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Antimicrobial Active Chitosan-Based Cotton Yarns: Effect of Chitosan Solution Concentration

Protimikrobno aktivna s hitozanom obdelana bombažna preja: vpliv koncentracije raztopine hitozana

Original scientific article/*Izvirni znanstveni članek*
Received/*Prispelo* 4-2023 • Accepted/*Sprejeto* 5-2023

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Abstract

Using the exhaustion-pad-dry-rinse method, chitosan was applied to alkaline-scoured and bleached cotton yarns in a solution with concentrations ranging from 0.2–1% to achieve good antimicrobial activity against the bacteria *Escherichia coli* (Gram-negative) and *Staphylococcus aureus* (Gram-positive). Studied samples were also assessed by measuring the amount of introduced chitosan, amount of accessible amino groups, mechanical properties, whiteness index and the *b** colour coordinate. Alkaline-scoured and bleached cotton yarns treated with all concentrations of the chitosan solution showed good antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus*. Better antimicrobial activity was achieved against *Escherichia coli*. Increasing the concentration of chitosan solution deteriorated the mechanical properties of chitosan-treated cotton yarns. The optimal concentration of chitosan solution incorporated in the exhaustion phase to obtain chitosan-treated yarns with good antimicrobial activity and mechanical properties was 0.6%. The best antimicrobial treatment should minimise potential economic costs while providing functionality.
Keywords: cotton, chitosan, antimicrobial activity, *Escherichia coli* bacteria, *Staphylococcus aureus* bacteria

Izvleček

Hitozan je bil v raztopini s koncentracijo od 0,2–1 % nanesen na alkalno izkuhano in beljeno bombažno prejo z izčrpalno-impregnirnim postopkom z naknadnim sušenjem in spiranjem, da bi dosegli dobre protimikrobne aktivnosti proti bakterijama Escherichia coli (Gram negativna) in Staphylococcus aureus (Gram pozitivna). Vzorce smo preučevali z merjenjem količine uporabljenega hitozana, količine dostopnih aminoskupin, mehanskih lastnosti, indeksa beline in barvne koordinate b. Kljub temu, da je alkalno izkuhana in beljena bombažna preja v vseh koncentracijah raztopine hitozana pokazala dobro protimikrobno aktivnost proti bakterijama Escherichia coli in Staphylococcus aureus, je bila protimikrobna aktivnost boljša proti bakteriji Escherichia coli. Mehanske lastnosti bombažne preje, obdelane s hitozanom, so se poslabšale, ko smo povečevali koncentracijo hitozana v raztopini. Optimalna koncentracija raztopine*

hitozana, ki je bila vključena v fazo izčrpavanja, da bi pridobili s hitozanom obdelano prejo, ki ima dobro protimikrobno aktivnost in mehanske lastnosti, je bila 0,6 %. Dobra protimikrobna obdelava bi morala zmanjšati morebitne stroške in hkrati zagotavljati funkcionalnost.
Ključne besede: bombaž, hitozan, protimikrobno delovanje, Escherichia coli, Staphylococcus aureus

1 Introduction

The need for environmentally justified antimicrobial textiles is particularly interesting, especially in the medical sector [1, 2]. The increase in the world's population, the interest in a safer, healthier and more comfortable environment, awareness and expectations regarding hygiene and healthcare standards (protection from microorganisms) significantly influence the daily development of bioactive materials [3]. Textile materials in the course of application in healthcare institutions, especially in operating rooms, for medical staff and hospital patients, represent a significant source of bacteria and infections [4]. Microbiologically contaminated textiles represent a potential opportunity for a further deterioration of patient's health status and disruption of medical personnel's health status.

