Determination of the Amino acid Composition, Structure and Properties of the Archaeological Leather Before and After Restoration

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Specimens of new and archaeological leather before and after restoration were investigated. To conduct qualitative and quantitative analyses of amino acid composition of the new and archeological leather before and after restoration, ion-exchange liquid-column chromatography was employed. It was determined that both the ageing of leather and its further restoration affect the amount of polar amino acids in the leather, primarily lysine, arginine, and asparagine and glutamine amino acids. TG, DTG studies of the new and archeological leather before and after restoration allowed a conclusion to be drawn that the prevailing process in the thermal destruction of the new leather is the breakage of cross-linking formed in tanning. In its turn, the prevailing process in the thermal destruction of the restored leather is oxidation of the products of reaction between collagen and organic substances introduced during the restoration.

Keywords: collagen, leather, amino acid composition, ion-exchange liquid-column chromatography thermal gravimetric studies

Leather has been used from ancient times for manufacturing heritage items. In the process of restoring and preserving leather heritage items, evaluating the degradation level of the material base is essential for the purpose of establishing both the degradation causes and the optimum intervention methods. Thus, various technologies are used to get information as complex and complete as possible, by means of non-destructive, discretionary and non-invasive.

Leather is composed mostly of the protein collagen, which displays a discrete structural hierarchy, from the molecular to microscopic levels [1-6]. Such visual macro- and microscopic damage as loss of strength, fraying, brittleness and fibre gelation in leather can correlate with the changes in collagen structure. In some cases, the changes can be connected with chemical transformations caused by the oxidation and hydrolysis processes which occur in protein chains and acid residues on a molecular level. It is known that there is a relationship between the visual damage of leather, which manifests itself in its physical properties on microscopic and macroscopic levels, and the changes revealed by structural, thermochemical, thermophysical and chemical analyses [5-11], in which the assessment is carried out in the leather fragments.

The goal of this work was the study of deterioration and degradation of archeological collagen based artefacts in order to develop science-based technologies for leather recovering, which would allow leather items to be stored and exhibited.

Experimental part

Samples of new and archaeological leather from Bakhchisarai site (15-16[th] century) before and after restoration were investigated. To carry out complex studies, leather specimens from Durbe Burial site of Khan's Hadji-Geray tomb no. 16 were used [9]. The cover of coffin from tomb no. 16 was covered with heavy lavishly-decorated greenish cloth with floral design. Under the cloth a leather covering was revealed (fig.1). It consisted of four separate pieces of leather from different raw-hide types (calfskin, sheepskin) nailed on the cover.

Restoration of the leather was conducted in the Army Museum of Romania and involved cleaning from dust and dirt, disinfection and conditioning. At first the leather was cleaned from dust and dirt with a soft brush. Then it was rinsed with a special composition consisting of nonionic SAS solution (0.1 %), thymol (0.5 %) and glycerine (15 %). For the leather conditioning a mixture of glycerine (15 %) and distilled water was used. Thymol was used for the disinfection. Glycerine acted as a plasticizer. The treatment of leather with the mixture of glycerine and distilled water was carried out several times.

To conduct qualitative and quantitative analyses of amino acid composition of the new and archeological

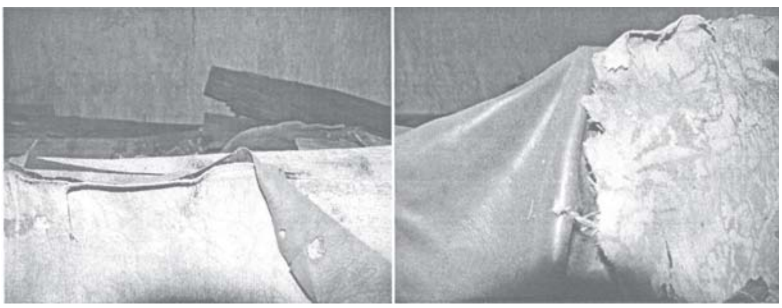

Fig. 1. Tomb no. 16 from Durbe Burial site of Khan's Hadji-Geray

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leather before and after restoration, ion-exchange liquid-column chromatography with the 339 M automatic analyzer (Microtechna, the Czech Republic) was employed.

The influence of ageing and restoration on the thermal properties of derma collagen was studied by differential thermal analysis. TG and DTG analysis were carried out by using the Paulig-Erdei derivatograph over the 22-760 °C temperature range in the air with simultaneous removal of destruction products. The temperature ranges of the specimens under study were evaluated from differential mass loss curves. The temperature range over which the shrinkage of collagen fibres takes place is visually determined by the MHT method [5, 6, 10-13].

