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Antimicrobial properties of pickled leather treated with zeolite

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Abstract

The quality of leather and its various properties depend on the preparatory work, as well as the tanning and finishing processes. Preparatory operations include soaking, liming, deliming, bating, and acidification (pickling), through which the leather acquires the characteristics necessary for tanning. After the preparatory stages, further treatment is carried out to prevent the growth of microorganisms on the leather surface. The development of microorganisms requires extra washing, which slows the regular leather-processing workflow and increases production costs. In this study, leather samples that had undergone standard factory preparatory procedures - including pickling - were additionally treated with zeolites. This paper describes the preparatory procedures and the process of treating pickled leather with zeolites, with the aiming to achieve improved antimicrobial protection as a more environmentally friendly solution for storing leather prepared in this way. The objective is to determine whether zeolite treatment provides an antimicrobial effect on pickled leather. The antimicrobial activity of the zeolite-treated leather against Candida albicans, Staphylococcus aureus, and Escherichia coli was tested in accordance with EN ISO 20645:2018. The results obtained from the zeolite-treated leather were compared with those of leather processed through the standard pickling procedure. The antimicrobial effectiveness of the zeolite-treated leather was confirmed.

***Keywords**: leather, zeolite, antimicrobial properties, pickling*

1. Introduction

Given the widespread use of bovine leather in clothing, footwear, and other industries, there is a strong need to improve leather processing and finishing. Legislative directives impose strict rules and regulations regarding to environmental protection. For this reason, this study considers the possibility of replacing an environmentally unfavourable fungicidal agent with zeolite. Microorganisms use nutrients present in hides as sources of food and energy and can spread uncontrollably throughout the hide - from slaughter to the production of finished leather. In the early stages of processing, bacteria predominate, while fungi develop after tanning. Their enzymes break down fats, proteins, and carbohydrates, and fungal growth on wet-blue hides can lead to visible defects in the finished leather. Therefore, the use of antimicrobial agents is essential. These may be specific (bactericides, fungicides) or broad-spectrum. Effective agents must demonstrate high activity, broad-spectrum effectiveness, compatibility with leather, stability, environmental acceptability, and low toxicity. When tanned leather is stored for some time in a moist state, it is necessary to include a fungicide during the preparatory processes. Without it, bacteria and mould develop, causing damage to the leather structure. Leather contains various nutrients, and industrial conditions are generally favourable for microbial growth [1–2]. In addition to high moisture content, leather is rich in fats, proteins, and carbohydrates, all of which significantly support the development of existing microorganisms, particularly bacteria and fungi. These microorganisms produce enzymes capable of breaking down macromolecules into smaller units that can be absorbed through cell membranes and used as nutrients and energy. Their activity begins as soon as the hide is removed from the animal and continues throughout the entire leather production process [3–6]. Fungi can attack salted, pickled, tanned, and finished leather. Fungal growth on salted hides can cause serious damage, such as a loosened structure in affected areas, deterioration of the collagen matrix, loss of the grain layer, detachment of the grain from the flesh side, discoloration, uneven grain visible in folds, and other defects. Semi-processed leather such as wet blue and wet white is particularly susceptible to fungal growth due to its high water content and low pH. Fungal development manifests as uneven dyeing resulting from tissue damage, which alters the physicochemical properties of the leather and affects its dye-binding capacity. Such defects often require additional dyeing to correct. Fatliquoring may also become problematic due to uneven fat absorption, leading to the formation of stains and necessitating further interventions, usually during finishing [3,7–8].

Ultimately, fungal growth can cause numerous undesirable defects that affect quality and reduce the value of the final product. In the leather industry, the proper use of antimicrobial agents is crucial to preserve leather quality. Damage to the leather surface can cause substantial direct and indirect economic losses. The impact on workers' health and the associated costs should also not be underestimated [7]. Highly toxic antimicrobial agents are increasingly being replaced by essential oils and chitosan-based derivatives in acidification, tanning, and finishing processes, as well as in the treatment of wet-blue leather.

In addition to these organic agents, nanoparticles of silver, zinc oxide, titanium dioxide, silicon dioxide, copper, and zeolite are also used for this purpose [9–10].