An antimicrobial textile is a textile material that acquires antimicrobial properties after being treated with an agent which carries antimicrobial properties. Textiles made of cotton fibres are in constant contact with microorganisms from the environment, especially in healthcare institutions. Due to their surface area and ability to retain moisture, they are an ideal substrate for developing microorganisms that reproduce and grow [5]. Textiles of natural origins, e.g. cotton, are more susceptible to the action of microorganisms than textiles of synthetic origin due to their porous hydrophilic structure that retains water, oxygen, and nutrients that represent ideal conditions for their growth and development. To date, various types of antimicrobial agents can be found on the market, e.g. inorganic salts and salts of organic compounds, iodoform, phenols, thiophenols, antibiotics, triclosan, polyhexamethylene biguanide (PNMB), quaternary ammonium compounds, derivatives of formaldehyde and amines [5, 6]. However, many of these agents are toxic and non-biodegradable; therefore, efforts are being made to replace them with agents with antimicrobial activity of natural origin. Natural antimicrobial agents can be of animal (chitin, chitosan, lysosomes and lactoperoxidase), vegetable (essential oils, aldehydes, esters, plants) and microbiological (nisin) origin [7].

As one of the antimicrobial agents of animal origin, chitosan has several advantages over other natural antimicrobial agents. These are high antimicrobial activity, a broad spectrum of action, a high degree of inhibition of microorganisms and low toxicity to human cells [8].

Sources of chitosan, with high economic justification, are the secondary products of the seafood processing industry [5]. It is produced on a large scale in different parts of the world (Japan, North America, Poland, Russia, Italy, Norway and India) [8]. Chitosan is obtained from chitin with its deacetylation. Structurally, chitin is a polysaccharide composed of N-acetyl-D-glucosamine, GlcNAc ($C_8H_{15}NO_6$) repeating units linked by β-(1→4) glycosidic linkages, while technically, the structure of chitin is similar to cellulose and can be considered as cellulose in which the hydroxyl group (-OH) of the C-2 atom is replaced by an acetamido group (-$NHCOCH_3$). Chitosan is a derivative of chitin, obtained with the deacetylation of chitin (40–45% NaOH, 120 °C, 1–3 h), and a linear polycationic heteropolysaccharide composed of N-acetyl-D-glucosamine, GlcNAc, and D-glucosamine, GlcN, linked by β-(1→4) glycosidic bonds. The reactivity of chitosan is due to the amino group on the C-2 atom and the two hydroxyl groups on the C-3 and C-6 atoms [9]. The ratio between GlcNAc and GlcN units in chitosan represents the degree of deacetylation and depends on the deacetylation conditions. It increases by increasing the NaOH concentration, deacetylation temperature and time, and decreasing molecular weight. Chitin solvents are toxic and corrosive; thus, chitosan is used for commercial use. Chitosan is obtained by deacetylating chitin above 60%, which dissolves in dilute acids. Chitosan is insoluble in water, alkalis and organic solvents, and forms water-soluble salts with organic (acetic, formic, lactic, glutamic and maleic) and inorganic (hydrochloric) acids. The solubility of chitosan is a consequence of amino groups [10] and is pH dependent. Chitosan forms a viscous solution depending on the degree of deacetylation, molecular mass,

concentration and type of solvent, pH environment and temperature [9]. Using chitosan in any physical form depends on its physicochemical properties, e.g. colour, degree of polymerisation, degree of deacetylation, crystallinity, molecular weight, solubility and chemical reactivity. The biological properties of chitosan arise from its polycationic nature, which depends on the degree of deacetylation and molecular weight.

Research groups have outlined the antimicrobial activity of chitosan and its derivatives, and the mode of antimicrobial action and application of chitosan and its derivatives [8–11]. Many studies have focused on the bonding of chitosan with cellulose, and the discussion on antimicrobial textiles has been primarily focused on defining the type of bond and condition for their formation [12–14]. For instance, several published studies have focused on viscose, lyocell and modal fibre to analyse and evaluate their antimicrobial activity after being treated with chitosan [15–17]. In that, mainly the tests with a 1% concentration of chitosan solution for treatment were performed [13, 15–18]. To properly inform the evaluation and selection of chitosan solution concentration for textiles, it should be noted that an adequately selected concentration of chitosan solution is desirable for achieving the required antimicrobial activity. Therefore, an optimal concentration of chitosan solution incorporated in the cotton yarns treatment bath needs to be obtained that will yield higher percentage of bacteria reduction while minimising potential economic costs.

