

Modification of Leather Surface Using Low-Pressure Plasma and Antimicrobial Reagent

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Abstract. Investigations, which are related to plasma efficiency on the treated leather surface, are significant in the development of ecologically and economically friendly processes in obtaining material of desired functional properties. Through the pretreatments using plasma different chemical-physical reactions in the surface layer of treated leather are occurred resulting in improved reactivity. In this paper, modification and functionalization of bovine leather using 1,2,3,4-butanetracarboxylic acid and chitosan were explored. Pretreatments of leather samples were realised using argon and oxygen plasma to assess various influence of chemically reactive oxygen and inert argon gas. Two different bovine leathers - chrome tanned leather and leather tanned with synthetic tanning agent (Cr-free) were chosen for treatments. Analyse of the surface morphology was conducted with SEM microscopy, while the chemical changes using ATR-FTIR spectroscopy. Antimicrobial effectiveness of treated leather was tested with qualitative Agar diffusion plate test against two bacterial *Staphylococcus aureus* and *Klebsiella pneumoniae*. Obtained results indicated how applied oxygen and argon plasma pretreatments in optimized process conditions contribute to the improvement of tested functional properties. Achieved surface changes positively affected on leather surface reactivity and antimicrobial effectiveness, particularly Cr-free leather.

1. Introduction

The leather industry is one of the huge polluters of the environment, because of that it is important to develop environmentally friendly procedures. A major problem is the proven treatments, which are mainly carried out by conventional procedures, and are most often very harmful to the environment. The use of plasma as a medium for property modification is an acceptable technique because its application could completely replace, or at least shorten the processing time and use of large amounts of water and chemicals in order to achieve the desired properties of leather product. The main advantages of cold plasmas are

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applicability to all kinds of materials without negative effects on the basic properties of the material, without high consumption of chemicals, water and energy. Plasma treatment impact to the modification surface layer of the treated leather, resulting in the surface cleaning and its activation thus achieving better hydrophilicity (absorptivity) for some new chemical reactions. At low-pressure plasma, it is possible to apply chemical agents in monomeric form, which have the ability to polymerize with the substrate, whereby cleavage or crosslinking of the agents on the surface can take place, in order to achieve and /or improve the functional properties [1].

Microorganisms have a significant, often negative impact on human health in the form of the development of unpleasant odours, stains and fungal diseases, so today there are a very large number of different antimicrobial agents. Recently, it has become important as an antimicrobial agent gaining chitosan due to its excellent biocompatibility and biodegradability, excellent antimicrobial properties and great availability in nature. Therefore, it was used in this work as an antimicrobial agent with environmentally friendly technology of cold low-pressure plasma on cowhide, which is coated with various agents in the production phase. The prepared chitosan solution was applied to treated bovine leather by the method of horizontal spraying of the agent with the aim of achieving antimicrobial efficacy against targeted gram-positive *Staphylococcus aureus* and gram-negative *Klebsiella pneumoniae* bacterial species [2, 3].

Leather making is one of the oldest human crafts present in history for more than 3000 years. Humans used the leather of slaughtered animals to protect their bodies from the weather and injuries, and gradually for other purposes, such as making boats, tents, and other products [4]. The leather is one of the fundamental importance to the animal because it has various physiological functions, the most important of which are the regulation of body temperature, storage of nutrients, protection and excretion of waste products [5]. The waterproof layer of the leather acts as a barrier against various infections, preventing the absorption of water from the outside and the loss of water from the inside. Animal leather consists of the three main layers: epidermis, dermis and hypodermis. Dermis contains intertwined collagen fibers, which represent the most important layer of raw leather for obtaining the finished product, while the remaining two layers (epidermis and hypodermis) are removed during the processes (Fig. 1.) [5, 6].