Zeolites are natural or synthetic aluminosilicates with a three-dimensional, crystalline, microporous structure. Their primary structural units are TO_4 tetrahedra connected by shared oxygen atoms to form secondary structural units. The crystal lattice of zeolites carries a negative charge due to the isomorphic substitution of tetravalent silicon with trivalent aluminium. This negative charge is balanced by cations such as Na^+, K^+,

Ca^{2+}, and Mg^{2+}, allowing zeolites to function as ion exchangers. The ion-exchange process is reversible and involves the formation of weak van der Waals bonds between the zeolite and metal ions. The crystal structure, pore size, and pore distribution influence adsorption and ion exchange within the zeolite framework. Chemical modification of zeolites is used to enhance their sorption properties (adsorption and ion exchange) and broaden their applications [11–16]. Such modifications are typically carried out using surfactants or inorganic salts. The role of fungicides is not only to protect the leather but also to reduce production costs associated with repairing damage caused by fungal contamination. The major disadvantages of conventional fungicides are their toxicity to human health and their negative environmental impact, and their use is legally restricted [1–3]. Environmental protection and human health are key priorities reflected in regulations and standards within the European Union and worldwide. For these reasons, alternative methods with reduced environmental and health risks have been explored.

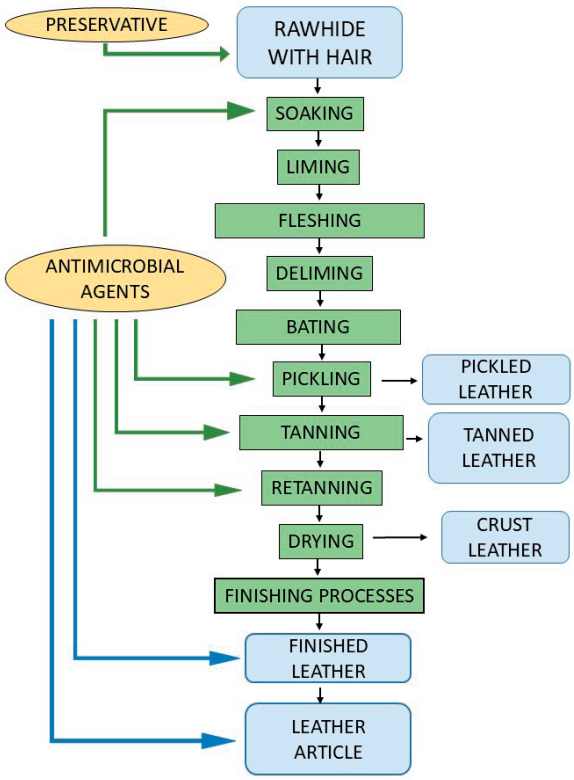


Figure 1. Scheme of the leather production process with highlighted stages of antimicrobial dosing

Preparatory work involves mechanical and chemical technological operations through which raw hides are prepared for the tanning process. During these operations, the epidermis, hair, fat, subcutaneous tissue with remnants of meat and blood, preservatives, and impurities are removed. The purpose of these technological procedures is to obtain loosened hides suitable for tanning. The preparatory work includes the following processes: soaking, liming, fleshing, deliming, bating, and pickling.

