PLASMA PRE-TREATMENTS IMPROVES ANTIMICROBIAL PROPERTIES OF BOVINE SPLITTED LEATHER

Sanja ERCGOVIĆ RAŽIĆ; Jadranka AKALOVIĆ; Tomislav IVANKOVIĆ; Jelena PERAN; & Katarina IŠTEF

Abstract: This paper presents the application of oxygen and argon plasma pre-treatments in combination with 1,2,3,4-butanetetracarboxylic acid (BTCA) and chitosan, as part of research of pre-treatment processes for bovine leather tanned with various tanning agent. Pre-treatments of leather-tested substrate were conducted using different gases in order to assess different impacts of chemically reactive oxygen and inert argon gas on leather surface properties. The tests were carried out on bovine chrome tanned cleaved leather. Simple drop test was used for testing hydrophilicity of the sample, while the surface morphological changes were analysed using SEM microscopy. In order to examine leather performance in conditions of usage, the permeability of alkaline sweat solution under defined conditions was measured. Antimicrobial efficacy of treated leather sample was tested according to agar diffusion plate test against two bacterial species Staphylococcus aureus and Klebsiella pneumoniae. Based on the obtained results it can be concluded that applied plasma pre-treatments in optimize process conditions can contribute to the improvement of functional (antimicrobial) properties as well as wearability in conditions of use, primarily intended for footwear insole and similar products.

Keywords: Plasma surface pre-treatments, semi-processed bovine splitted leather, chitosan, SEM analysis, sorption properties, antibacterial activity.

1. Introduction

Natural leather as a unique natural biological material consists of several layers (epidermis - a thin cellular outer layer, dermis and subcutaneous layer - inner layer) and represents a heterogeneous nano-fibril system (Figure 1a). The dermis layer contains intertwined collagen fibers, which represent the most important layer of raw leather for obtaining the finished product. Collagen fibers are made of fibrils - elemental fibers placed in parallel, slightly bended and interconnected, and contribute to different thicknesses of collagen fibers. The dermis is additionally composed of two layers - papillar (upper layer composed of thinner collagen fibers) and reticular (lower layer composed of thicker bundles of collagen fibers). The reticular layer is a more complex and metabolically active layer consisting of thicker bundles of collagen fibers, intertwined in a complex three-dimensional network. The unique property of the natural leather is strength of the leather that is determined by the orientation of the fibrils - high strength leather has fibrils mostly parallel to the surface and a smaller interlacing angle, while the weaker leather has a higher interlacing angle with fibrils that are not so parallel to its surface (Figure 1b) [1-5].

The industry of leather is one of the biggest polluters of the environment, so it is necessary to develop environmentally friendly leather processing methods. A major problem is the implementation of satisfactory treatments, which are mostly carried out by conventional procedures, and are usually very harmful to the environment. The use of plasma as a medium for processing is an acceptable technique to achieve desired properties. One of the advantages of cold plasma is its applicability to all types of materials, and the possibility of various modifications without negative effects on the basic (mechanical) properties of the material, and without high consumption of chemicals and energy [6].

Plasma treatment of the leather leads to modifications in the surface layer, so-called surface cleaning and its activation thus achieving better hydrophilicity for some new chemical reactions. Using plasma technology is possible to apply chemical agents in monomeric form with the ability to polymerize with the substrate, resulting in cleavage or crosslinking of the agent on the activated surface, in order to achieve or improve the desired functional properties [6].

The emphasis of this paper is application of an ecologically acceptable technique of cold plasma, in achieving of satisfactory sorption and antibacterial properties of the leather under the usage conditions (for footwear insole, primarily), by surface modifications of semi-processed bovine chrome tanned splitted leather, with plasma and ecological bio-agent chitosan.
2. Experimental

2.1 Material and methods

Industrially prepared semi-processed bovine chrome tanned leather of hydrophobic properties was supplied by Viviani company (Rešetari, Croatia). The specification of the bovine leather is listed in Table 1. 1.2.3.4 - butantetraacrylic acid (BTCA) used in pre-treatment was purchased from Sigma-Aldrich. Chitosan (C56H103N9O39, medium molecular weight, 100 – 300 kDa) used in treatment of pre-treated leather (labelled as CH, 1% solution dissolved in 0.1 mol/L acetic acid) was purchased from Acros Organics, respectively. Bacterial species *Staphylococcus aureus* and *Klebsiella pneumoniae* were used for antibacterial efficiency tests.

Table 1: Specification of tested leather

<table>
<thead>
<tr>
<th>Sample name</th>
<th>Thickness [mm]</th>
<th>Sample description</th>
<th>Sample appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>chrome/crust bovine splitted leather - natural</td>
<td>1.0 - 1.2</td>
<td>semi-processed bovine chrome tanned, hydrophobic leather</td>
<td></td>
</tr>
</tbody>
</table>

2.2 Plasma pre-treatment

For plasma pre-treatments, we used oxygen and argon gases (purity 99.998%, by Messer) at a defined pressure, gas flow and constant frequency of 40 kHz in a low-pressure plasma system (NANO LF, Diener electronic), Table 2. To determine the effect of exposure time and power on leather surface properties, samples were treated for 10 minutes under 500 and 800 W.

