ANTIBACTERIAL AND ANTIOXIDANT PROPERTIES OF CELLULOSE/CHITOSAN COMPOSITE TEXTILE MATERIALS FUNCTIONALIZED WITH BEECH WOOD STEAMING CONDENSATE

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The main objective of this study was to obtain cellulose/chitosan composite textile materials with improved antibacterial and antioxidant properties using a circular economy approach. In this regard, viscose fabric and viscose fabric coated with 2,2,6,6-tetramethylpiperidine-1-oxy radical (TEMPO)-oxidized cellulose nanofibrils (TOCN) were functionalized with chitosan or chitosan nanoparticles. These cellulose/chitosan composite textile materials were subsequently functionalized with beech wood steaming condensate (BSC), a wood industry process effluent rich in phenolic acids and flavonoids. The volumetric method was used to determine the content of carboxy and aldehyde groups in TOCN-coated viscose fabrics. UHPLC-QToF-MS was used to identify and characterize compounds in the BSC. The surface chemistry of cellulose/chitosan composite textile materials before and after BSC functionalization was characterized by FTIR spectroscopy. Their antibacterial and antioxidant properties were determined by the agar diffusion and DPPH methods, respectively. BSC functionalization enhanced inhibition of *S. aureus* and *E. coli* and markedly increased antioxidant activity (ranging from 67.74% to 92.58%).

Keywords: viscose fabric, chitosan, beech wood steaming condensate, antibacterial activity, antioxidant activity

INTRODUCTION

Increased environmental awareness, the predicted depletion of fossil resources, and the increasing costs of their extraction have fueled a surge in interest in renewable and sustainable resources on a global scale. Recently, polysaccharides have attracted the greatest interest due to their renewability, recyclability, and biodegradability, as well as the fact that they represent the most abundant part of biomass.[1] Among them, cellulose and chitosan are particularly attractive for textile applications because they combine complementary characteristics. Cellulose primarily possesses good structural, mechanical, and hydrophilic properties, making it a suitable carrier for bioactive substances, while on the other hand, chitosan possesses a wide range of bioactive properties, making it a suitable bioactive substance.[2-4]

When cellulose and chitosan are combined, their interactions are typically governed by hydrogen bonding and Van der Waals forces. Therefore, to achieve more intense and irreversible binding between cellulose and chitosan, it is necessary to introduce additional functional groups into/onto the cellulose, which are then potential anchoring sites for chitosan molecules.[5]

These additional functional groups can be introduced into/onto cellulose by numerous chemical modifications.[6] Unfortunately, most of them lead to a deterioration in the mechanical properties of textile materials based on cellulose.[7–9] Considering this fact, Korica *et al.*[10] found a way to introduce additional functional groups onto textile materials based on cellulose without deteriorating their mechanical properties. Specifically, Korica *et al.*[10] were the first to coat cellulose-based textile materials with 2,2,6,6-

tetramethylpiperidine-1-oxy radical (TEMPO)-oxidized cellulose nanofibrils (TOCN). Namely, by TEMPO oxidation of cotton fibers and successive mechanical and ultrasound treatments, nanofibrillated cellulose with an increased content of carboxy and aldehyde groups, the so-called TOCN, was obtained. After coating cellulose-based textile materials with TOCN, the introduction of additional functional groups (carboxy and aldehyde) onto their surface was achieved without deteriorating their mechanical properties.[10]

It has been reported that cellulose/chitosan-based textile materials exhibit bioactive properties.[2,11,12] However, there is a need for their improvement because most of them show strong antibacterial activity against Gram-positive bacteria, but moderate antibacterial activity against Gram-negative bacteria and weak antioxidant activity.[10,13-18] This limitation can be overcome by subsequent functionalization of cellulose/chitosan-based textile materials with some additional natural compounds that possess bioactive properties.[19-23]

Natural compounds that possess bioactive properties are widespread. However, those that can be found in industrial wastewater are increasingly being considered. The reason for this lies in the tendency to reduce the harmful impact of industrial wastewater on the environment by using it as a valuable resource of different compounds that can be used in the textile industry.[24-26] Wood steaming is an established hydrothermal treatment in the wood industry that generates a colored process effluent (steaming condensate) rich in phenolic acids, flavonoids, and related compounds that possess bioactive properties.[27-30] Beech is the most abundant broad-leaved tree in Central Europe,[31] and its use has increased significantly in recent years. Consequently, the volume of beech wood steaming condensate (BSC), *i.e.*, a colored industrial wastewater, has also increased.[32] To address the environmental challenges associated with this wastewater in Europe and promote the use of naturally derived compounds with bioactive properties in the textile industry, this study investigates the use of BSC for the functionalization of textile materials. To the best of our knowledge, this is the first study to employ BSC to functionalize cellulose/chitosan-based textile materials, more precisely, to improve their antibacterial and antioxidant properties. According to the available literature data, so far, only eucalyptus wood steaming condensate, which is most common in Brazil, India and China, has been used to functionalize textile materials. Namely, Rossi *et al*.[24] used this condensate to functionalize cotton, wool and polyamide 6.6. However, the antibacterial and antioxidant properties of these textile materials have remained unexamined.