The present work aimed to study the antimicrobial activity of alkaline-scoured and bleached cotton yarns treated with different concentrations of chitosan solution incorporated in the application method. In addition to the antimicrobial activity, the tested samples were also examined for the amount of introduced chitosan, the amount of accessible amino groups, mechanical properties, whiteness index and the b^* colour coordinate. The surface of examined samples was also monitored with SEM and FTIR-ATR techniques.

2 Materials and methods

2.1 Materials

Piled, ring-worsted cotton yarns with 30 × 2 tex linear density and 330 twists/m were used. Commercially available chitosan samples with low molecular weight of 50000–190000 Da (g/mol) and deacetylation degree (DD) of 75–85% (denoted as ChL) were used to treat cotton yarns. Chitosan was used without further purification. *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 29219) bacteria obtained from American Type Culture Collection (ATCC) were used for the antibacterial activity test. The acetic acid and C.I. Acid Orange 7 were analytical grades purchased from Sigma-Aldrich (USA). Cotoblanc HTD (N-anionic surfactant) and Kemonecer NI (nonionic surfactant) were purchased from CHT and Kemo (Croatia), respectively.

2.2 Treatment Process

Pre-treatment of cotton yarns

Cotton yarns were pre-treated with alkaline scouring and bleaching processes before being treated with chitosan [19, 20]. Scouring was conducted in a bath with 30 : 1 LR using 20 g/dm^3 NaOH in the presence of 2 cm^3/dm^3 Cotoblanc HTD-N and 1 cm^3/dm^3 Kemonecer NI at 95 °C for 90 min. Bleaching was done in a bath with 30 : 1 LR, using 6 cm^3/dm^3 H_2O_2 (30%), 1 cm^3/dm^3 Kemonecer NI, 2 cm^3/dm^3 Na_2SiO at pH 11.2 at 95 °C for 30 min.

Chitosan treatment

The chitosan solution with 0.2, 0.6, and 1% (w/v) concentrations was prepared in 1% (v/v) CH_3COOH for 60 min at 60 °C followed by constant stirring; pH was adjusted to 4–4.5 by adding 95% acetic acid. The prepared chitosan solution was applied to the pre-treated cotton yarns using the exhaustion-pad-dry-rinse (EPDR) application method. A pre-treated sample (10 g) was immersed in the 300 cm^3 chitosan solution with stirring at 60 °C for 120 min. After the exhaustion phase, the sample was padded at 80% on a padding machine, dried at 60 °C for 12 h, rinsed five times at 20 °C for 10 min and dried at room temperature.

2.3 Methods

Determination of weight add-on

Before the testing, all samples were conditioned in a standard atmosphere (temperature 20 °C and 65% relative humidity) for 24 h to be identically acclimatised. The percentage add-on of cotton yarns after the chitosan treatment (i.e. the increase in sample weight relative to original weight) was determined with the gravimetric method with equation 1.

$$\Delta W\ (\%) = \frac{(W_2 - W_1)}{W_1} \times 100 \quad (1)$$

where: ΔW is weight add-on, W_1 is sample weight before the treatment with chitosan, and W_2 is sample weight after the treatment with chitosan.

Spectrophotometric C.I. Acid Orange 7 method

This method is based on the absorption of the dye C.I. Acid Orange 7 with the principle of reducing the dye concentration in the dye bath following the Lambert-Beer Law [16]. Sulfonic groups (SO_3^-) of the dye form within the acidic medium ionic bonds in the ratio 1 : 1 with the positively charged amino groups of chitosan (NH_3^+); therefore, the amount of the dye bound to fibres corresponds to the amount of accessible amino groups [16]. A 0.2 g sample was soaked in 100 cm^3 acetate buffer at pH 4 (0.5 g/dm^3 CH_3COONa+0.5 g/dm^3 CH_3COOH glac.) in the presence of 0.02 g/dm^3 C.I. Acid Orange 7 at 30 °C for 3 h. The maximum wavelength of absorbency (λ_{max} = 484 nm) of C.I. Acid Orange 7 dye solution was measured using a Perkin Elmer Lambda 2 UV-VIS spectrophotometer. For the calibration of standard solutions, a dye stock solution was prepared.