Table 2: Plasma pre-treatment conditions

<table>
<thead>
<tr>
<th>Pre-treatment/gas</th>
<th>t [min]</th>
<th>P [W]</th>
<th>p [Pa]</th>
<th>q [cm³/min]</th>
<th>l x d [mm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. oxygen</td>
<td>10</td>
<td>500</td>
<td>32</td>
<td>220</td>
<td>100x100</td>
</tr>
<tr>
<td>2. oxygen</td>
<td>10</td>
<td>800</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. argon</td>
<td></td>
<td>500</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

To remove moisture and consequently accelerate vacuum acquirement, samples were dried at 50°C for 24 hours prior to plasma pre-treatment.
2.3 Plasma treatment

For plasma polymerization process, we used pure BTCA (Sigma-Aldrich) as reagent (in monomer bottle) at a pressure of 50 Pa, gas flow of 200 cm$^3$/min and frequency of 40 kHz in a low-pressure plasma system (NANO LF, Diener electronic). To determine the effect of exposure time and power on polymerization rate, samples were treated for 30 minutes under 100 W.

2.4 Spraying method

In the second part of the experiment, 1% chitosan solution was applied on the plasma treated leather samples by spray method in order to achieve antibacterial effectiveness. For enhanced deposition of chitosan as antibacterial agent, plasma pre-treated leather substrate was sprayed with chitosan solution for 5 second and dried at 65°C for 15 minutes.

2.5 Surface morphology

The surface morphology of untreated and plasma pre-treated leather substrates was analysed with a Jeol scanning electron microscope (SEM) (JEOL LV-6060) at 100x and 2000x magnifications. In order to obtain conductivity, samples were coated with gold for 20 minutes, using a sputter coater before analysis. Results are presented at Figures 1.-3.

2.6 Sorption properties

The effect of plasma pre-treatment on wicking (hydrophilicity) properties was evaluated by determining absorption time of the water drop according to AATCC 79-2000 standard. Results are presented in Table 3.

2.7 Permeability of alkaline sweat solution

For examining the behaviour of the leather under the usage conditions (for footwear insole), the permeability of the alkaline sweat solution is measured under defined conditions. The testing of absorbency and permeability of alkaline sweat solution under simulated laboratory conditions was performed on untreated and pre-treated samples. A previously weighed sample dimensions 100 mm x 100 mm was placed on a Petri dish containing an alkaline sweat solution with a volume of 40 ml (pH 8 ± 0.2). The test was performed in an oven at 37 °C for 8 hours. At the end of the test, the mass of the samples was determined (m$_2$, g), the remaining volume of the sweat solution, while the amount of sweat leaked (ΔV, ml) was calculated.

2.8 Antibacterial activity

Antibacterial activity against S. aureus (ATCC 25 923) and K. pneumoniae (ATCC 11 296) in treated leather samples was determined with the qualitative agar diffusion plate test following ISO 20645:2004 standard and was compared with an untreated leather sample. 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

The micrographs of untreated chrome tanned splitted leather sample (Figure 2) shows that the collagen fibers are almost vertical oriented to the surface, and of different fineness with visible tangled fibrils along the visible surface. After plasma pre-treatments and treatments with BTCA/CH solutions, collagen fibers are oriented parallel to the surface with more bundles sticker together due to the agent applied during treatments. The overall structure of the leather surface is smoother with a residual agent on the surface of the bundles of collagen fibers, presented at Figures 3 and 4.

This orientation of the fibers is present in leather of higher strength in which the fibers are oriented parallel to the surface of the leather with a smaller angle of interlacing compared to leather of lower strength, and will be the focus in future research.
**Figure 2**: SEM micrographs of untreated leather samples; observed at 100x and 2000x magnifications.

**Figure 3**: SEM micrographs of splitted leather samples; observed at 100x and 2000x magnifications: a), a1) - O₂ plasma pre-treatment; b), b1) - treatment with O₂/BTCA/CH.

- a) untreated_100x
- a1) untreated_2000x
- a) O₂ plasma pre-treated_100x
- a1) O₂ plasma pre-treated _2000x
- b) O₂/BTCA/CH treatment_100x
- b1) O₂/BTCA/CH treatment_2000x
- a) Ar plasma pre-treated _100x
- a1) Ar plasma pre-treated _2000x
3.2 Hydrophilicity

Table 3: Water absorption of the leather surface after pre-treatments

<table>
<thead>
<tr>
<th>Leather sample – pre-treatments</th>
<th>t [s]</th>
<th>Evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td>chrome tanned splitted leather</td>
<td></td>
<td></td>
</tr>
<tr>
<td>untreated</td>
<td>280.0</td>
<td>hydrophobic</td>
</tr>
<tr>
<td>O₂ plasma</td>
<td>8.6</td>
<td>hydrophilic</td>
</tr>
<tr>
<td>Ar plasma</td>
<td>1.4</td>
<td>hydrophilic</td>
</tr>
</tbody>
</table>

According to the results of water absorption (Table 3), plasma pre-treatments carried out under tested conditions resulted in an increase of hydrophilicity of the leather sample. These changes can be attributed to changes of the collagen fibers surface during plasma pre-treatments. Plasma-activated species bombard the surface with active species, which react with the surface and cause etching and ablation of the surface that results in the increases surface micro-roughness, and finally could improve wetting of the sample.