In the proposed concept, viscose fabric was coated with TOCN to introduce carboxy and aldehyde groups onto its surface, which are necessary for more intense and irreversible binding of chitosan and chitosan nanoparticles.[5,10] Although chitosan and chitosan nanoparticles possess antibacterial and antioxidant activity,[2,3,4] cellulose/chitosan-based textile materials exhibit moderate antibacterial activity against Gram-negative bacteria and weak antioxidant activity.[10,13-18] Therefore, these textile materials were subsequently functionalized with BSC, which is rich in additional natural compounds with antibacterial and antioxidant properties such as phenolic acids and flavonoids,[27-30] to improve their antibacterial and antioxidant properties. The content of carboxy and aldehyde groups in unmodified and TOCN-coated viscose fabrics was determined by the calcium acetate method. For the identification and characterization of compounds in BSC, an ultra-high-performance liquid chromatography system coupled with a quadrupole time-of-flight mass spectrometry (UHPLC-QToF-MS) was used. The surface chemistry of cellulose and chitosan/chitosan nanoparticles-based textile materials, before and after functionalization with BSC, was examined by FTIR spectral analysis. Their antibacterial properties were determined by the agar diffusion method, while antioxidant properties were determined by the 1,1-diphenyl-2-picrylhydrazil (DPPH) method.

EXPERIMENTAL

Materials

Viscose fabric (15A23 sandy-white viscose) provided by IGR Agence was used as a base material (with mass per unit area of 82 g/m^2, yarn count in the warp and in the weft direction of 9.6 tex and, 9.9 tex (respectively), number of threads per unit length of 400 warp threads/10 cm and 350 weft threads/10 cm). Beech wood steaming condensate was provided by Megadrvo Company (Bijeljina, Bosnia and Herzegovina). Chitosan (CH) from crab shells (with low molecular weight (Aldrich, 448869), 75%–85% deacetylated), TEMPO, sodium tripolyphosphate (TPP), sodium bromide (NaBr), sodium hypochlorite (NaClO), sodium chlorite ($NaClO_2$), sodium hydroxide (NaOH), and 13% sodium hypochlorite (NaClO) solution were from

Sigma-Aldrich (Vienna, Austria), and applied without further purification.

Preparation of TEMPO-oxidized cellulose nanofibrils

TOCN was prepared by defibrillation of TEMPO-oxidized cotton fibers according to the method reported by Korica *et al*.[5]

Preparation of chitosan solution and chitosan nanoparticles dispersion

A 0.5% w/v CH solution was prepared by suspending CH in distilled water. The pH of CH suspension was adjusted to 2.5 using 1 M HCl. The resulting CH suspension was then stirred for 24 h under room temperature until achieving a complete dissolution of the CH. The final pH of the CH solution was adjusted to pH 5.5 with 0.5 M NaOH.[10]

CH solution (0.5% w/v) was prepared by suspending CH in distilled water, after which the pH was adjusted to 2.5 using 1 M HCl. The resulting CH suspension was then stirred for 24 h under room temperature until achieving a complete dissolution of the CH. The chitosan nanoparticles were prepared through the ionotropic gelation of chitosan with sodium tripolyphosphate (TPP) as a crosslinking agent. TPP solution (1 mg/mL) was prepared by dissolving TPP in distilled water. Finally, chitosan nanoparticles were formed spontaneously by dropping TPP solution into the CH solution (in a ratio of 1:1 (w/w)) during 1 h under stirring at room temperature. The pH of the chitosan nanoparticle dispersion was adjusted to pH 5.5 by addition of 0.5 M NaOH.[13]

Preparation of beech wood steaming condensate

Beech wood steaming condensate (BSC) originated from an industrial indirect steaming process, in which steam is generated by heating water within the chamber. The steaming was performed at 95 ℃ for 12 hours per cycle, including the heating and cooling phases. After two consecutive 12-hour cycles, the condensate sample was collected from different points of the tank in the chamber floor. The pH of BSC was 4.6, dry matter was 0.46%, and total phenolic content was 24.3 mg GAE/20 mL. The sample was stored in a sealed glass container in a refrigerator at 4 °C, protected from light, until use to minimize the risk of additional bacterial or fungal contamination.