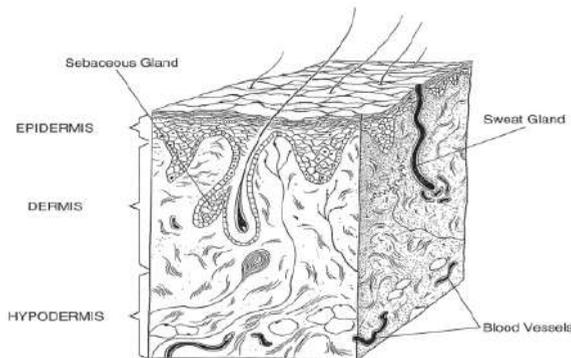


Fig. 1. Cross section of animal leather [6]

Finished leather is a product that is created by processing of raw animal leather in the tanning process by various means, of which the most widely used are chrome tanning, during which it becomes more durable and applicable. Tanning agents are agents that

chemically react with the collagen molecule that builds the leather structure, stabilizing the triple helical structure of the collagen core, thus achieving leather resistance to chemical, thermal, and microbiological degradation. It has a complex morphological structure and its processing is extremely complex and requires the implementation of many process steps, of which the emphasis is on tanning in which it acquires its characteristic and unique properties and can be used for different purposes [5-7].

The emphasis of this paper is an introducing of environmentally friendly processes of the surface modification of semi-processed bovine tanned leathers, by application of plasma and ecological bio-agent chitosan, in order to achieve satisfactory functional (antimicrobial) properties of treated samples. The main goal is to achieve the desired properties by using of environmentally friendly processes with the lowest possible consumption of energy, water and chemicals to maximally preserve natural resources, with the purpose of protecting the environment and human health.

2. Materials and Methods

2.1 Material

The tests were carried out on two different semi-processed bovine leathers - chrome tanned leather and leather tanned with synthetic tanning agent (Cr-free). The basic characteristics of the tested leather samples are shown in Table 1.

Table 1. Basic characteristics of the examined leather samples

Sample name and label	Thickness [mm]	Sample description	Sample face appearance
Chrome tanned leather (sample label: <i>Cr-leather</i>)	1,1 - 1,3 mm	semi-processed bovine chrome tanned and hydrophobic leather full of natural face	
Leather tanned with synthetic tanning agent (sample label: <i>Cr-free leather</i>)	1,1 - 1,3 mm	semi-processed cowhide with a full natural face, synthetically tanned; not hydrophobic	

2.2. Methods of treatments

2.2.1 Plasma pretreatment

Plasma pretreatments were realized using oxygen and argon gases (high purity 99.998%, by Messer) at a defined parameters of pressure and gas flow rate, at constant frequency of 40 kHz in a low-pressure plasma system (NANO LF, Diener electronic), Table 2. For determination of the effect of exposure time and power on leather surface properties, time of 10, 20 or 40 minutes under 500 and 800 W were explored. All samples were dried at 50 °C for 24 hours prior to plasma pretreatment to remove the moisture. Plasma pretreated samples were treated with BTCA immediately upon completion of pretreatment.

Table 2. Plasma pretreatment conditions

Sample label	Gas	Time, t [min]	Power, P [W]	Pressure, p [Pa]	Gas flow, q [cm ³ /min]	lxd [mm]
<i>Cr-leather</i>	oxygen	20	500	32	220	100x100
<i>Cr-leather</i>	oxygen	20	800			
<i>Cr-free leather</i>	oxygen	10	500			
<i>Cr-free leather</i>	oxygen	10	800			
<i>Cr-leather</i>	argon	40	800			
<i>Cr-free leather</i>	argon	40	500			

* Frequency of low-pressure plasma system is constant - 40 kHz.

2.2.2 Treatment with BTCA

Polymerization process directly in the plasma chamber, was used with BTCA as reagent (in monomer bottle) at a pressure of 50 Pa, gas flow of 200 cm³/min and frequency of 40 kHz in a plasma system. The effects of exposure time for 30 minutes under the power of 100 W, on the polymerization rate were explored.