The new vegetable tanning leather treated with mimosa was used to determine changes in physico-chemical properties of leather and compare them according to ICOM CC recommendations.

Results and discussions

Visual evaluation of the archeological leather showed that the leather had such faults as brittleness, rigidness, warping (buckling), cracks on the grain and notched edge, noticeable damage caused by moisture, mineral residue and moldy bloom, traces of blue metallic residue (which is likely to be due to copper oxidation).

Visual evaluation of the leather after restoration showed that the leather became softer and more elastic, with smooth grain, without warping, though some topographical sections of the leather demonstrated stripping of the retiform layer from the papillary one.

According to [12] the destruction of vegetable-tanned leather results from two processes: acid hydrolysis and oxidative degradation of collagen and tannins. In oxidative degradation of collagen there is observed transformation of positively charged amino acid residues into negatively charged ones, whereas in undamaged collagen the positively charged and negatively charged amino acid residues are balanced. A change in this balance, including that caused by oxidative degradation of leather, leads to leather destruction. The relationship $B = \frac{\sum(Arg, Lys)}{\sum(Asp, Glu)}$ between the amounts of basic and acidic amino acids reflects the degree of oxidation decomposition of collagen. In this study the content of basic amino acids in the new and archeological leather was determined with results being presented in figure 2.

It was determined that both the ageing of leather and its further restoration affect the amount of polar amino acids in the leather, primarily lysine, arginine, and aspartic and glutamic amino acids. Basically, the oxidation decomposition of collagen decreases the amount of basic amino acids proline and oxyproline, whereas the amount of acidic acids increases, which is partly due to an increasing amount of collagen decomposition products. In the case of oxidation mechanism of collagen decomposition transformation of positively charged amino acid residues into negatively charged ones occurs. Hydrolytic decomposition of collagen gives an increase in the amount of basic amino acids due to the peptide bond breakage.

The total amount of arginine and lysine for new leather is 7.11 %, whereas for old leather it is 6.18% that is the

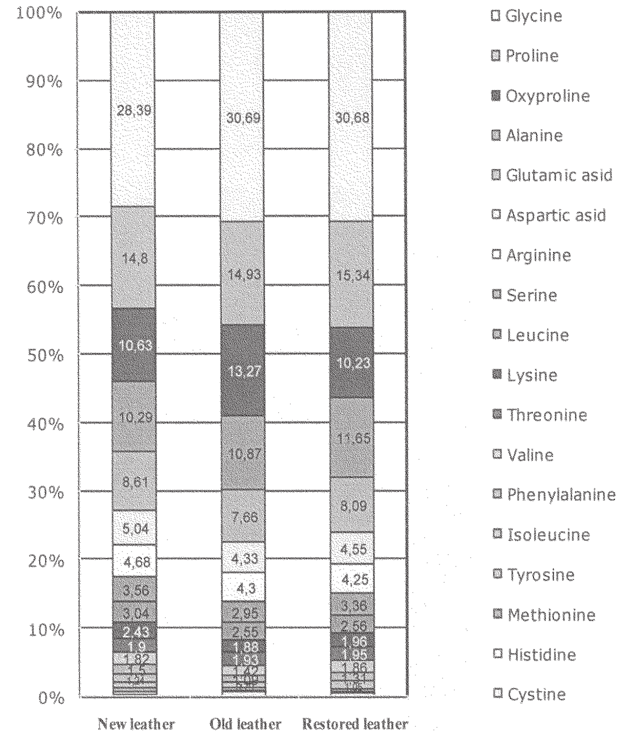

Fig.2. Results of an ion-exchange liquid-column chromatography

difference is 0.93%. Correspondingly, if there was only the process of oxidation destruction in collagen, the content of acidic amino acids would have to increase by the same value. However, in fact the content of amino acids decreases by 1.66%. This proves that ageing of leather results from both oxidation and hydrolysis. The B value for the new leather is 0.52, whereas for the old and restored leather it is 0.49-0.51, which reflects the degree of oxidation decomposition of collagen.

Leather restoration is accompanied by a considerable increase in the amounts of substances extracted by organic solvents, which influences favorably the thickness of leather and its elasticity. However, the shrinkage temperature of the leather gets somewhat lower (table 1).

A similar phenomenon was reported by Dr. Rene Larsen [12], but it has not been consistently explained yet. One of the possible causes is increased heterogeneous of derma structure, which must result from the breakage of a certain number of bonds caused by the separation of structural elements of derma, which can be proved by thermal analysis.

Thermal gravimetric studies allowed a conclusion to be drawn that thermooxidation destruction of the new and archeological leather before and after restoration is a multistage process which involves at least three stages (fig. 3). The maximum rate of the first stage of thermal oxidation, $d\Delta m/dt_I$, min^{-1} relates to the degree of leather destruction. The sample of archeological leather has the highest degree of destruction that reveals the highest rate of destruction at the first stage.