Viscose fabric pretreatment

Preparation of viscose fabric coated with TEMPO-oxidized cellulose nanofibrils

This step was carried out to introduce carboxy and aldehyde groups onto the surface of the viscose fabric, given that the introduced carboxy and aldehyde groups are necessary for more intense and irreversible binding of chitosan and chitosan nanoparticles. Namely, viscose fabric was soaked in a 0.5% (w/v) dispersion of TOCN with a material-liquid bath ratio of 1:50 and a wet pick-up of 100%. Soaking lasted for 30 min at room temperature. The viscose fabric was then squeezed onto a laboratory padder (Rapid, Istanbul, Turkey) at a pressure of 2 bars. After excess liquid was removed, the fabric was dried in a laboratory oven (Instrumentaria, Zagreb, Croatia) at 40 °C for 30 min. The fabric was then conditioned at T = 20 ± 2 °C and RH = 65 ± 4% before being functionalized with chitosan and chitosan nanoparticles.

Functionalization of fabrics with chitosan and chitosan nanoparticles

Subsequent functionalization of cellulose-based textile materials with chitosan and chitosan nanoparticles was carried out to achieve their antibacterial and antioxidant properties. The unmodified and TOCN-coated viscose fabrics were soaked in a chitosan solution or a chitosan nanoparticle dispersion with a material-liquid bath ratio of 1:50. Soaking lasted for 30 min at room temperature. Effective concentrations of CH and NCH during soaking were 25% and 12.5% (on the weight of fabric), respectively. The fabrics were then squeezed onto a laboratory padder (Rapid, Istanbul, Turkey) at a pressure of 2 bars to achieve a wet pick-up of 100%. After excess liquid was removed, the fabrics were dried in a laboratory oven (Instrumentaria, Zagreb, Croatia) at 40 °C for 30 min. The fabrics were then conditioned at T = 20 ± 2 °C and RH = 65 ± 4% before being analyzed further.

Functionalization of fabrics with beech wood steaming condensate

To further improve the antibacterial and antioxidant properties of cellulose/chitosan-based textile materials, their functionalization with BSC was carried out.

A 15% solution of beech wood steaming condensate was prepared by diluting 15 mL of the initial solution of this condensate with 85 mL of distilled water. The final pH of the aforementioned 15% condensate solution was adjusted to pH 3 by adding 0.1 M HCl.

Unmodified and TOCN-coated viscose fabric, as well as unmodified and TOCN-coated viscose fabric functionalized with chitosan and chitosan nanoparticles, was subsequently functionalized with a 15% solution of beech wood steaming condensate.

The aforementioned fabrics were soaked in a 15% solution of condensate with a material-liquid bath ratio of 1:50 for 24 h, under frequent shaking at 35 °C (Memmert, Schwabach, Germany). Afterwards, the fabrics were thoroughly washed with warm distilled water and air dried. The fabrics were then conditioned at T = 20 ± 2 °C and RH = 65 ± 4% before being analyzed further.

The sample denotations are listed in Table 1.

Carboxy group content

The carboxy groups of cellulose react with salts of weaker acids, such as calcium acetate, forming a salt of

the cellulose and releasing an equivalent amount of the weaker acid. Based on this and a modification of the calcium acetate method,[34] the following method was applied to determine the carboxy group content in unmodified and TOCN-coated viscose fabric.

Table 1
Denotation of the fabric samples

Sample description	Sample denotation
Unmodified viscose fabric	V
TOCN-coated viscose fabric	T-V
V+ chitosan	V-CH
T-V+ chitosan	T-V-CH
V+ chitosan nanoparticles	V-NCH
T-V+ chitosan nanoparticles	T-V-NCH
V+ beech wood steaming condensate	V- BSC
T-V+ beech wood steaming condensate	T-V-BSC
V-CH+ beech wood steaming condensate	V-CH-BSC
T-V-CH+ beech wood steaming condensate	T-V-CH-BSC
V-NCH+ beech wood steaming condensate	V-NCH-BSC
T-V-NCH+ beech wood steaming condensate	T-V-NCH-BSC

0.5 g of fabric was treated with 0.01 M HCl during 1 h and then thoroughly washed with distilled water. Afterwards, the fabrics were soaked in 50 mL of distilled water and 30 mL 0.25 M of calcium acetate solution. Soaking lasted for 2 h with frequent shaking to facilitate completion of the interchange. Portions of 30 mL of this liquid were taken and titrated with 0.01 M sodium hydroxide, using an 848 Titrino Plus (Metrohm UK Ltd., Buckingham, UK) device. The carboxy content was calculated as follows (1):

$$Carboxy\ content = \frac{\frac{80}{30} 0{,}01 \cdot V(NaOH)}{m(1-\frac{w}{100})}, mmol \cdot g^{-1}\ cellulose$$

where 0.01 M is the concentration of NaOH, V(NaOH) is the volume (mL) of the NaOH solution used for titration, m is the weight of the fabrics (g) and w is the moisture content (%).