2.2.3 Spraying method with Chitosan

Second part of the experiment was realised using 1% chitosan (C₅₆H₁₀₃N₉O₃₉, medium molecular weight 100-300 kDa) solution on the plasma pretreated and BTCA treated leather samples by spraying method for achievement of antibacterial effectiveness. For enhanced deposition process of antibacterial agent, leather was sprayed with chitosan solution for 5 second and dried at 65 °C for 15 minutes.

2.3 Methods of Analysis

2.3.1 Surface morphological analysis

Surface changes of morphology were analysed using scanning electron microscope (SEM) (type JEOL LV-6060) at 100x and 2000x magnifications. The scanning electron microscope works by using a focused electron beam to scan the surface of the test sample to give information about the characteristics of the sample surface. When the electron beam is focused on the sample, various interactions occur, such as the emission of secondary and return primary electrons. A beam of electrons erupts electrons that are part of the cause atoms. The electron energies from the sample are collected and measured by detectors, and a pseudotwodimensional image is created with the help of a microprocessor. In order to obtain conductivity, samples were coated with gold for 20 minutes, using a sputter coater prior to analysis [8].

2.3.2 Chemical changes

The chemical changes were analysed using ATR-FTIR spectroscopy (Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy). Infrared spectroscopy is based on the interaction between IR radiation and molecules, i.e. groups of two or more atoms connected by a chemical bond. The material absorbs infrared radiation, and the amount of absorbed radiation is measured as a function of energy that can be expressed in the form of frequency. IR radiation is absorbed only if its frequency coincides with the vibration frequency of the molecular bond. The absorbed radiation is reflected as a "jump" on the spectrogram. The analysis of the samples on the device was performed by the method of

Attenuated Total Reflectance (ATR), in which the IR light was completely reflected when it reached the surface of the ATR crystal. When the sample is placed on the crystal surface, the IR beam penetrates the sample to a certain depth depending on the angle of incidence of the beam and the refractive index of the ATR crystal, as well as the type of sample [9].

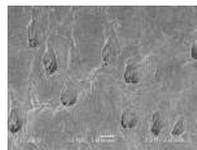
2.3.3 Antimicrobial efficacy

Antimicrobial effectiveness of treated leather samples was conducted using qualitative Agar diffusion plate test against two bacterial species *Staphylococcus aureus* (ATCC 25 923) and *Klebsiella pneumonia* (ATCC 11 296). The test procedure for antimicrobial efficacy of treated samples was performed in accordance with the standard HRN EN ISO 20645:2008, as well as the final assessment of antibacterial activity included the inhibition zone and the growth of the bacteria under the specimen.

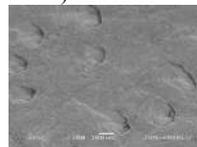
3. Results and discussion

3.1 SEM analysis of leather surface

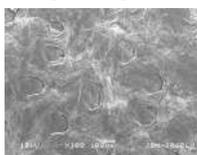
Based on the obtained images taken by scanning electron microscopy, the surface morphology characteristic of tested samples with observable follicles visible at a magnification of 100x is observed (Fig.2 a-d). By comparing images of the leather surface before and after pretreatment with O₂ plasma at a magnification of 2000x, a certain cleaning of the surface with residual surface "damage" of the face is observed (Fig. 2. b1). After treatment with polycarboxylic acid (BTCA) and chitosan on the pretreated surface with oxygen plasma (Fig. 2. c1 and d1), a slight smoothing and covering of the damaged parts with the applied agent was observed, which may indicate crosslinking of polycarboxylic acid on the sample surface and bonding.



