CHITOSAN-TREATED COTTON YARNS: IMPACT OF APPLICATION METHOD ON ANTIMICROBIAL ACTIVITY

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Abstract: Textiles with antimicrobial activity are necessary to prevent the action of pathogenic microorganisms on textiles, as well as their spread and transmission to protect the health of medical personnel and patients. There is a need for an application method that will provide textiles with the greatest resistance to the action of pathogenic microorganisms. This research presents data regarding the effect of chitosan application methods on bacteria reduction, which is useful for obtaining cotton yarns with antibacterial activity. Low molecular weight chitosan was used to pre-treated (alkaline scoured and bleached) cotton yarns in four application methods. Each application method changed the exhaustion, padding, drying, and rinsing phases to obtain antibacterial yarns against Staphylococcus aureus and Escherichia coli. Determining the applied chitosan amount and accessible amino groups was used to analyze their effect on antibacterial activity. Differences in surface morphology and chemistry between samples were analyzed by FTIR-ATR and SEM analysis. The result indicated that the methods in which the drying phase precedes the rinsing phase have a higher efficiency in reducing bacteria than the methods in which the rinsing phase precedes the drying phase. The method with the highest efficiency in reducing bacteria is the method in which the exhaustion phase is followed by a phases pad, dry, and rinse.

Keywords: yarn, chitosan, cotton, application methods, pathogenic bacteria, antimicrobial activity.

PAMUČNA PREĐA TRETIRANA HITOSANOM: UTICAJ METODA PRIMENE NA ANTIMIKROBNU AKTIVNOST

Apstrakt: Tekstil sa antimikrobnim dejstvom je neophodan za sprečavanje patogenih mikroorganizma na tekstil, kao i njihovo širenje i prenošenje radi zaštite zdravlja medicinskog osoblja i pacijenata. Postoji potreba za metodom koja će tekstilu obezbediti najveću otpornost patogenih bakterija. U ovom istraživanju prikazani su podaci o uticaju metoda nanošenje hitozana na smanjenje bakterija, što je korisno za dobijanje pamučnih prediva sa antimikrobijskim dejstvom. Hitosan male molekulske težine je korišćen za prethodno tretirano (alkalno očišćeno i beljeno) pamučno predivo u četiri metode nanošenje. Svaki metod nanošenje menjao je faze iscrpljivanja, fulardiranja, sušenja i ispiranja da bi se dobilo antibakterijsko predivo na bakterije Staphylococcus aureus i Escherichia coli. Uočavanje razlika u površini i hemiji između uzorka analizirane su FTIR-ATR i SEM analizom. Rezultat je pokazao da metode u kojima faza sušenja prethodi fazi ispiranja imaju veću efikasnost u smanjenju bakterija od metoda u kojima faza ispiranja prethodi fazi sušenja. Metoda nanošenja sa najvećom efikasnošću u smanjenju bakterija je metoda u kojoj se faza iscrpljivanja prati fazaima fulardiranjem, sušenjem i ispiranjem.

Ključne reči: predivo, hitozan, pamuk, metode primene, patogene bakterije, antimikrobna aktivnost.
1. INTRODUCTION

One of the sources of microorganisms, in hospitals and medical institutions, is the textiles used by the staff and patients [1]. A textile material that is not resistant to the action of microorganisms has a significant impact on the health of a person, regardless of whether he is a staff, patient, or visitor in a hospital or health institution. The textile on which pathogenic microorganisms have already developed is a potential problem for viruses, bacteria, and infections with the possibility of their recurrence, which delays the time of treatment and also increases the cost of the period required for treatment [2,3]. Antimicrobially active textile materials have resistance to the action of pathogenic microorganisms, with the possibility of a simultaneous healing effect [4,5]. Nowadays, obtaining textiles with good resistance to microorganisms, with ecological and economic justification is of particular interest. The functionalization of cotton with chitosan is an excellent opportunity in response to this challenge. The effectiveness and efficiency of production processes for obtaining textiles with antimicrobial activity, concerning the choice of antimicrobial agents and textiles, have advantages and disadvantages [6,7]. Obtaining antimicrobial textiles is possible through an antimicrobial agent introduced into the polymer melt during polymer extrusion and fiber formation or through the surface of the textile material being functionalized with an antimicrobial agent [8].