The carboxy group content of samples was determined in triplicate.

Aldehyde group content

The aldehyde content in unmodified and TOCN-coated viscose fabric was determined according to a modified method described by Parks and Hebert.[35] The aldehyde groups of the fabrics were selectively oxidized to carboxy groups with sodium chlorite under acidic conditions (pH 3-4), adjusted with acetic acid. More precisely, a solution of 0.905 g $NaClO_2$ in 50 mL distilled water was acidified by adding 10 mL of 5 M CH_3COOH. After that, 1 g of fabric was immersed in this mixture. The oxidation lasted for 48 h with stirring of the mixture at room temperature, followed by washing with water and filtration. Then, the carboxy group content was determined by the above-mentioned calcium acetate method.

The carboxy groups formed in this way were considered to be aldehyde groups present in the fabrics. The aldehyde group content was calculated as the increase in carboxy group content after sodium chlorite oxidation relative to the initial carboxy group content.

The aldehyde group content of samples was determined in triplicate.

UHPLC-QToF-MS analysis

Beech wood steaming condensate evaporated to dryness was reconstituted in 20 mL of 80% methanol containing 0.1% HCl, assisted by ultrasound treatment. After that, BSC was filtered through 0.22 μm syringe filters and analyzed by UHPLC Q-ToF MS.

The analyses were carried out on Agilent 1290 Infinity ultra-high-performance liquid chromatography (UHPLC) system coupled with a quadrupole time-of-flight mass spectrometry (6530C Q-ToF-MS) from Agilent Technologies, Inc., CA, USA, as previously described by Kostić *et al*.[36] The chromatographic separation was performed at 40 °C on a Zorbax C18 column (2.1 × 50 mm, 1.8 μm) from Agilent Technologies, Inc., CA, USA. The mobile phase mixtures comprised: (A) ultrapure water and (B) acetonitrile (MS grade), both A and B containing 0.1% HCOOH (MS grade). The flow rate was constant and set to 0.3 mL min^{-1}, while the injection volume was 5 μL. The gradient elution program started with 2% solvent B for the twice minute, which then reached 98% B over the next 17 minutes, and for the next 5 minutes the gradient was returned to initial conditions (2% B) to re-equilibrate the column to initial conditions.

The QToF-MS system was equipped with a Dual Agilent Jet Stream electrospray ionization (ESI) source, operating in both positive (ESI+) and negative (ESI-) ionization modes. The operation parameters for ESI were set as follows: nebulizer pressure of 45 psi, a drying gas temperature of 225 °C and a flow rate of 8 L/min, sheath gas temperature of 300 °C and sheath gas flow 10 L/min, capillary voltage of 2500 V, fragmentor energy of 175 V, skimmer voltage of 65 V, octopole RF Peak at 750 V. The QToF-MS system was recorded spectra over the m/z range 100–1700, with a scan rate of 2 Hz. Data-dependent acquisition (DDA) was

employed for suspect screening, using the Auto MS/MS acquisition mode with collision energy at 30 eV. Parameters for the Auto MS/MS mode were as follows: m/z = 100–1700, scan rate 1 spectra/second. Agilent MassHunter software was used for instrument control, data evaluation and analysis.

Phenolic compounds were identified based on their monoisotopic mass, MS fragmentation and previously reported literature data.[37-39] Accurate masses of components were calculated by using ChemDraw software (version 12.0, CambridgeSoft, Cambridge, MA, USA).

Target compounds, expected retention time (RT), molecular formula, calculated mass, exact mass, and MS^2 fragments are presented in Table 3.

FTIR analysis

Before FTIR analysis, the fabrics were dried at 40 °C for 24 h in a laboratory oven (Instrumentaria, Zagreb, Croatia) and stored in a desiccator. FTIR spectra of the fabrics were recorded using a Nicolet™ iS™ 10 FTIR Spectrometer (Thermo Fisher Scientific) with a Smart iTX™ Attenuated Total Reflectance sampling accessory in the wavenumber range of 400–4000 cm^{-1}, at a resolution of 2 cm^{-1}, and in 32 scan mode.

Antibacterial activity

The antibacterial activity of the fabrics was evaluated using an agar diffusion test against the Gram-positive bacterium *Staphylococcus aureus* ATCC 25923 (*S. aureus*) and the Gram-negative bacterium *Escherichia coli* ATCC 25922 (*E. coli*). The test medium consisted of tryptone soy medium supplemented with yeast extract (6 g/L) and agar (16 g/L). Overnight bacterial cultures containing approximately 10^8 CFU/mL were diluted 10-fold to obtain a suspension containing approximately 10^7 CFU/mL. An aliquot of 200 µL of the diluted bacterial suspension was added to 6 mL of molten agar medium, thoroughly homogenized, and poured into Petri dishes, resulting in a final bacterial concentration of approximately 3×10^5 CFU/mL. After solidification, circular fabric samples (10 mm in diameter) were placed onto the inoculated agar and incubated at 37 °C for 24 h. The antibacterial activity of samples was determined in duplicate.