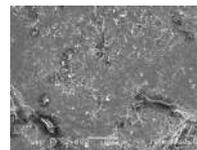
a) untreated



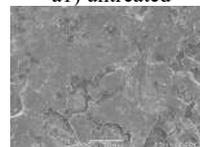
b) O₂ plasma pretreated



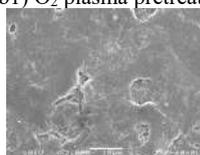
c) O₂/BTCA treatment



a1) untreated



b1) O₂ plasma pretreated



c1) O₂/BTCA treatment



Fig. 2. SEM images of chrome tanned leather with 100x (left) and 2000x (right) magnifications: a, a1) untreated; b, b1) O₂ plasma pretreated; c, c1) O₂/BTCA treatment and d, d1) O₂/BTCA/CH treatment

By analysing microscopic images of chromium tanned samples, after pretreatment with argon plasma, granular segments are visible on the surface of tested sample, and the surface is rougher with more pronounced and open follicles. Treatment with chitosan after pretreatment with Ar plasma makes the surface smooth and the follicles are closed (Fig. 3. b and b1). After treatments with BTCA and chitosan, an increased amount of agent is observed on the leather surface (Fig. 3. c1).

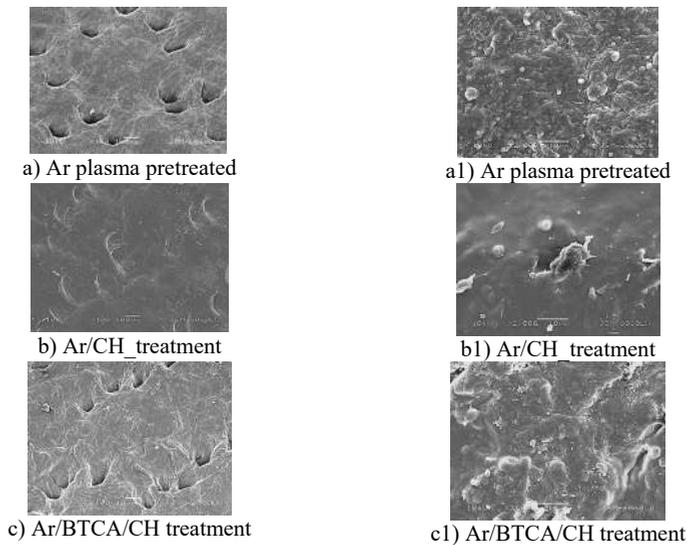


Fig. 3. SEM images of a chromium tanned leather sample with magnifications of 100x (left) and 2000x (right): a, a1) argon plasma; b, b1) Ar/CH treatment and c, c1) Ar/ BTCA/CH treatment

By analysing the surface of the tanned sample with synthetic tanning agent, after pretreatment with oxygen plasma (Fig. 4), the surface of the sample was cleaned of present impurities visible on the untreated sample (Fig. 4. a1), but also additionally "damaged" surface layer (face) below which collagen fibres (Fig. 4. b1). After the treatments with BTCA and chitosan, the surface is closed, smoothed and covered with the visible content of the applied agent (Fig. 4. c1, d1).

