [3]. In this case, one of the most important factors that determine the resistance of parchment material to damage is the method of treatment of hides and the type of used reagents. Therefore, in order to improve the producing technique, to expand the possibilities of using and preserving this specific material, it is important to be aware of the change in its properties over time under different conditions.

In view of the above, processes of artificial aging and sweat action of leather parchment, obtained by different technologies was investigate in many previous researches [4-7]. As determinative factors in this research, the effect of accelerated aging by high temperature and action of artificial sweat were selected which sufficiently reflect the influence that parchment can undergo during storage and exploitation.

2. Materials and methods

As investigated materials, samples of leather parchment (hereinafter simply parchment), obtained from sheep's skin by several known methods were used: group 1 (parchment for writing) according to the modern method, which involves two-stage liming with a lower (26 g/l) consumption of calcium hydroxide [4]; group 2 (parchment for restoration works) by the modern method on the basis of two-



stage liming with a higher (35 g/l) consumption of calcium hydroxide [5]; group 3 (transparent parchment) according to the ancient sulphide-lime method of liming [6]; group 4 (parchment for general purpose) by accelerated method of oxidative liming [7]. Common to all methods was the presence of two consistently performed technological blocks: the first which consisted of beam house processes and operations (washing, soaking, liming, flashing), and the second which consisted of finishing processes and operations in the form of drying, grinding, plasticization; in addition, in groups 2 and 3, bleaching was carried out, and in group 3 the filling with potassium alum salt [8].

In order to studying the behaviour of parchment in time-accelerated methods of artificial aging and determination of sweat action were used. The method of artificial aging consisted of keeping the samples in climate test chamber (70°C, 0% rH, and 72 h). Sweat resistance test was carried out by keeping the samples in a solution of artificial sweat which included: sodium chloride, ammonium carbonate, disodium phosphate, carbamide, lactic acid (25°C, 72 h) and after, samples dried under norm-climate condition (23±1°C, 60% rH) [10].

To establish the biochemical changes in collagen structure, the methods of gelatine yield and disc electrophoresis in polyacrylamide gel with the adding of sodium dodecyl sulphate were used [11-13]. For the yield of gelatine, the crushed parchment weighing about 0.5 g (in recalculation of absolute dry substance) was placed into a flask (100 ml volume) and 50 ml of distilled water was added. The flask was incubated in a thermostat for 2 hour at 90°C. After cooling, the contents of the flask were filtered and washed with distilled water. A Biuret reagent was added to the measuring flask and diluted with distilled water to the mark on a 100 ml volumetric flask. After holding the flask in a dark place for 20 minutes the optical density of gelatine solution was determined on a spectrophotometer (U-LAB102) at absorption mode 520 nm. The concentration of gelatine (%) was determined according to standard calibration curve [11].

For the analysis of proteins in solutions, in which samples of parchment were incubated disc electrophoresis method was used in 10% polyacrylamide gel (PAAG) in the presence of sodium dodecyl sulphate (SDS) in the Lemley system beneath denaturing conditions. Electrophoresis was carried out in a device for vertical preparative disc electrophoresis (BioRad, USA) in glass plates 1 mm thick with a current of 19 mA for concentrating gel and 35 mA for separating gel. The gels were stained with a 0.125% (w/v) Coomassie G-250 solution (dissolved in 25% (w/v) propan-2-ol and 10% (w/v) acetic acid), excessive colour washed with 0.8% (w/v) acetic acid. At staining the ratio of volumes of liquid and gel was 3:1, at washing was 5: 1 with two changes of solvent. A marker mixture of proteins with a molecular weight of 94 kDa (phosphorylase B), 67 kDa (albumin), 43 kDa (ovalbumin), 30 kDa (anhydrase), 20.1 kDa (soybean trypsin inhibitor) and 14.4 kDa (lactalbumin) were used (Amersham Biosciences) [12].

The concentration of protein in the solution was determined using Bradford method. To this end, 10 µl of 10% sodium hydroxide, 70 µl of distilled water and 2 ml of the working solution were added to 20 µl of sample. The working solution was prepared by mixing of 6 ml a stock solution (containing 10 ml of 95% ethanol, 20 ml of 85% phosphoric acid, 35 mg of Coomassie blue), 3 ml of 95% ethanol, 6 ml of 85% phosphoric acid diluting with distilled water to the mark on a 100ml volumetric flask. The concentration of the solution was measured on a Smartspec spectrophotometer (BioRad) at absorption mode 595 nm compared to control sample with distilled water. The amount of protein in solution (mg/ml) was determined according to standard calibration curve [13].