Antibacterial activity was evaluated qualitatively by visual examination of bacterial growth beneath the fabric samples and in the immediate contact area around their edges. Growth inhibition was observed beneath the samples and in the contact area adjacent to the fabric, but no distinct inhibition zone extending away from the samples was formed. Therefore, inhibition-zone diameters were not measured, and the antibacterial effect was evaluated based on the presence of growth inhibition in the direct contact area.

Antioxidant activity

The free radical scavenging potential of the fabrics was evaluated using DPPH free radical method. For the DPPH free radical scavenging analysis, a methanolic solution of DPPH was prepared by dissolving 4 mg of DPPH in 100 mL of methanol. Then, 50 mg of fabric was added to 10 mL DPPH solution and kept in the dark for 3 h. The fabrics were removed from the DPPH solution after 3 h. Then, the absorbance of DPPH solutions was measured at 517 nm (Shimadzu 1700 Spectrophotometer, Shimadzu Corp., Kyoto, Japan). The same test without fabric was used as a control. The antioxidant activity of fabrics was calculated using the following equation:

$$Antioxidant\ activity\ (\%) = \frac{A_0 - A_T}{A_0} \times 100 \quad (2)$$

where A_0 and A_T are the absorbance values for the control and fabric sample solution, respectively.

Sample-specific blanks, consisting of V, T-V, V-CH, T-V-CH, V-NCH, and T-V-NCH incubated individually in methanol in the absence of DPPH, were used to correct for the absorbance of components released from samples functionalized with CH, NCH, and BSC. The antioxidant activity of samples was determined in triplicate.

Statistical analysis

The experimental results for antioxidant activity were statistically analyzed using the t-test. The parameter t for independent samples was determined using Equation 3, whereas for dependent samples, it was calculated through Equation 4:[40]

$$t = \frac{\overline{x_1} - \overline{x_2}}{\sqrt{\frac{\sigma_1^2(n_1-1) + \sigma_2^2(n_2-1)}{n_1+n_2-2} \cdot \frac{n_1+n_2}{n_1 \cdot n_2}}} \quad (3)$$

$$t = \frac{\overline{d}}{\frac{\sigma_d}{\sqrt{n}}} \quad (4)$$

where $\overline{x_1}$ and $\overline{x_2}$ are the samples' mean values of antioxidant activity, σ_1 and σ_2 are the samples' standard deviation of antioxidant activity, n_1 and n_2 are their corresponding sample sizes (n_1=n_2=3), $\overline{d}$ is the sample mean value of the differences in antioxidant activity between (a) unmodified viscose fabric and the fabric after different modifications, (b) TOCN-coated viscose fabric and the fabric after modification with chitosan and chitosan nanoparticles, (c) viscose fabric functionalized with BSC and the fabric after different modifications, and (d) TOCN-coated viscose fabric functionalized with BSC and the fabric after modification with chitosan and chitosan nanoparticles, σ_d is the sample standard deviation of these differences, while n is the sample size (n=3).

RESULTS AND DISCUSSION

Content of carboxy and aldehyde groups in unmodified and TOCN-coated viscose fabric

During TEMPO oxidation of cotton, its primary hydroxy groups are converted into carboxy groups (in the form of sodium carboxylate groups) via aldehyde intermediates. The introduced carboxy and aldehyde groups are crucial for the successful mechanical defibrillation of TEMPO-oxidized cotton to TOCN. In an earlier study by Korica *et al*.,[10] it was determined that according to the applied procedure, TOCN was obtained with a content of carboxy groups of 0.830 mmol/g of cellulose and a content of aldehyde groups of 0.090 mmol/g of cellulose. By coating unmodified viscose fabric with TOCN, the introduction of carboxy and aldehyde groups on its surface is achieved. As can be seen from Table 2, the coating of unmodified viscose fabric with TOCN resulted in an increase in the content of carboxy groups by 0.024 mmol/g cellulose and the content of aldehyde groups by 0.007 mmol/g cellulose, in this work.

Chitosan and chitosan nanoparticles were immobilized on V and T-V samples. After that, V and T-V samples with and without immobilized chitosan and chitosan nanoparticles were functionalized with BSC.