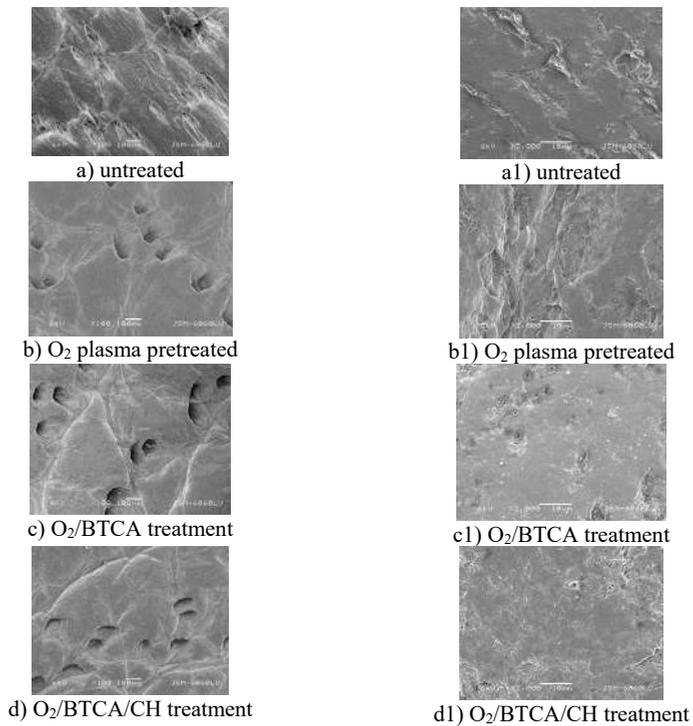


Fig. 4. SEM images of synthetic tanned leather with 100x (left) and 2000x (right) magnifications: a, a1) untreated; b, b1) O₂ plasma pre-treated; c, c1) O₂/BTCA treatment and d, d1) O₂/BTCA/CH treatment

By microscopic analysis of the surface of the tanned sample with synthetic tanning agent, after pretreatment with argon plasma, the surface of the sample is more open in the presence of surface craters formed due to the action of argon gas (Fig. 5. b1). After application of the crosslinking agent BTCA and antibacterial agent chitosan, the agents bound to the collagen fibres of the sample, which is visible at image with magnification of 2000x after treatment Ar/BTCA/CH (Fig. 5. c1).



a) Ar plasma pretreated

a1) Ar plasma pretreated

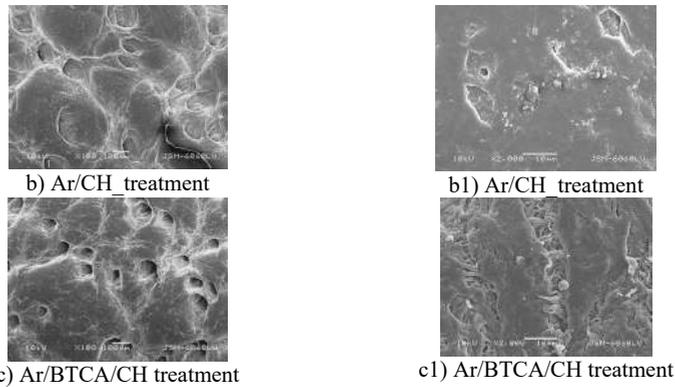


Fig. 5. SEM images of synthetic tanned leather with 100x (left) and 2000x (right) magnifications: a, a1) argon plasma; b, b1) Ar/CH treatment and c, c1) Ar/BTCA/CH treatment

3.2 Results of FTIR-ATR functional group analysis

The chemical structure of the surface of untreated and treated samples was analysed, i.e. the presence of characteristic wave bands (peaks) corresponding to functional groups. For better comparison, each graph shows the spectra of untreated samples and the spectra of samples after plasma pretreatments/BTCA and chitosan treatments in the spectral range of the mean IR spectrum from 4000 cm^{-1} to 600 cm^{-1} .

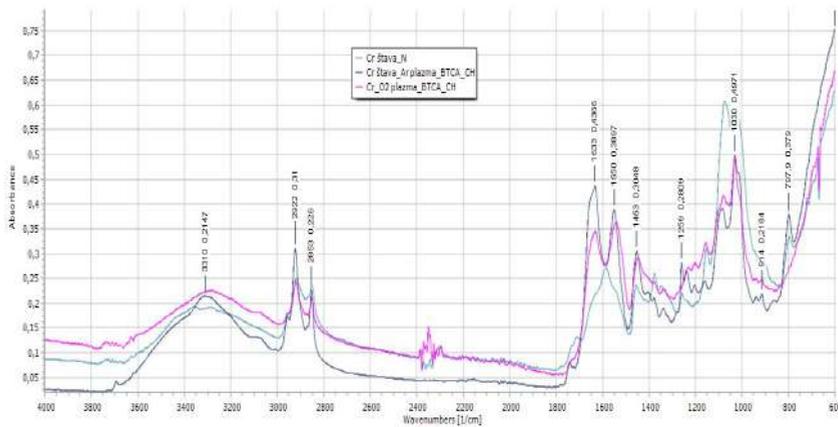


Fig. 6. Absorption FTIR spectra of chromium tanned leather sample before and after treatment with O_2 or Ar/BTCA/CH