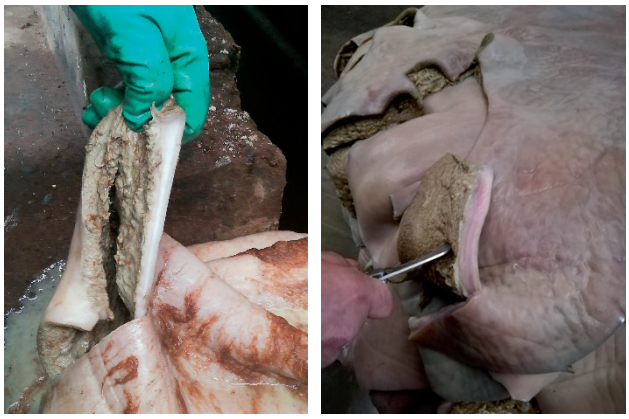
The soaking process restores the necessary moisture to the hide, removes impurities, blood, and preservatives, breaks down fats and proteins on the flesh side, and - with the addition of antimicrobial agents - prevents bacterial growth and the degradation of the collagen structure. Liming, carried out using lime, sulphides, or enzymes, removes the hair and epidermal layers, saponifies natural fats, and eliminates unstructured proteins that interfere with the tanning process, producing loose and swollen hide tissue. This is followed by fleshing, a mechanical operation that removes subcutaneous tissue along with remaining muscle and fat. It is performed on rollers equipped with sharp blades that separate unwanted tissue from the hide. After liming and fleshing, rinsing is performed to remove residual lime as well as hydrolysed proteins, fats, and minerals. To avoid defects in the finished leather, most of the lime and sulphides must be removed, which is achieved through deliming. This process also reduces the swelling of the hide. Bating is conducted using enzymatic preparations that eliminate remaining hair roots, elastic fibres, unstructured proteins, fats, pigments, and lime. As evident from the above, substances of natural origin that promote microbial growth and development are removed during these stages. The purpose of bating is to loosen the hide tissue and partially separate the fibres. Pickling is an operation in which the leather is treated with an acid solution in the presence of neutral salt. The acid diffuses into the leather, removing residual bound lime, and is absorbed by the active groups of collagen, displacing water. Through acidification, collagen becomes more receptive to chromium tanning complexes, allowing for better penetration and more uniform tanning. Acid causes protein hydrolysis depending on the acidity and duration of the process. The addition of salt reduces the negative effects of the acid on the leather, increases acid absorption into collagen and its degree of acidification, reduces swelling, and simultaneously increases hydration. Salt also prevents acid-induced protein hydrolysis and coagulates proteins that have already been hydrolysed. Acidification produces properties characteristic of tanned leather—the so-called “pseudo-tanning” [17–18].

2. Materials and methods

Raw hides after storage are dry, stiff, and prone to cracking. They retain hair, curing salt, and impurities acquired during the animal’s life, at the moment of flaying, and during transport. In this condition, they lack the necessary flexibility and moisture (Figure 1a). Preparatory work prepares the hides for the tanning process, which in this study was carried out under conventional industrial conditions, as previously described.

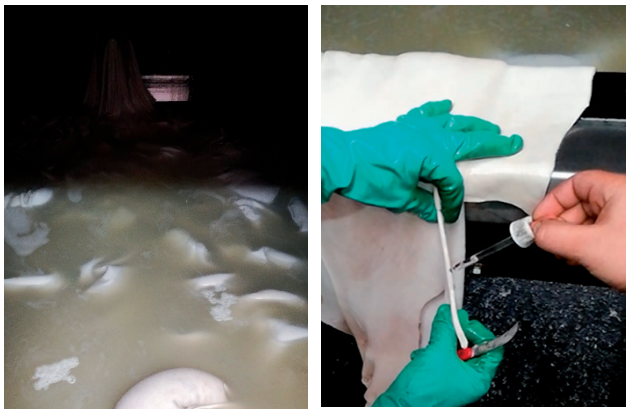
a b

Figure 2. Raw bovine hide with hair: a) before the soaking process, b) after the soaking process



a b

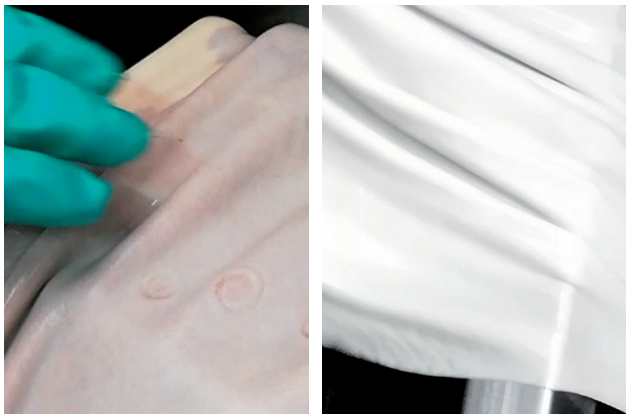
Figure 3. Raw bovine hide, cross-section control a) during the leaching process, b) after the leaching process



a b

Figure 4. Deliming process a) leather in the deliming bath, b) control of the deliming process, testing the cross-section of leather with an indicator