Table 2
Content of carboxy and aldehyde groups in unmodified and TOCN-coated viscose fabrics

Sample	Carboxy groups, mmol/g cellulose	Aldehyde groups, mmol/g cellulose
V	0.063±0.002	0.016±0.001
T-V	0.087±0.002	0.023±0.001

Functionalization of viscose fabrics with beech wood steaming condensate

Beech wood steaming creates a condensate that is a rich source of water-soluble organic compounds released from the beech wood during hydrothermal treatment. This condensate usually has a brown color that comes from hydrolyzed lignin, phenolics, and carbohydrates that are released when hemicelluloses and lignin are broken down under the influence of heat.[30,41] Figure 1 shows fabric samples, both before and after functionalization with BSC. From Figure 1, it can be seen that all fabric samples turned brown after functionalization with BSC due to the binding of components of BSC that contribute to the coloring (the chemical composition of BSC is presented in Table 3).

By visual observation, it can be seen that this coloring is more intense on samples of fabrics with immobilized chitosan and chitosan nanoparticles. Given that the amino groups of chitosan and chitosan nanoparticles at pH 3, at which functionalization with BSC was carried out, are completely in the protonated form,[42] this trend is likely due to the greater affinity of the BSC components that contribute to the coloring (Table 3) towards positively charged groups. This assumption is supported by several previous studies in which it was reported that components of plant extracts show a greater affinity towards fibers with positively charged groups (such as wool and silk) compared to fibers with negatively charged groups (such as cotton and viscose). The reason for this lies in the fact that fibers with negatively charged groups in aqueous solution have negatively charged surfaces and generate electrostatic repulsion with most components of plant extracts, which makes it difficult for them to bond together.[43-46] To address this limitation, some research has already been devoted to the cationization of fibers with negatively charged groups using chitosan as a natural polycationic aminopolysaccharide.[43,47]

If we visually compare the coloring of the samples with immobilized chitosan and chitosan nanoparticles after functionalization with BSC in Figure 1, it is clearly noticeable that the samples with immobilized chitosan were colored more intensely. The more intense coloring of the samples with immobilized chitosan might be explained by the higher amount of immobilized chitosan, *i.e*., positively charged groups ready to react with BSC components. Namely, in earlier research by Korica *et al*.,[10,13] it was determined that according to the applied procedure of immobilization of chitosan and chitosan nanoparticles, 0.77 g of chitosan/100 g of cellulose was immobilized on V and 1.13 g of chitosan/100 g of cellulose on T-V, 0.43 g of chitosan nanoparticles/100 g of cellulose on V, and 0.63 g of chitosan nanoparticles/100 g of cellulose on T-V. Considering these facts, the assumption that the components of BSC that contribute to the

coloration show a higher affinity towards positively charged groups is further supported. The UHPLC-QToF-MS analysis confirmed that BSC contains a range of water-soluble extractives, including phenolic acids and derivatives, flavonoid-related compounds (*e.g.*, taxifolin glycosides), lignans, and other constituents (Table 3). This composition provides a chemical basis for improving the antioxidant and antibacterial activity of cellulose/chitosan composite textile materials after functionalization with BSC.



Figure 1: Samples of fabrics before and after functionalization with BSC

Table 3
Compounds in beech wood steaming condensate (target compounds, expected retention time (RT), molecular formula, calculated mass, exact mass, and MS^2 fragments)

Compound name	RT, min	Formula	Calculated mass	m/z exact mass	mDa	Fragments (MS^2)
Phenolic acids and derivatives						
Dihydroxybenzoic acid hexoside isomer I	2.76	$C_{13}H_{15}O_9$-	315.07216	315.07328	-1.13	108.02198(100), 109.02888(38), 152.01164(61), 153.01795(15)
Vanillic acid hexoside isomer II	3.70	$C_{14}H_{17}O_9$	329.08781	329.08870	-0.90	108.02180(100), 109.02519(8), 123.04515(41), 152.01139(72), 153.01605(9), 167.03482(36)
Dihydroxybenzoic acid hexoside isomer II	4.58	$C_{13}H_{15}O_9$	315.07216	315.07353	-1.38	108.02194(13), 109.02962(100), 110.03266(7), 123.04529(8), 152.01126(20), 153.01916(59)
Methyl-galloyl hexoside	4.65	$C_{14}H_{17}O_{10}$	345.08272	345.08560	-2.88	123.00993(3), 124.01672(52), 168.00644(100), 183.03012(79)
Hydroxybenzoic acid isomer I	4.71	$C_7H_5O_3$-	137.02442	137.02479	-0.37	/
Dihydroxybenzoic acid isomer I	4.98	$C_7H_5O_4$-	153.01933	153.01991	-0.58	108.02166(100), 109.02654(11)
Syringic acid hexoside	5.52	$C_{15}H_{19}O_{10}$	359.09837	359.09965	-1.28	123.00874(32), 138.03233(100), 153.05573(26), 182.02217(59), 197.04559(25)
Dihydroxybenzoic acid isomer II	6.20	$C_7H_5O_4$	153.01933	153.01965	-0.31	108.02152(100), 109.02964(99)
Hydroxybenzoic acid isomer II	8.36	$C_7H_5O_3$-	137.02442	137.02487	-0.45	/
Vanillin	9.17	$C_8H_7O_3$	151.04007	151.04118	-1.11	107.05046(100)
Flavan-3-ols and procyanidins						
(Epi)catechin-3-O-hexoside	6.06	$C_{21}H_{23}O_{11}$	451.12459	451.12699	-2.41	125.02451(17), 179.03459(12), 203.07107(16), 205.05081(16), 245.08171(50), 289.07156(100)