By comparing the spectra shown in Fig. 6., the intensity of the absorption bands of the treated samples is observed in relation to the untreated ones at 2922 cm^{-1} , 1633 cm^{-1} , 1550 cm^{-1} , 1453 cm^{-1} and 797 cm^{-1} , which indicate that intense vibrations occur on the $-\text{NH}$, $-\text{NH}_2$, $-\text{CH}$, $-\text{CH}_2$ groups and $-\text{C}=\text{O}$ groups, which show that polycarboxylic acid (BTCA) and chitosan are very likely to bind to the substrate surface. The presence of absorption

peaks of the treated samples at 1633 cm^{-1} and 1550 cm^{-1} , which are not present in the untreated sample, and which correspond to the -C=O and -NH_2 groups is observed. That may indicate the binding of chitosan to the substrate surface. There is also a decrease in the intensity of the absorption peak at 1030 cm^{-1} , which indicates reduced tensile vibrations in the -C=O group [10-13].

The FTIR-ATR spectrum of the tanned sample with synthetic tanning agent (Fig. 7.) has marked absorption bands in the spectral range at characteristic wavenumbers where at 3311 cm^{-1} and 3083 cm^{-1} they indicate tensile vibrations -NH group of amino acids, at 2921 cm^{-1} -CH asymmetric deformation vibrations, at 1630 cm^{-1} tensile vibrations -C=O group. The higher intensity of the absorption bands of the processed samples was expressed at wavenumbers at 2921 cm^{-1} for -CH groups, at 2852 cm^{-1} -CH_2 groups, at 1630 cm^{-1} for -C=O groups, at 1551 cm^{-1} for -NH_2 deformation vibrations, at 1315 cm^{-1} for -COC- tensile vibrations, at 1236 cm^{-1} for -C=O groups and at 780 cm^{-1} for aromatic -CH groups. The intensity of the absorption bands of the sample treated with argon plasma, with BTCA and chitosan is higher at 1630 cm^{-1} , 1551 cm^{-1} and 780 cm^{-1} , which may indicate better binding of chitosan and BTCA to the substrate after pretreatment with argon plasma. The intensity of the absorption bands at the wavenumbers at 1158 cm^{-1} , 1103 cm^{-1} and 1032 cm^{-1} , which indicate reduced vibrations at -C=O , and -CH groups, was reduced [10-13].

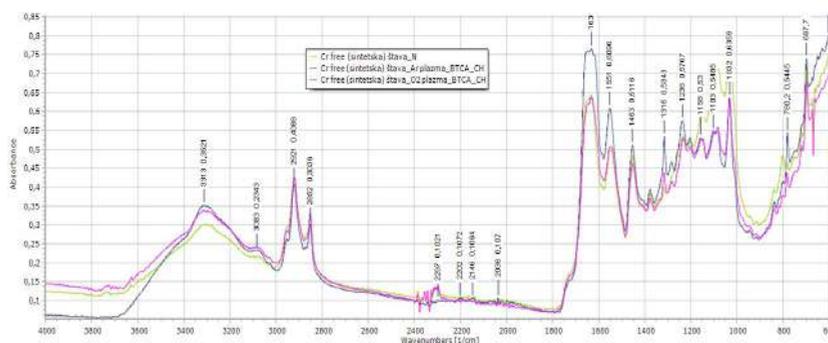


Fig. 7. Absorption FTIR spectra of a synthetic tanned leather sample before and after treatment with O_2 or Ar/BTCA/CH

3.3 Antibacterial activity against selected bacteria

The results of antimicrobial efficacy of untreated and treated leather samples against specified bacteria *Staphylococcus aureus* and *Klebsiella pneumoniae* are shown in Table 3.