Measurement of colour strength (K/S)

The colour strength (K/S) of samples dyed with C.I. Acid Orange 7 was measured by using X-Rate Color i7 at maximum wavelength of absorbency (λ_{max}). It was calculated using the built-in software of the computer colour matching system according to the Kubelka-Munk equation given in equation 2.

$$\frac{K}{S} = \frac{(1-R)^2}{2R} \quad (2)$$

where: K is the absorption coefficient, S is the scattering coefficient and R is the reflectance value of the dyed sample at the wavelength at maximum absorption.

Fourier transform infrared spectroscopy-attenuated total reflectance (FTIR-ATR)

The FTIR-ATR spectroscopy was performed on a FTIR-ATR Perkin Elmer Spectrum GX 69876 spectrometer. 16 scans at the resolution of 4 cm^{-1} were recorded for each sample between 4000 cm^{-1} and 650 cm^{-1}.

Scanning electron microscopy (SEM)

The characterization of surface morphology with scanning electron microscopy (SEM) was conducted on a JEOL JSM-6060 LV (Japan) electron microscope operating at an accelerating voltage of 10 kV and magnification of 5000× on samples previously coated with gold in a scatter coater.

Antimicrobial activity

The antimicrobial activity of samples against *Escherichia coli* ATCC 25922 (Gram-negative bacteria) and *Staphylococcus aureus* ATCC 29219 (Gram-positive bacteria) was quantitatively determined according to the standard AATCC Test Method 100:2004. A 2 g sample was soaked in 57 cm^3 nutrient broth inoculated with the bacterium ($1.5–3.0 \times 10^5$ CFU/ml) and incubated at 37 °C for 60 min. After a series of dilutions of the bacterium, the plates were inoculated and incubated at 37 °C for 24 h. Later, surviving cells were counted. The average values of duplicates were converted to CFU/ml in flasks by multiplying by the dilution factor. The antimicrobial activity was expressed in the percentage reduction of organisms after contact time with the test specimen compared to the number of bacterial cells surviving after the contact with the control according to equation 3.

$$Reduction\ (\%) = \frac{B - A}{B} \times 100 \quad (3)$$

where: A is CFU/ml after contact (end test), and B is CFU/ml at zero contact time.

Mechanical properties

Tensile strength (F_a), elongation at break (ε) and work of rupture (A) were measured on a Tinus Olsen (SDL ATLAS) instrument according to the EN ISO 2062:2009 standard using the test speed of 100 mm/min and gauge length of 300 mm. The presented results are the mean value of 10 measurements at a confidence level of 95%.

Whiteness index and b^* colour coordinate

The whiteness index and b^* colour coordinate (yellowness) were measured according to the ISO 105-J02:1999 and ISO 105-J01:1997 standards, respectively, on an XRate 7i spectrophotometer under illuminant D65 and using the 10° standard observer. For each fabric sample, 10 measurements were performed at a confidence level of 95%, and the

mean value (M) and standard deviation (SD) were presented as the result.

3 Results and discussion

The pre-treatment process removed non-cellulose components from cotton yarns and influenced their surface functional group; therefore, it was proven that alkaline-scoured cotton yarns possess lower carboxyl and aldehyde group amounts than enzymatic-scoured cotton yarns [21, 22]. Thus, to remove all non-cellulosic components from cotton yarns, rigorous alkaline scouring and bleaching processes were conducted, and the amount of carboxyl and aldehyde groups of these yarns determined with the calcium acetate method was 60 and 10 mmol/kg, respectively [19, 20]. The alkaline-scoured and bleached cotton yarns used in our previous research were employed in the present work for monitoring the effect of chitosan solution concentration incorporated in the exhaustion phase on the antimicrobial activity of chitosan-treated cotton yarns. The exhaustion phase was the first phase of the chitosan application method used in this research, which included the following phases: exhaustion, pad, dry and rinse. The amount of introduced chitosan onto alkaline-scoured and bleached cotton yarns after the treatment with different concentrations of chitosan solution (from 0.2 to 1%) is shown in Figure 1. Chitosan has an affinity to absorb onto cellulose due to its similar nature [23]. It was observed that the amount of introduced chitosan onto pre-treated cotton yarns increased by increasing the concentration of chitosan solution from 0.2 to 1%. Pre-treated cotton yarns showed after the chitosan treatment (Figure 1) an introduced amount of chitosan of 2.5% at 0.2% concentration of chitosan solution. The amount of introduced chitosan onto pre-treated cotton yarns increased up to 7.51% with the increase in the concentration of chitosan solution in the exhaustion bath at 1%.