Flavonol aglycones and glycosides						
Taxifolin 3-O-hexoside	7.21	C21H21O12	465.10385	465.10658	-2.73	107.0144(10), 151.00392(100), 151.03505(45), 152.01073(14), 180.00652(8), 285.04079(66)
Taxifolin 3-O-pentoside	7.61	$C_{20}H_{19}O_{11}$-	435.09329	435.09421	-0.92	107.01429(14), 125.02454(15), 151.00406(100), 177.01935(3), 180.00637(14), 285.04063(57
Lignans						
Coniferin (+ HCOOH)	6.53	$C_{17}H_{23}O_{10}$-	387.12967	387.13046	-0.79	146.03719(73), 161.06031(31), 164.04799(58), 179.07155(100)
Syringaresinol-4-O-hexoside	8.36	$C_{28}H_{35}O_{13}$-	579.20832	579.21052	-2.20	166.02754(11), 181.05084(100), 387.11092(7), 402.13246(21), 417.15576(89)
Other detected compounds						
Quinic acid	1.75	$C_7H_{11}O_6$	191.05560	191.05659	-0.99	101.06130(100), 111.00925(16), 115.04062(30), 129.05670(4)
Abscisic acid	9.37	$C_{15}H_{19}O_4$	263.12830	263.13007	-1.77	122.03765(58), 136.05309(50), 138.06800(37), 153.09113(67), 203.10781(100), 219.13802(18)

Changes in surface chemistry

To elucidate the changes in surface chemistry of the treated fabric samples, FTIR spectral analysis was employed. FTIR spectra of the fabric samples, before and after treatment with BSC, are shown in Figures 2 and 3.

From Figure 2a, it can be seen that the FTIR spectra of V and T-V showed characteristic absorption bands of cellulose: at 892–896 cm^{-1} for C–O–C valence vibration of the β-glycosidic linkage or deformation at C(1) in cellulose II, at 996 cm^{-1} for C–O valence vibration at C(6)), at 1024 and 1067 cm^{-1} for skeletal vibrations involving C–O stretching, at 1158 cm^{-1} for C–O–C asymmetric from β-glycosidic link in cellulose II), at 1200 and 1336 cm^{-1} for OH in-plane bending vibrations, at 1225–1235 cm^{-1} for O–H in-plain deformation at C(6), at 1316 cm^{-1} for CH_2 wagging vibration, at 1372 cm^{-1} for C–H deformation in cellulose II, at 1418 cm^{-1} for CH_2 scissoring at C(6) in cellulose II, at 1636 cm^{-1} for adsorbed water, at 2893 cm^{-1} for C–H stretch in cellulose II and CH_2, CH_2OH in cellulose from C6 and at the range of 3000–3600 cm^{-1} for hydrogen-bonded OH groups.[13] It is known that the appearance of a new band at 1600 cm^{-1} for C=O stretching vibration in the FTIR spectra of viscose fabric indicates the introduction of sodium carboxylate groups at the C6 position of the cellulose molecules.[10] However, a new band at 1600 cm^{-1} did not appear in the T-V spectrum, probably due to the low content of carboxylate groups in this sample (Table 2), as well as the fact that this absorption band is located in the spectral region where the band of adsorbed water could mask its signal. A slight decrease in the intensity of the band at 996 cm^{-1} observed in the T-V spectra (Fig. 2b) might be explained by the introduction of aldehyde groups at C(6).[10]

It is known that the main bands of chitosan are at 1560 cm^{-1} (for stretching vibration of the amino group), at 1590 cm^{-1} (for N–H bending), and at 1651 cm^{-1} (for carbonyl stretching of acetyl groups).[47] However, these bands did not appear in the V and T-V spectra after functionalization with chitosan and chitosan nanoparticles (Fig. 2). The reason for this probably lies in the fact that these bands are located in the spectral region where the band of adsorbed water masks their signal, as well as the formation of hydrogen bonds between cellulose and chitosan. After functionalization of V and T-V with chitosan and chitosan nanoparticles, the band in the range of 3000–3600 cm^{-1} was slightly shifted to higher wavenumbers and showed lower intensity, which also indicates the formation of hydrogen bonds between cellulose and chitosan.[48,49]