3.3 Results of permeability of alkaline sweat solution

The test results expressed as the change of sample weight, \( \Delta m \) [%], the proportion of permeable sweat solution through the sample into the environment was expressed as the difference between the initial volume and the residual volume, \( \Delta V \) [%], and are shown in Table 4.

Table 4: Permeability of alkaline sweat solution of untreated and pre-treated samples

<table>
<thead>
<tr>
<th>Leather sample – pre-treatments</th>
<th>( m_1 ) [g]</th>
<th>( m_2 ) [g]</th>
<th>( \Delta m ) [%]</th>
<th>( P_{th} ) [g]</th>
<th>( V_{th} ) [ml]</th>
<th>( \Delta V ) [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>chrome tanned splitted leather</td>
<td>6.7249</td>
<td>6.9309</td>
<td>3.06</td>
<td>0.2060</td>
<td>26.9</td>
<td>-32.75</td>
</tr>
<tr>
<td>O₂ plasma</td>
<td>6.7190</td>
<td>7.1283</td>
<td>6.09</td>
<td>0.4093</td>
<td>26.4</td>
<td>-34.00</td>
</tr>
<tr>
<td>Ar plasma</td>
<td>6.1415</td>
<td>6.5336</td>
<td>6.38</td>
<td>0.3921</td>
<td>25.0</td>
<td>-37.50</td>
</tr>
</tbody>
</table>

Based on the results presented in Table 4, it can be concluded that the weight of leather samples increases over a period of 8 hours, and at the same time the permeability of the sweat solution through the structure of the pre-treated samples was increased, compared to the untreated one. Trend of increasing the ability to absorb and release the solution of sweat of pre-treated samples into the environment is evident.

3.4 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 5 and Figure 4.

Table 5. Antibacterial activity of untreated and plasma pre-treated leather samples

<table>
<thead>
<tr>
<th>Leather sample – treatment</th>
<th>Growth under the specimen</th>
<th>Assessment*</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>untreated</td>
<td>Slight</td>
<td>Limit of efficacy</td>
</tr>
</tbody>
</table>

Figure 4: SEM micrographs of splitted leather samples; observed at 100x and 2000x magnifications: a), a1) - Ar plasma pre-treatment; b), b1) - treatment with O₂/BTCA/CH.
Considering the obtained results, the sample treated with O<sub>2</sub> or Ar/BTCA/CH shows a good effect against S. aureus bacteria. Untreated chromium tanned cleaved leather, obtained a slight growth of bacteria, which indicates a limit of antimicrobial efficacy, which is after treatments manifested (although there is no zone of inhibition) by inhibition of bacteria below the sample and indicates a good antimicrobial effect. The lack of an inhibition zone can be aggravated by the specific fibrillary structure of the leather surface, which can act as barrier to the better contact of bacteria and antimicrobial agent.

4. Conclusions

The following conclusions are proposed:

- pre-treatment conditions with O<sub>2</sub> and Ar plasma are optimized; the ability to absorb water increase after pre-treatments. Achieving a higher level of hydrophilicity is important for wearing comfort due to the change in liquid moisture of the leather product;
- morphological changes of leather after plasma pre-treatment analysed with SEM microscope indicate on higher binding of collagen fibers into bundles, which can affect on better transport of water from the surface through the structure and into the environment;
- the results of the permeability of the alkaline sweat solution indicate on a trend of increasing absorption capacity, and the transfer of the sweat solution through the samples treated with plasma;
- satisfactory antimicrobial efficacy of the tested samples against the selected bacteria was achieved after the performed treatments.

ACKNOWLEDGEMENTS

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).

References


Authors:

Assoc. prof. Sanja ERCEGOVIĆ RAŽIĆ, PhD. (corresponding author)
Jadranka AKALOVIĆ, dipl. ing.; Jelena PERAN, mag.ing.techn.text. PhD student and Katarina IŠTEF, mag.ing.techn.text.
University of Zagreb Faculty of Textile Technology
Prilaz baruna Filipovića 28a, 10 000 Zagreb, Croatia
Phone: +(385) (01) 3712 523 Fax: +(385) (01) 3712 577 E-mail:sanja.ercegovic@ttf.unizg.hr
Phone: +(385) (01) 3712 500
E-mail:jadranka.akalovic@ttf.unizg.hr

Assist. prof. Tomislav IVANKOVIĆ
University of Zagreb Faculty of Science
Rooseveltov trg 6, 10 000 Zagreb, Croatia
Phone: +(385) (01) 6189 712 Fax: +(385) (01) 4826 260 E-mail: tomislav.ivankovic@biol.pmf.hr