The amount of accessible amino group of chitosan-treated cotton yarns was determined with dyeing with Acid Orange 7 (an acidic dye that reacts with protonated amino groups). The amount of accessible amino groups determined by the colour strength after the dyeing of chitosan-treated cotton yarns (K/S) and the amount of accessible amino groups determined with the exhaustion method of the same dye (NH_2, mmol/kg) are shown in Table 1.

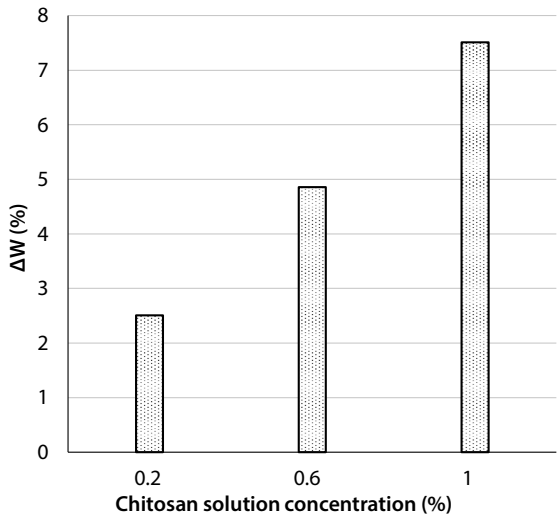

Figure 1: Amount of introduced chitosan onto alkaline-scoured and bleached cotton yarns after treatment with different concentrations of chitosan solution

The results indicate that increasing the concentration of chitosan solution increased the amount of accessible amino groups onto chitosan-treated cotton yarns. A larger amount of accessible amino groups gives a higher K/S value [24]. Chitosan-treated samples having lower K/S values indicated less accessible amino groups that can react with the negatively charged groups of the anionic dye.

Table 1: Amount of accessible amino groups onto alkaline-scoured and bleached cotton yarns treated with different concentrations of chitosan solution

Concentration of chitosan solution (%)	K/S	NH_2 (mmol/kg)
0.2	1.03	19.78
0.6	3.70	20.27
1.0	5.93	20.75

The samples were analysed by SEM concerning their surface morphology. Comparing the SEM images of alkaline-scoured and bleached cotton yarn and chitosan-treated yarn, as reported in Figure 2, the presence of chitosan was clear. The alkaline-scoured and bleached cotton yarn (Figure 2, AB) had clearly expressed fibrils on the surface of the fibre or completely removed the cuticle, while after the treatment with chitosan, the surface became smooth (Figure 2, AB-ChL). On chitosan-treated samples, no agglomerated chitosan was present on the surface, denoting the good