To obtain an apparent understanding of changing the properties of parchment as a result of accelerated aging (high temperature and action of artificial sweat) such important physical and mechanical indices of leather as hydrothermal stability, strength, percentage elongation, stiffness and thickness had been analysed which were evaluated by standard methods of testing leather materials on standard equipment's [14-17]. During the production of parchment to exclude the influence of topographical areas, which are different, the groups of samples have been complete by the asymmetric method [11]. The elastic-plastic properties of parchment were determined using indices of percentage elongation. The uniformity of leather properties on the area, presented as coefficient uniformity of tensile strength C_{unif} , which is the ratio of average values relatively index of tensile strength cross-

vertebral direction (σ_{cv}) to the average values index of tensile strength the longitudinal direction (σ_l), calculated according to formula [11]:

$$C_{unif} = \sigma_{cv} / \sigma_l$$

Reliability of obtained results was assessed by traditional methods of mathematical statistics, using the mean square deviation and arithmetic mean [18].

3. Results and discussion

Test results of parchment samples both after accelerated aging and after resistance, testing was given in Table 1 space required from which it follows that after the heat treatment and action of sweat the yield of gelatine decreases for specimens of groups 1, 2, 4 (and especially for group 4 by 28.2 and 53.1% respectively), and increases in group 3 by 69.8 and 8.7% respectively. The least (3.6-4.0%) changes in the indicator are observed in group 1 after action of sweat and group 2 after artificial aging. Particularities of structure of investigated parchment samples, caused by a greater degree of opened up of structure, broken or weakening of the intramolecular and intermolecular bonds, are found because of deeper yield of gelatine in groups 2, 3 (19.1 and 24.9%) after artificial aging, as well as in groups 1, 2 (21.1 and 17.5%) after influence of sweat.

Table 1. Results of physical and mechanical tests of parchment

Parchment after stage	Group 1	Group 2	Group 3	Group 4
1	2	3	4	5
Yield of gelatine (%)				
Initial sample	21.9	19.9	14.3	22.2
After aging	13.9	19.1	24.3	15.9
After sweat action	21.1	17.5	15.5	10.4
Shrinkage temperature (°C)				
Initial sample	94.0	87.0	93.0	70.0
After aging	65.5	64.0	68.0	68.5
After sweat action	65.0	63.0	66.0	67.0
Tensile strength σ (N/mm ²)				
Initial sample	39.4	65.8	24.8	49.3
After aging	25.9	32.6	34.4	59.5
After sweat action	29.8	24.5	30.6	39.5
Coefficient of uniformity of tensile strength C_{unif}				
Initial sample	0.65	0.74	0.71	0.77
After aging	0.45	0.67	0.62	0.65
After sweat action	0.29	0.50	0.56	0.53
Percentage elongation at the strain N/mm ² (%)				
Initial sample	16.0	15.0	18.0	17.3
After aging	11.6	10.0	13.5	11.0
After sweat action	12.5	11.5	13.0	10.5
Percentage elongation at tear load (%)				
Initial sample	41.0	40.5	46.5	33.5
After aging	19.3	21.7	28.8	24.2
After sweat action	23.0	23.5	29.0	29.0
Thickness (mm)				
Initial sample	0.44	0.50	0.48	0.40
After aging	0.37	0.42	0.45	0.30
After sweat action	0.48	0.48	0.61	0.39

Hydrothermal stability of parchment was determined by shrinkage temperature, which decreases in all cases: for groups 1-3, significantly almost a threefold (26.4%-30.9%), for group 4 only by few percent (2.1-4.3%). It should be noted that initially for group 4 this indicator was the lowest ($\leq 70^{\circ}\text{C}$) of all analysed groups for which it was at level of $87-94^{\circ}\text{C}$.

As with any other types of leather, one of the important functional properties of parchment is its strength, determined by the method of biaxial stretching. As a result of the test, the decrease in this index by 24.4-62.8% was found for group 1 and 2 after high temperature and action of artificial sweat and for group 4 only after action of sweat (by 19.9%). The strength of transparent parchment (group 3), on the opposite, increased by 23.4-38.7% for test specimens subjected to sweat aging and dry aging, respectively. Strength of the parchment made by the accelerated method of liming (group 4) changed ambiguously after the manipulations: increased by 20.7% after artificial aging and decreased by 19.9% after sweat action.

The least value index of the coefficient of uniformity of tensile strength indifferent directions of leather is observed in samples of group 1 (two-stage liming with lower consumption of calcium hydroxide) at all stages of the study; most of all in samples of group 2 (two-stage liming with higher consumption of calcium hydroxide) before and after artificial aging. In all cases, the influence of artificial aging and action of sweat is observed in reducing of the coefficient of distribution uniformity index of parchment within 9.5-30.8 and 21.1-55.4% in this order.