After the preparatory work, the pickled leather is soft, supple, swollen, light in colour, and has a clean surface (Figure 5b).



a b

Figure 5. a) control of the bathing process by pressing a finger into the face of the leather, b) bovine leather after the pickling process

After the bovine hide had undergone preparatory work, it was treated with zeolite 5A using a Mathis Turbomat P4502 laboratory apparatus. The amount of chemical agents was dosed in relation to the volume of water, which corresponded to 50% of the weight of the pickled leather. The first pickled leather sample was treated for 1 hour at 30 °C in a bath with the following composition: citric acid (Sigma Aldrich, St. Louis, USA) 70 g dm^{-3}, zeolite 5A 65 g dm^{-3}, and the soaking agent Felosan RG-N (CHT, Switzerland) 1 g dm^{-3}. The second pickled leather sample was treated using the same procedure, except that the treatment time was extended to 2 hours. The temperature and pH of the bath were measured throughout the process, and the values are presented in Table 1. After the treatments, antimicrobial tests were conducted according to EN ISO 20645:2018 Textile fabrics – Determination of antibacterial activity – Agar diffusion plate test on the following strains: the fungus *Candida albicans* (*C. albicans*) and the bacteria *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*). As the standard method is intended for testing textile materials, certain modifications were applied to objectively evaluate the leather samples. The tests were carried out at the Teaching Institute for Public Health "Dr. Andrija Štampar", Zagreb. Samples measuring 1 cm × 1 cm were prepared from untreated leather and from leather treated with zeolite for 1 hour and 2 hours. Six pieces were cut from each leather sample, two pieces for each tested strain. The samples were examined from both the grain side and the flesh side.

3. Results and discussion

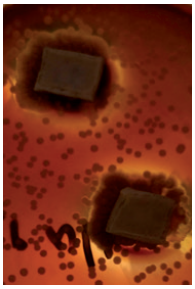
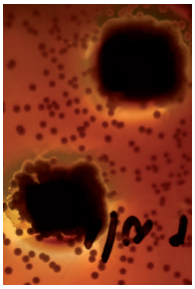
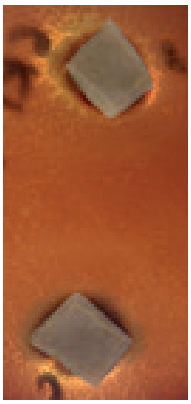
Table 1 shows the temperatures and pH values of the bath in which the leather samples were processed. The pH value of the bath did not vary significantly while the bath temperature was maintained automatically according to the set program throughout the process.

Table 1. pH value and bath temperature

Measurement moment	pH	temperature, °C
before starting processing, after adding the agents	pH=2,74	T=22,9 °C
1 h after processing	pH=2,78	T=25,7 °C
2 h after processing	pH=3,05	T=26,9 °C

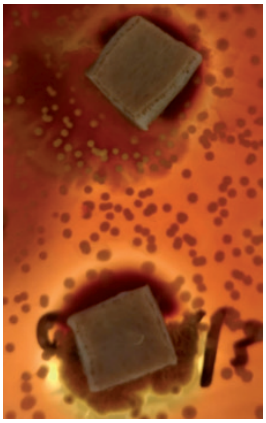
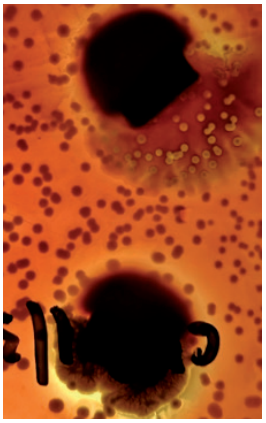
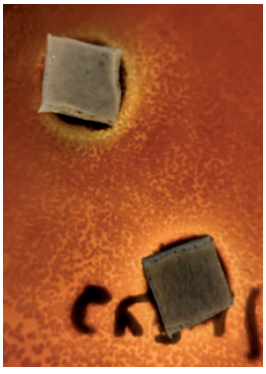
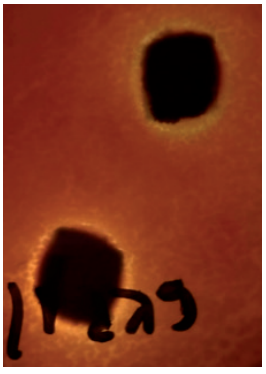
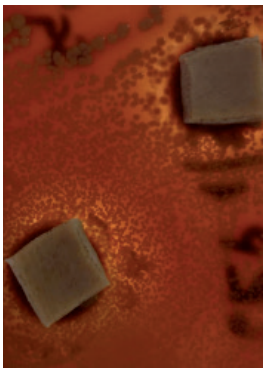
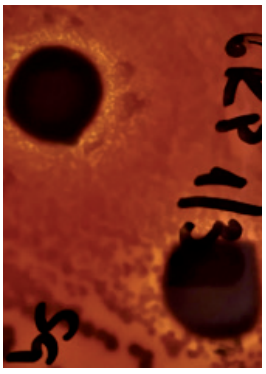
To evaluate the antimicrobial effectiveness of the zeolite treatment, leather samples were subjected to antimicrobial testing using the ISO 20645:2018 method against the fungus *Candida albicans* (*C. albicans*), the Gram-negative bacterium *Escherichia coli* (*E. coli*), and the Gram-positive bacterium *Staphylococcus aureus* (*S. aureus*).