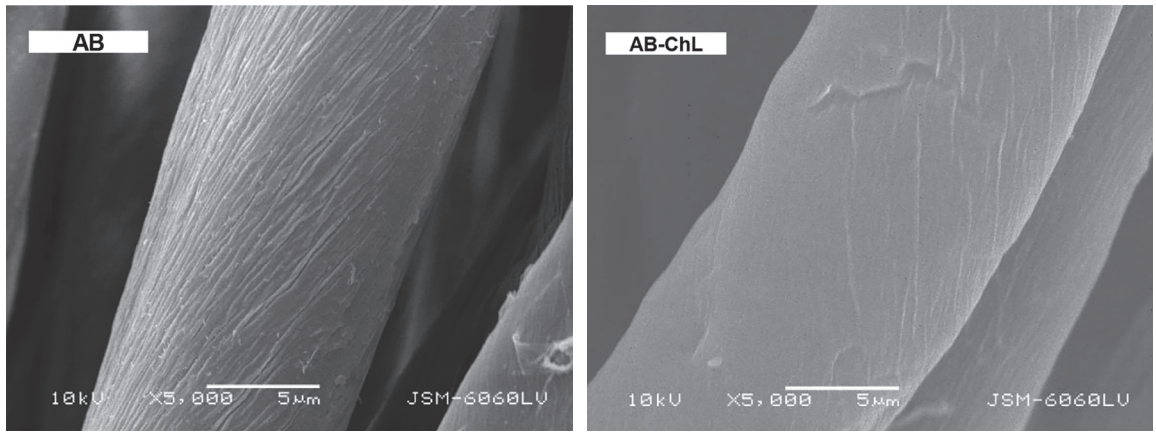

Figure 2: SEM images of surface of alkaline-scoured and bleached cotton yarns (AB) treated with 0.6% concentration of chitosan solution (AB-ChL)

quality of the chitosan solution and the effectiveness of the treatment.

The surface changes of pre-treated cotton yarns after the chitosan treatment were also observed from the FTIR-ATR spectra, which are given in Figure 3. Chitosan and cotton have similar FTIR-ATR spectra [23]. Alkaline-scoured and bleached cotton yarns (AB) showed characteristic FTIR-ATR spectra of cellulose. The presence of chitosan was confirmed by the increase of the 3295 cm^{-1} peak characteristic of ν(NH), ν(OH), and ν(NH_2) and the increase of 2922 cm^{-1} and 2854 cm^{-1} peaks characteristic of ν(C-H). In addition, the increase in the peaks at 1655 cm^{-1} (C = O, amide I) and 1550 cm^{-1} (N-H, amide II) (Figure 3), characteristic of the chitosan presence [25, 26], was observed. Depending on the degree of deacetylation, the peak at 1550 cm^{-1} can also appear at 1604 cm^{-1}, 1598 cm^{-1} or 1592 cm^{-1} [27]. The addition of chitosan to the pre-treated sample showed the appearance of the peak at 1720 cm^{-1} attributable to the $–COO^-$ anion of the carboxyl group suggesting the existence of a polyelectrolyte complex formed by the interaction between the chitosan amino groups and the carboxylic group of alkaline-scoured and bleached cotton samples. The scouring and bleaching processes remove non-cellulosic components, making acidic groups more available to increase the negative values of ζ-potential [20]. The peak at 1720 cm^{-1} originates from a combination of asymmetric COO^- and NH_3^+ stretching vibrations of the formed carboxylate complex [27].