The data from the figure 4 show that the lowest mass loss for the samples of archeological leather at the II stage of destruction is related to partial splitting of collagen macromolecules and, therefore, increasing hetero-

Table 1
QUALITATIVE INDEXES OF LEATHER

Parameter	New leather	Old leather	Restored leather
Shrinkage temperature, °C	80	56	52
Thickness, mm	1.20	0.85	1.18

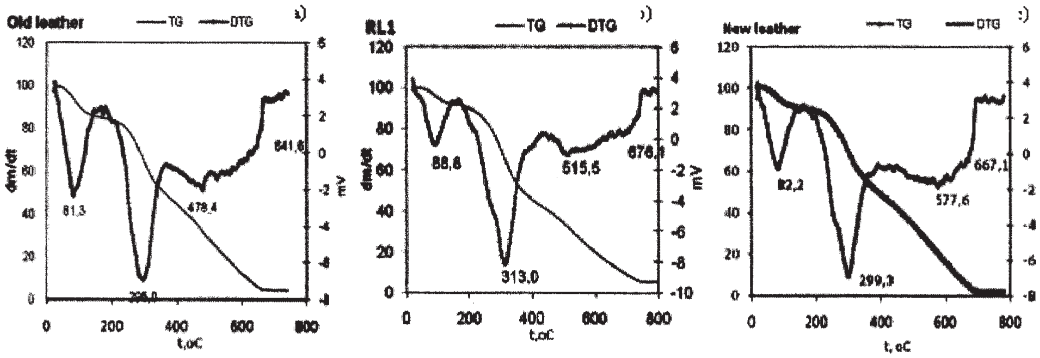

Fig. 3. The results of thermogravimetric studies of leather: (a) before, (b) after restoration and new (c)

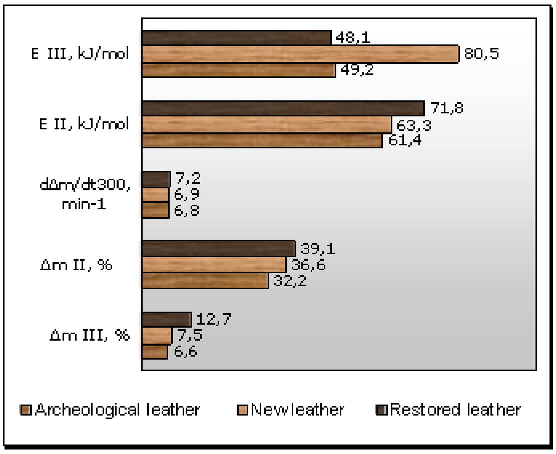

Fig. 4. Parameters of the thermooxidation destruction for leather before and after restoration

geneousness of its structure. The prevailing process in the thermal destruction of the restored leather is oxidation of the products from reaction between collagen and organic substances introduced during the restoration as evidenced by high activation energy at the II stage of destruction E_{II}. In its turn, the prevailing process in the thermal destruction of the new leather is the breakage of cross-linking formed in tanning as evidenced by high activation energy at the III stage of destruction E_{III}.

The change of the destruction mechanism from hydrolytic to hemolytic over a wide range of temperatures, 163.2 and 160.6°C for the old and restored leather, proves that the increasing heterogeneous of the structure is caused by ageing and restoration. Thus, determining the effects of restoration on structural-phase transformations of derma collagen and physico-chemical properties of leather will promote creating science-based technologies for leather restoration, which will enable leather articles to be properly stored and exhibited.

Conclusions

It was determined that both the ageing of leather and its further restoration affect the amount of polar amino acids in the leather, primarily lysine, arginine, and asparagine and glutamine amino acids. Basically, the oxidation decomposition of collagen decreases the amount of basic amino acids, whereas the amount of acidic acids increases, which is partly due to an increasing amount of collagen decomposition products. Hydrolytic decomposition of collagen gives an increase in the amount of basic amino acids due to the peptide bond breakage.

TG, DTG and DTA studies of the new and archeological leather before and after restoration allowed a conclusion to be drawn that the prevailing process in the thermal destruction of the new leather is the breakage of cross-linking formed in tanning. In its turn, the prevailing process in the thermal destruction of the restored leather is oxidation of the products from the reaction between collagen and organic substances introduced during the restoration.

The shrinkage temperature of the leather after restoration gets somewhat lower. One of the possible causes is increased heterogeneous of derma structure, which must result from the breakage of a certain number of bonds caused by the separation of structural elements of derma.

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