The antimicrobial agent needs to match the chosen fiber production technology and method, as well as working parameters. The effectiveness of the process for obtaining antimicrobial textiles depends on matching the choice of the antimicrobial agent and its chemical composition with the fiber production method and technology. To obtain fibers with good antimicrobial activity, the antimicrobial agent must be evenly distributed in the mass of the fiber along its entire length. For this purpose, it is necessary to apply an agent that will have good solubility in the polymer or to disperse it in the polymer. The antimicrobial activity of the textile obtained in this way is the result of the possibility of the antimicrobial agent being released gradually into the skin and thus acting to prevent the growth and development of pathogenic bacteria. The functionalization of the surface of the textile with an antimicrobial agent is done as finishing. Regardless of the raw material composition, all textile materials are suitable for surface functionalization. This type of antimicrobial surface activation is an excellent solution for obtaining antimicrobial textiles because antimicrobial textiles offer a large contact surface with the environment. Functionalization with chitosan on the surface of cotton textiles can be through the resin, which cross-links with cotton [9], functionalization with micro and nanoparticles of chitosan [10,11], and through the formation of bonds with the surface (covalent, ionic, etc.) [12,13]. Chitosan functionalization methods with resin tend to be avoided due to toxicity [9]. Functionalization with micro and nanoparticles of chitosan is not a fully developed method, its development is still being worked on, which is a big challenge for scientists. Possible techniques for functionalization with chitosan include foaming, printing, exhaustion etc.

Every day, science and industry are dedicated to obtaining textiles with good antibacterial properties, mainly from cotton and chitosan, due to their biodegradability, non-toxicity, etc. Different methods are options for functionalization with chitosan. The methods may include multiple stages (drying, rinsing, exhausting, and padding) of the fabric, each of which may have a different sequence [14-16]. It is considered that the application method of chitosan, which researchers and manufacturers require to be cheap, quick, and easily applied in industry and laboratory, can affect antimicrobial activity.

This work aimed to apply chitosan to pre-treated cotton yarns by using different application methods and to analyze the effect of the application method on the antibacterial properties of cotton yarns functionalized with chitosan. For this purpose, applied chitosan amount, accessible amino groups, and antibacterial activity were examined. Fourier-transform infrared spectroscopy (FTIR-ATR) and scanning electron microscopy (SEM) were used to analyze the composition and structure of the sample surface. Industry and researchers will use the data from this research to choose the most appropriate method for functionalizing cotton yarns with chitosan to obtain textiles with good activity against pathogenic microorganisms. The method has the possibility of application in industrial conditions, and it is also simple to handle.

2. EXPERIMENTAL PART

2.1. Materials

Worsted cotton yarns obtained with ring spinning system (linear density of 30 x 2 tex) were used for functionalization with chitosan. For the functionalization of samples, chitosan with deacetylation degree of 75-85% and 50000-190000 g/mol (low molecular weight, ChL) was purchased from Sigma-Aldrich (USA). A gram-negative bacteria, *Escherichia coli* (*E.coli*), and a gram-positive bacteria, *Staphylococcus aureus* (*S.au-
2.2 Pre-treatment and functionalization with chitosan process used to treat cotton yarns

Alkaline scouring and bleaching were used to pre-treated cotton yarns (Control marked) \cite{17,18}. Scouring was performed using 20 g/dm³ sodium hydroxide, 1 cm³/dm³ Kemonecer NI and 2 cm³/dm³ Cotoblanco HTD-N in a bath with liquor-to-ratio of 30:1 for 90 min at 95°C. Bleaching was conducted using 2 cm³/dm³ Na₂SiO₃, 1 cm³/dm³ Kemonecer NI, and 6 cm³/dm³ H₂O₂ (30%) at pH 11.2 in a bath with liquor-to-ratio of 30:1 for 30 min at 95°C. After pre-treating the yarns, they were functionalized with chitosan. Chitosan was prepared as follows: 0.6 g chitosan was placed in 100 cm³ 1% (w/v) CH₃COOH at 60°C for 60 min at pH 4-4.5. The pre-treated cotton yarns were functionalized with chitosan solution by the following application methods:

- Method A consisted of the following phases: pre-treated yarns were immersed in chitosan solution pH 4-4.5 for 2 hours at 60°C with 30:1 liquor-to-ratio (exhaustion phase). After the exhaustion phase, the samples were dried for 12 hours at 60°C (drying phase), rinsed with deionized water at ambient temperature for 10 min (five times) (rinsing phase), and dried at ambient temperature.

- Method B consisted of the following phases: pre-treated yarns were immersed in chitosan solution pH 4-4.5 for 2 hours at 60°C with 30:1 liquor-to-ratio (exhaustion phase). After the exhaustion phase, the samples were rinsed with deionized water for 10 min at ambient temperature (five times) (rinsing phase) and dried at ambient temperature.