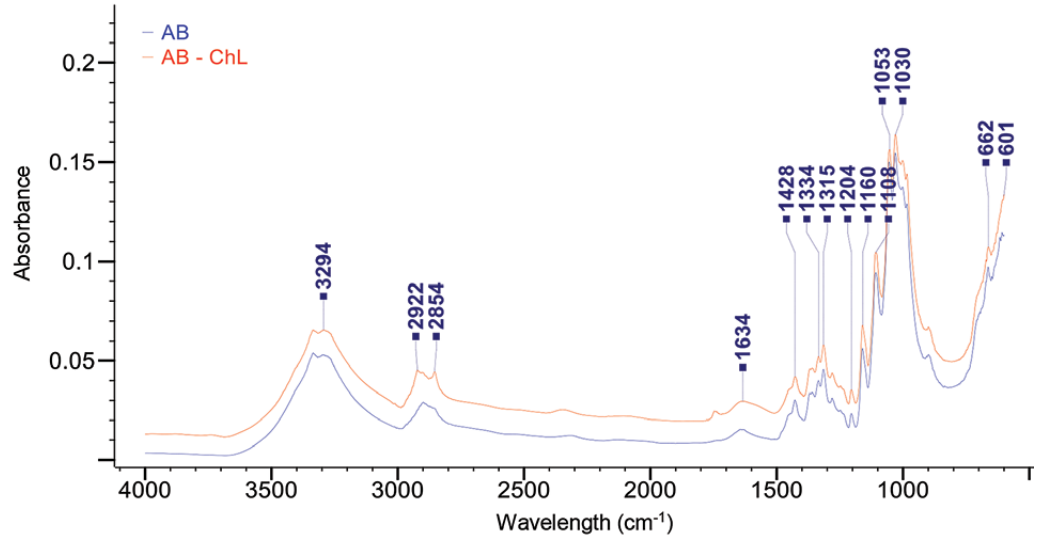

Figure 3: FTIR-ATR spectra of surface of alkaline-scoured and bleached cotton yarns (AB) treated with 0.6% concentration of chitosan solution (AB-ChL)

Chitosan contains amino groups responsible for its reactivity and antimicrobial activity [9]. The antimicrobial activity of chitosan-treated cotton yarns on the percentage reduction in CFU against *Escherichia coli* (Gram-negative bacteria) and *Staphylococcus aureus* (Gram-positive bacteria) is shown in Figure 4. Textile materials with an effective antimicrobial activity reduce the Gram-negative and Gram-positive bacteria by more than 70% [16, 28]. It was observed that regardless of the chitosan solution concentration, the chitosan-treated cotton yarns showed over 75% reduction in the number of CFU against both *Escherichia coli* and *Staphylococcus aureus*, indicating excellent antimicrobial activity of chitosan solution even at a low concentration of 0.2% (Figure 4). At 0.6% concentration of chitosan solution, the reduction rate increased to 91.74% for *Staphylococcus aureus*. The reduction increased up to 99.64% against *Escherichia coli* with the increase in the concentration of chitosan solution to 1%. The accessible amino groups of chitosan react with hydrogen ions and give NH_3^+ cations, which react with the negative charge from the surface of the bacterial cell, leading to the emission of intracellular components and disruption of cell functions [11]. The antimicrobial activity was greater against *Escherichia coli* than against *Staphylococcus aureus* (Figure 4). The mode of the antibacterial activity is a complex process that differs in Gram-positive and Gram-negative bacteria due to the differences in the bacterial cell surface [9]. According to some studies, cotton treated with chitosan has a higher antimicrobial activity against Gram-positive (*Staphylococcus aureus*) bacteria compared to Gram-negative (*Escherichia coli*) bacteria [28]. According to other research, the general trend is that chitosan-based antimicrobial textiles inhibit the growth of Gram-negative bacteria more than of Gram-positive bacteria [11].

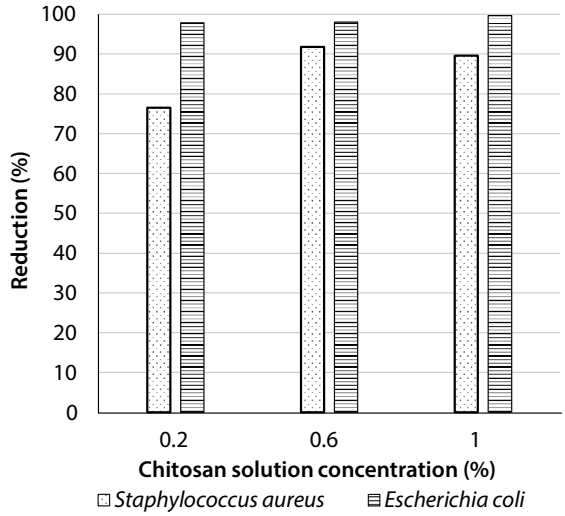

Figure 4: Reduction percentage in number of CFU against Staphylococcus aureus and Escherichia coli of cotton yarns treated with different concentrations of chitosan solution

The effect of chitosan treatment with different concentrations of chitosan solution on the mechanical properties of alkaline-scoured and bleached cotton yarns is shown in Table 2. Increasing the amount of chitosan introduced onto pre-treated cotton yarns was accompanied by decreased tensile strength, elongation at break and work of rupture after the chitosan treatment. Compared with alkaline-scoured and bleached cotton yarns, the tensile strength and work of rupture of chitosan-treated cotton yarns obtained with 0.2% concentration of chitosan solution decreased by 20%, while elongation at break decreased by 1%. The loss in tensile strength of pre-treated cotton yarns after the chitosan treatment was related to the acidity of the solution of chitosan treatment (chitosan dissolved in 1% acetic acid solution), which causes the depolymerisation of cellulose macromolecules [18].