Table 2. Antimicrobial efficiency results of untreated pickled leather samples

Candida albicans	
grain side	flesh side
	
Escherichia coli	
	
Staphylococcus aureus	
	

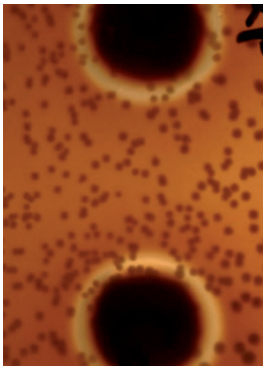
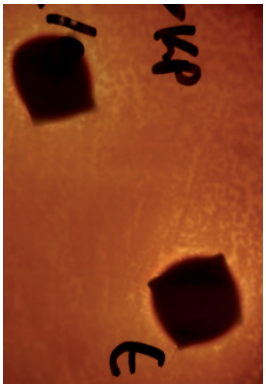
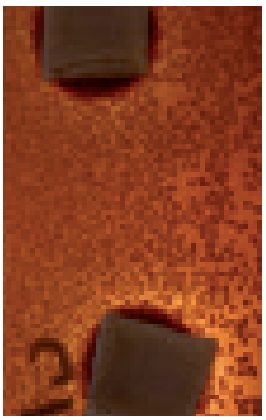
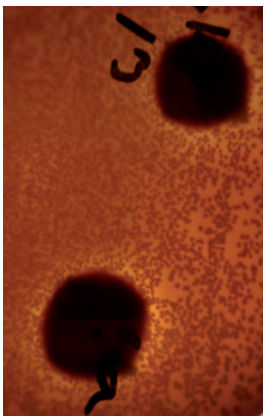
From the results presented in Table 2, it can be seen that the untreated pickled leather samples show no inhibition zone against the fungus *C. albicans* or the bacteria *E. coli* and *S. aureus*, on either the grain side or the flesh side. Although an apparent inhibition zone seems visible in all samples, microbial growth is present both around and under the samples (as seen in the images of the flesh side), indicating that a true inhibition zone is not present. The grain side of the samples was not affected by *E. coli*, whereas *S. aureus* and *C. albicans* partially affected the grain side as well. In the case of *E. coli*, bacterial growth was less intense compared to the other two strains. Based on these observations, the untreated pickled leather sample does not show antimicrobial effectiveness.

Table 3. Results of antimicrobial efficiency of pickled leather treated with zeolite for 1 hour

Candida albicans	
grain side	flesh side
	
Escherichia coli	
	
Staphylococcus aureus	
	

For the pickled leather treated with zeolite for 1 hour (Table 3), a distinct inhibition zone is visible against *E. coli*. No bacterial growth is present within the inhibition zone, around the samples, under the samples, or on the grain surface. An apparent inhibition zone is also visible for *S. aureus* and *C. albicans*. However, in the areas surrounding the samples and on the flesh side beneath them, growth of *C. albicans* and *S. aureus* is still present, although with significantly reduced intensity compared to the untreated leather. The grain surface of the samples was not affected. Based on these findings, the leather sample treated with zeolite for 1 hour demonstrates partial antimicrobial efficacy against *S. aureus* and *C. albicans*, and full antimicrobial efficacy against *E. coli*.