- Method C consisted of the following phases: pre-treated yarns were immersed in chitosan solution pH 4-4.5 for 2 hours at 60°C with 30:1 liquor-to-ratio (exhaustion phase). After the exhaustion phase, the samples were padded with around 80% wet pickup (padding phase), dried for 12 hours at 60°C (drying phase), rinsed with deionized water at ambient temperature for 10 min (five times) (rinsing phase), and dried at ambient temperature.

2.3 Testing methods

2.3.1. Determination of applied chitosan amount

According to equation 1, a gravimetric method was used to determine the weight increase for the sample after functionalization with chitosan. Before testing, the samples were conditioned at 20±2°C and 65±4% relative humidity (standard atmosphere) for 24 hours.

\[
\Delta W(\%) = \left( \frac{W_2 - W_1}{W_1} \right) \cdot 100
\]

where: \(\Delta W\) represents the applied chitosan amount (%), \(W_1\) represents the initial weight of the sample (g), and \(W_2\) represents the weight of the chitosan functionalized sample (g).

2.3.2. Determination of accessible amino groups

The determination of accessible amino groups through acid dye dyeing from acid dye's adsorption on the tested sample. The amino groups of chitosan (positively charged, NH\(_3^+\)) react with the sulfonic groups (negatively charged, SO\(_3^-\)) of the acid dye, so the more amino groups accessible to bond with the acid dye, the more color is on the surface \cite{19}. A 0.2 g sample was soaked in 100 cm³ acetic buffer pH 4 (0.5 g/dm³ CH\(_3\)COONa+0.5 g/dm³ CH\(_3\)COOH; ≥99%) and 0.02 g/cm³ Acid Orange 7 at temperature of 30°C for 180 min. After dyeing, the samples were dried at room temperature. The color strength value of the dyed pieces with acid dye was determined using X-Rate Lab Colorimeter Color i7. Color strength was determined with the Kubelka-Munk equation (equation 2).

\[
\frac{K}{S} = \frac{(1 - R)^2}{2R}
\]

where: \(K\) represents the adsorption, \(S\) represents the scattering, and \(R\) represents the reflection of light.
2.3.3. Fourier transform infrared spectroscopy-attenuated total reflectance (FTIR-ATR)

FTIR-ATR (Fourier transform infrared spectroscopy-attenuated total reflectance) spectra of the un-functionalized and chitosan-functionalized samples were obtained using an FTIR-ATR spectrophotometer (Perkin Elmer Spectrum GX 69876). The instrument's resolution was 4 cm\(^{-1}\) for 16 scans, and the spectra were recorded from 4000 cm\(^{-1}\) to 650 cm\(^{-1}\).

2.3.4. Scanning electron microscopy (SEM)

The surface morphology was characterized by scanning electron microscopy (SEM) on a JEOL JSM-6060 LV (Japan). The instrument operates with a 10 kV accelerating voltage and 5000x magnification. Before the test, samples were coated with gold.

2.3.4. Antibacterial activity

The antibacterial activity of samples was evaluated using the AATCC Test Method 100:2004. Samples were tested for Staphylococcus aureus and Escherichia coli. A sample (2 g) was immersed in 57 cm\(^3\) inoculated and incubated nutrient broth. Inoculation of the nutrient broth was performed with 1.5-3.0 \(\times\) 10\(^5\) CFU/ml bacteria, and incubation was performed at a temperature of 37 \(^\circ\)C for 60 min. After a series of dilutions of the nutrient broth with incubated bacteria, they were applied to plates, which were inoculated and incubated for 24 hours at 37 \(^\circ\)C. Then, the cells that survived were counted. A CFU/ml in the flasks was obtained by multiplying the CFU/ml determined by the dilution factor. Antibacterial activity was represented by the reduction of bacteria in the control sample expressed in percentage, according to equation 3.

\[
R(\%) = \frac{(C - E)}{C} \cdot 100
\]

where: \(R\) represents reduction (\%), \(E\) represents the colony forming unit (CFU) per ml after contact (end test), and \(C\) represents the colony forming unit (CFU) per ml at zero contact.

3. RESULTS AND DISCUSSION

Various procedures can be used to functionalize cotton with chitosan. Most often, the procedures include several stages (drying, exhaustion, padding, rinsing) whose schedule can be changed [20,21]. The interest was focused on defining a procedure that would give the best antimicrobial activity, after the optimal concentration of chitosan was defined [22]. Pre-treated cotton yarns are functionalized with chitosan by different application methods (methods A-D). The applied amount of chitosan after the chitosan functionalization process through the application of the methods used in this study is shown in Figure 1. It can be observed that samples functionalized with application methods A and C showed significantly higher amounts of applied chitosan than samples functionalized with application methods B and D. This may be due to the removal of some of the applied chitosan by the rinsing phase followed by the drying. The small amount of applied chitosan, which may indicate the removal of chitosan by the rinsing phase, was not characteristic of methods in which the drying phase precedes rinsing. Samples functionalized with application methods B and D showed neglected differences in the chitosan applied amount.