A decrease of percentage elongation at the strain after artificial aging by 25.0-36.4% and after action of sweat by 21.9-39.3% was experimentally established. The greatest decrease of this index 36.4-39.3% is observed for group 4 (accelerated method of liming), the most for group 3 (transparent parchment). After artificial aging and action of sweat, the percentage elongation at tensile strength of samples of all leather decreased: most for group 1 (53.0 and 43.9%), the least for group 4 (27.8 and 13.4%). By the way, the initial samples of parchment were the least flexible in group 4, and conversely in group 3, were from the beginning it had been more flexible and retained this ability after additional manipulations.

The thickness of initial parchment was at a level of 0.40-0.50 mm. The influence of artificial aging has been founds in decreasing the thickness of samples of all groups: least amount (6.3%) for group 3, most (25.0%) for group 4 and almost the same amount (15.9-16.0%) for groups 1 and 2. The influence of action of sweat on the thickness of parchment was completely different. Thus, for samples of groups 2 and 4 there is only a slight decrease (2.5-4.0%) of this index, while for group 1 and especially for group 3 samples there is a more significant increase by 9.1% and 27.1% respectively.

To assess the change in structure of parchment as a result of aging procedure the process of releasing proteins and their fragments into surrounding solution, in which the samples of this biopolymer were incubated, was investigated by determining the amount of total protein and qualitative composition of proteins-protein fragments in the solution using disc electrophoresis method. As can be seen from table 2, artificial aging and action of sweat have significant impact on native structure of parchment, resulting in a certain release of protein macromolecules and their fragments into surrounding solution. At the same time, the minimal manipulative influence on this process was found in group 2, maximal in group 1.

Table2. Total protein concentration (mg/ml) in incubated parchment solution

Stage of research	Group 1	Group 2	Group 3	Group 4
Initial sample	5.50	3.75	8.00	7.00
After aging	13.25	1.62	7.37	9.37
After sweat action	10.37	4.50	6.25	3.62

Based on the analysis of samples electrophoregram obtained by using the method of disc electrophoresis in PAAG, it is established (Table 3) that aging procedure used has different influence on the qualitative composition of proteins that are released from the parchment structure into solution. Thus, the complete absence of proteins and their fragments with a molecular weight <35 kDa and the

most content of heterogeneous molecular weight group of proteins in the range of 150-100 kDa was found. Perhaps this is due to the fact that the parchment consists mainly of collagen polymer structures and the manipulations carried out lead to a partial destruction of collagen and release it into solution. This in turn, can be a reason for changing the stability and integrity of parchment. Also, a certain amount of protein with a molecular weight in the range of 100-67 and 67-35 kDa was detected. The last group, most probable, mainly consists of fragments of those fibrillar protein complexes that are part of the structure of the biopolymer.

Table3. Results of electrophoresis analysis of incubated parchment

Stage of research	Group 1	Group 2	Group 3	Group 4
1	2	3	4	5
Molecular weight >150 kDa				
Initial sample	163	164,155,151	156	151
After aging	161	159	152	152
After sweat action	161	158	150	–
Molecular weight 150-100 kDa				
Initial sample	143, 118, 112	133, 119, 114, 104	141, 115, 107	122, 115, 107
After aging	146, 125, 116	146, 123, 111, 103	141, 117, 107	128, 117, 110
After sweat action	143, 121, 111, 104	141, 115, 108, 100	120, 110	124, 115, 104
Molecular weight 100-67 kDa				
Initial sample	79	90, 82, 73	79	–
After aging	–	84	89, 78	95
After sweat action	78	94, 75	92, 84	94, 84
Molecular weight 67-35 kDa				
Initial sample	65, 45	63, 58, 52, 49, 46	63	–
After aging	–	66	62	–
After sweat action	65	62, 44, 42	62, 51, 46	64, 51, 46
Molecular weight < 35 kDa				
Initial sample	–	–	–	–
After aging	–	–	–	–
After sweat action	–	–	–	–

4. Conclusions

The processes of artificial aging by high dry temperature and sweat of the samples of parchment, obtained from sheep's skin by different methods were investigate.

To obtain an apparent understanding of changing the properties of parchment after carried out aging such important physical and mechanical indices as hydrothermal resistance stability, strength and percentage elongation were analysed. Methods of gelatine yield and gel electrophoresis in a polyacrylamide gel were used to determine the biochemical changes of the collagen structure, as the main component of the parchment.

Experimentally it was found that in respect to the indices of hydrothermal stability and percentage elongation samples of parchment produced by accelerated method of liming showed highest resistance to the influences of high temperature and sweat while in respects of strength for transparent-parchment. The obtained data may be due to different degrees of separation of structural elements during the liming process, the presence of plasticizing component that can prevent the influence of external factors. By evaluating the process of release of parchment proteins in the surrounding solution, considerable influence of aging on the structure of parchment was revealed that could predict the probability of losing the integrity of parchment and, consequently, its resistance to wear.

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