Table 2: Tensile strength, elongation at break, and work of rupture of alkaline-scoured and bleached cotton yarns treated with different concentrations of chitosan solution

Concentration of chitosan solution (%)	Mechanical properties					
	F_a (N)		ε (%)		A (mJ)	
	M	SD	M	SD	M	SD
0.0	9.67	0.72	16.22	2.31	159.40	0.02
0.2	7.77	0.64	16.09	1.02	127.93	0.02
0.6	8.49	0.54	14.97	2.09	133.62	0.01
1.0	7.98	0.38	14.91	2.31	122.29	0.02

Table 3: Whiteness index and b^* colour coordinate of alkaline-scoured and bleached cotton yarns treated with different concentrations of chitosan solution

Concentration of chitosan solution (%)	WICIE		b^*	
	M	SD	M	SD
0.0	76.24	1.02	0.95	0.06
0.2	73.15	1.84	1.73	0.07
0.6	60.43	0.88	3.27	0.07
1.0	40.72	0.97	4.85	0.09

The changes in whiteness index and b^* colour coordinate (yellowness) of alkaline-scoured and bleached cotton yarns treated with different concentrations of chitosan solution were observed as well. The whiteness index and yellowness of tested samples are shown in Table 3. Increasing the concentration of chitosan solution reduced the whiteness index and increased the yellowness of chitosan-treated cotton yarns, which was noticed from the increased values of the b^* colour coordinate. The thermal stability of chitosan may also contribute to reducing the whiteness index in chitosan-treated cotton yarns. As the temperature increases, the colour of chitosan changes, indicating that heating causes the degradation of chitosan [29]. In our research, the temperature of the drying phase was 60 °C. This drying temperature decreased the whiteness index and increased the yellowness (b^* value) of cotton yarns treated with a higher concentration of chitosan solution.

4 Conclusion

The antimicrobial textile obtained from chitosan and cotton yarns was prepared using the exhaustion-pad-dry-rinse application method. Different concentrations of chitosan solution incorporated in the exhaustion phase were used. The chitosan-treated cotton yarns were investigated using the introduced chitosan, the amount of accessible amino groups, and the antimicrobial activity against *Escherichia coli* (Gram-negative bacteria) and *Staphylococcus aureus* (Gram-positive bacteria). SEM and FTIR-ATR techniques were used to examine the surface of tested samples. The mechanical properties, whiteness index and b^* colour coordinate were determined as well. The data confirmed a significant increase in the amount of introduced chitosan and the amount of accessible amino groups when the concentration of chitosan solution from 0.2% to 1% was applied on alkaline-scoured and bleached cotton yarns, which also led to a smooth surface as illustrated by SEM and increased peaks (amide I and amide II) corresponding to the presence of chitosan shown by FTIR-ATR. All concentrations of chitosan solution showed good antimicrobial activity of chitosan-treated cotton yarns against *Escherichia coli* and *Staphylococcus aureus* (by more than 75%). The antimicrobial activity was found to be more effective against *Escherichia coli*. Chitosan caused changes in the mechanical properties, whiteness index and b^* colour coordinate (yellowness) of pre-treated cotton yarns; thus, a slight decrease in the mechanical properties and whiteness index and, consequently, an increase in the b^* value was noticed. The optimal concentration of chitosan solution for applying chitosan and obtaining antimicrobial-active chitosan-based cotton yarns with good antimicrobial activity was 0.6%. A successful treatment of chitosan onto cellulose is opening the way for several applications where the properties of biocompatibility and antimicrobial activity may be combined.

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