The assessable amino groups after functionalized with chitosan, depending on the method used for its application, and their coefficients of variation are shown in Figure 2. During the acid dyeing process, a reaction occurs between the acid dye’s negatively charged groups and the chitosan’s positive amino groups [23]. Chitosan-functionalized samples having higher K/S values indicate more amino groups that can react with the dye. The chitosan-functionalized samples obtained by methods B and D showed much less accessible amino groups. It can be observed that the chitosan-functionalized yarns obtained by methods A and C possessed significantly more accessible amino groups than B and D methods. However, they showed different coefficients of variation for the accessible amino groups. Functionalization with chitosan by an application method in which the rinsing phase precedes the drying phase may lead to removing some of the applied chitosan from the samples.
Hence, the sample obtained by methods B and D showed less accessible amino groups. The smaller coefficient of variation obtained by the C method indicated more excellent uniformity of the applied chitosan and accessible amino groups introduced with it than by the A method.

**Figure 2:** Accessible amino groups after functionalization with chitosan depending on application method and its coefficient of variation

SEM analysis was performed to assess the surface sample's morphology. The micrographs (a-e) in Figure 3 present unfunctionalized and chitosan-functionalized yarns with different application methods. The micrographs of the surface of the pre-treated sample in Figure 3(a) revealed a coarse surface texture with pronounced parallel lines, indicating that the pre-treatment process had a peeling effect on the non-cellulose components. The chitosan-functionalized samples, Figure 3(b-e), showed a difference in surface compared to unfunctionalized ones. The surface roughness characteristic of unfunctionalized ones was reduced. It can be observed that there were no major significant differences between the chitosan-functionalized samples with the application methods used.

Fourier-transform infrared spectroscopy (FT-IR-ATR) technique was used to analyze the surface before and after functionalization with chitosan (Figure 4). Spectra indicated that with methods B and D, the samples show lower intensity for the characteristic peaks than methods A and C. This indicates that the samples obtained by methods B and D have a lower amount of applied chitosan.

The increase in the spectra peaks for amide I (C=O) and amide II (N-H) at 1655 cm⁻¹ and 1550 cm⁻¹, respectively, noticed in alkaline-scoured and bleached cotton yarns, indicated the presence of chitosan [24-26]. The increase in peaks observed for ν(NH), ν(OH), and ν(NH) at 3294 cm⁻¹, as well as ν(CH) (2922 cm⁻¹ and 2854 cm⁻¹) also indicate the presence of chitosan.

Chitosan application methods influence the reduction percentage against *E.coli* and *S. aureus* of the chitosan-functionalized samples (Figure 5). The textile
with over 70% effective reduction of pathogenic bacteria possesses effective antibacterial activity [19]. The sample functionalized with chitosan obtained using the A method showed a reduction percentage of \textit{S. aureus} bacteria not greater than 70%. The sample obtained using the C method had the highest antibacterial activity against \textit{S. aureus}. The reaction between the cations of chitosan, \textit{NH}_3^+, and anions located in the membrane of bacterial cells causes components to leak out of the bacterial cell, decreasing vital cell functions. It can be noticed that the antibacterial activity of all samples against \textit{E. coli} was greater than \textit{S. aureus} (Figure 5). The different percent reduction in gram-negative and gram-positive bacteria is due to differences in cell wall thickness and their chemical composition [26].

4. CONCLUSION

Pre-treated cotton yarns were functionalized with chitosan by four different methods in which the phases of exhaustion, padding, drying, and rinsing were alternated. The method with the highest degree of bacteria reduction is singled out. After functionalization with chitosan with the methods in which the drying phase was before the rinsing phase, it was found that samples had higher resistance to bacteria and also had higher applied chitosan amount and more accessible amino groups compared to the samples obtained with the methods in which the rinsing phase precedes the drying phase. The application methods in which the rinsing phase precedes the drying phase result in a significantly lower amount of applied chitosan and less accessible amino groups on the chitosan-functionalized cotton yarns, and consequently, a lower antibacterial activity, especially against \textit{Staphylococcus aureus}. Regardless of the application method, pre-treated cotton yarns functionalized with chitosan showed excellent efficiency in reducing \textit{Escherichia coli} bacteria compared to the reduction of \textit{Staphylococcus aureus} bacteria. The chitosan-functionalized samples led to the highest efficiency in reducing bacteria obtained by a method in which the exhaustion phase was followed by padding, drying, and rinsing.

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