Table 3. Antibacterial activity of untreated and treated leather samples.

Sample label	Treatment	Growth under the specimen	Assessment*
<i>Staphylococcus aureus</i>			
<i>Cr-leather</i>	Untreated	slight	limit of efficacy
	O_2 /BTCA/CH	none	good effect
	Ar/BTCA/CH	slight	limit of efficacy
<i>Cr-free leather</i>	Untreated	none	good effect
	O_2 /BTCA/CH	none	good effect

	Ar/BTCA/CH	none	good effect
<i>Klebsiella pneumoniae</i>			
<i>Cr-leather</i>	Untreated	none	good effect
	O ₂ /BTCA/CH	none	good effect
	Ar/BTCA/CH	medium	insufficient effect
<i>Cr-free leather</i>	Untreated	none	good effect
	O ₂ /BTCA/CH	none	good effect
	Ar/BTCA/CH	none	good effect

*Assessment is based on HRN EN ISO 20645:2008 - "Determination of antibacterial activity - Agar diffusion plate test", where "good effect" is described as "no growth" under the specimen, "limit of efficacy" as "slight growth (only some restricted colonies or growth nearly totally suppressed)" under the specimen and "insufficient effect" is described as "growth" of bacterial colonies under the specimen.

Both of samples show a good effect against *Staphylococcus aureus* after treatments. Tanned sample with synthetic tanning agent show significant antimicrobial activity prior to plasma pretreatment and treatment with agents (BTCA and chitosan). In the untreated chromium tanned sample there is a slight growth and without present zone of inhibition, which indicates that the antimicrobial effect is limited. Although, after treatment with plasmas and agents, zone of inhibition is not evident, bacterial growth below the sample is not present indicating good antimicrobial effect. The chromium tanned sample after pretreatment with argon plasma and treatment with the agents resulting in growing of tested bacteria, and it's assumed that no chitosan particles have bound to its surface, or the sample is contaminated during the antibacterial testing.

The obtained results in which the antimicrobial effect of the treated leather samples against the negative bacteria *Klebsiella pneumoniae* were tested show a generally good antimicrobial effect, which is manifested without bacterial growth and without a visible zone of bacterial inhibition. Slightly poorer results are shown by the chromium tanned sample after pretreatment with argon plasma and treatment with agents whose presence reduced its antimicrobial efficacy.

4. Conclusions

Morphological changes of leather samples after pretreatment with oxygen and argon plasma were confirmed by analysis of SEM images where the 'cleaning' of the surfaces of the chromium tanned and Cr-free tanned sample is visible. After treatment with polycarboxylic acid (BTCA) and chitosan on pretreated plasma surfaces, a slight smoothing and covering of the damaged parts with applied agents is visible, which may indicate on crosslinking of polycarboxylic acid on the sample surface and binding of chitosan particles.

By analyzing the chemical structure of the surfaces of untreated and treated samples using FTIR-ATR spectroscopy, absorption peaks at 1630 cm⁻¹ and 1550 cm⁻¹ corresponding to the -C=O and -NH₂ groups are observed. An increase in the intensity of the absorption peaks may indicate the binding of chitosan to the surface of the substrate.

The applied treatments achieved satisfactory antimicrobial efficacy of both tested leather samples, especially the Cr-free tanned sample after pretreatment with oxygen and argon plasma and treatment with chitosan as antibacterial agent.

This work has been fully supported by the Croatian Science Foundation under the project (IP-2016-06-5278). Work (SEM analysis) was also financed by Slovenian Finance Agency, Slovenia (Infrastructure Centre RIC UL-NTF).

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