Table 4. Results of antimicrobial efficiency of pickled leather treated with zeolite for 2 hours

Candida albicans	
grain side	flesh side
	
Escherichia coli	
	
Staphylococcus aureus	
	

In the leather samples treated with zeolite for 2 hours (Table 4), as well as in those treated for 1 hour, an inhibition zone is visible, comfirming antimicrobial effectiveness against the bacterium *Escherichia coli*. For strains of *Staphylococcus aureus* and *Candida albicans*, an apparent inhibition zone is also present. In the area around and under the samples (flesh side), the presence of *Candida albicans and Staphylococcus aureus* is visible, but at a significantly reduced intensity compared to untreated leather and leather treated with zeolite for 1 hour. The grain side of the samples was not affected. According to these results, the leather sample treated with zeolite for 2 hours shows partial antimicrobial efficacy against *Staphylococcus aureus* and *Candida albicans,* with the growth of these strains almost completely suppressed.

Extending the leather treatment time with zeolite for 1 hour improved the results of partial antimicrobial protection. Based on the observations obtained from antimicrobial efficacy testing of pickled leather treated with zeolite, it was determined that zeolite exhibits an inhibitory effect on the growth of microorganisms, particularly against the Gram-negative bacterium *Escherichia coli.* The obtained results suggest that the presence of zeolite within the leather structure contributes to the suppression of microbial growth and proliferation, thereby demonstrating a bacteriostatic effect.

Table 5. Antimicrobial efficacy of leather samples before and after treatment

Sample description	***Ca***	***Ec***	***Sa***
untreated pickled leather	-	-	-
pickled leather treated for 1 hour	+/-	+	+/-
pickled leather treated for 2 hours	+/-	+	+/-

- no zone of inhibition, strains present; + zone of inhibition present; +/- no zone of inhibition, no strain on the leather surface but partially in the apparent zone of inhibition

Table 5 shows that both zeolite treatments provide antimicrobial efficacy against *E. coli* and partial antimicrobial efficacy against *S. aureus* and *C. albicans*. Leather not treated with zeolite after pickling shows no antimicrobial efficiency against the tested strains. Untreated pickled leather does not exhibit any contribution to antimicrobial properties. Considering the processing duration, no significant differences were observed among the tested samples. Since pH does not substantially affect the structure of collagen and leather, it is justified to continue research aimed at validating processing parameters, including pH, treatment duration, and temperature, in order to achieve antimicrobial effectiveness against all tested strains.

4. Conclusion

The results show that untreated pickled leather does not exhibit inhibition zones for any of the tested strains, indicating a lack of antimicrobial efficacy. It is important to note that leather contains residual formic acid pickling, which has partial antimicrobial properties. This can create the appearance of an inhibition zone around the sample due to acid diffusion, even though microbial growth is not truly inhibited. Pickled leather treated with zeolite demonstrated antimicrobial activity against *E. coli*, as evidenced by a clear inhibition zone and the absence of bacterial growth. Partial antimicrobial activity was observed against *S. aureus* and *C. albicans*. Although growth of these strains was still present, its intensity was significantly reduced compared to untreated leather. Extending the zeolite treatment time further improved the results. After two hours of treatment, the growth of *S. aureus* and *C. albicans* was almost completely suppressed, indicating greater antimicrobial effectiveness. The experiments were conducted on bovine pickled leather, which is thicker than leather obtained from smaller animals. Because such leathers have a lower thickness, a similar effect can be expected, and it may even be more pronounced due to easier penetration of the treatment into the material. However, additional experiments on different types of leather are needed to confirm the broader applicability of this approach and to establish whether process parameters should be optimized separately for each leather type.

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This research was conducted as part of a project assignment for the Doctoral Program in Textile Science and Technology at the University of Zagreb, Faculty of Textile Technology.

Acknowledged

The work was prepared and financed by the short-term support of the University of Zagreb, Faculty of Textile Technology, entitled Functionalization and characterization of textiles and leather to achieve protective properties, TP/7, Head: Sandra Flinčec Grgac