In the FTIR spectra of the samples treated with BSC, no new bands can be seen (Fig. 3). However, some differences were also observed. Typically, band shifts in the FTIR spectra of BSC-treated samples occur due to interactions between cellulose/chitosan and BSC components. The shift of the bands in the range of 3000–3600 cm^{-1} and at 2893 cm^{-1} to a lower wavenumber suggests the possible binding of -O-H and -C-H groups of cellulose/chitosan with BSC components.[50] Moreover, in the case of BSC-treated samples, the band at 1024 cm^{-1} shifted to lower wavenumber values after the incorporation of BSC components, which also indicates the existence of hydrogen

interactions between cellulose/chitosan and the BSC components. Besides the obvious coloration of the BSC-functionalized viscose fabrics, this phenomenon also indicated that the BSC components were successfully incorporated into the samples. Intermolecular hydrogen bonds between cellulose/chitosan and components of some other plants extracts have also been previously reported by several authors.[50-53]

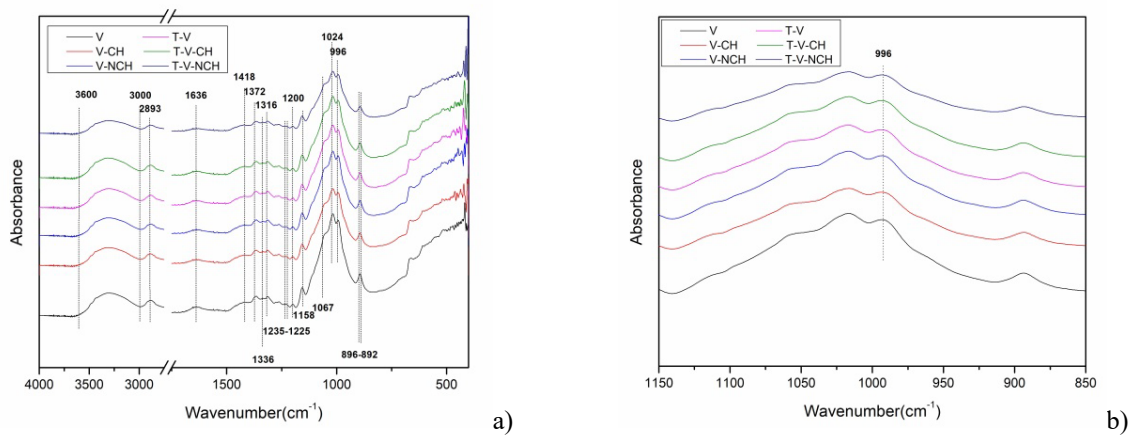


Figure 2: FTIR spectra of fabrics before functionalization with BSC, in the wavenumber range of a) 400–4000 cm^{-1} and b) 550-1150 cm^{-1}

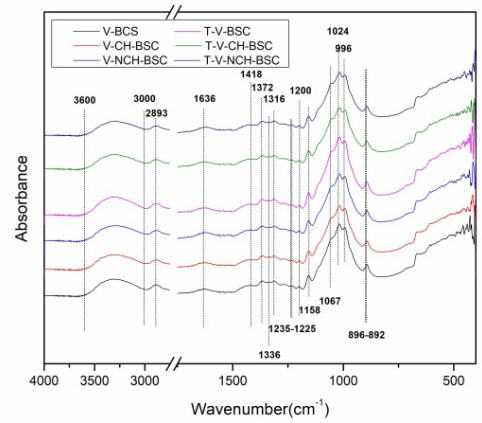


Figure 3: FTIR spectra of fabrics after functionalization with BSC, in the wavenumber range of 400–4000 cm^{-1}

Antibacterial activity of fabrics

The presence of pathogenic bacteria on textile materials leads to their degradation, the development of unpleasant odors, and a significant danger to human health because they can cause severe skin infections and, in certain cases, even heart problems and pneumonia. Therefore, there is increasing interest in textile materials that have antibacterial activity.[54]

The antibacterial activity of fabric samples was qualitatively tested using the diffusion method on an agar plate. The antibacterial activity of fabric samples, before and after functionalization with BSC, against Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) bacteria is shown in Figure 4 and Figure 5.

Given that viscose, as well as other cellulose-based textile materials, contains nutrients and energy sources, it represents a suitable substrate for the growth of bacteria.[55] In accordance with this fact, it can be seen from Figure 4 that V (1) and T–V (4) samples did not show antibacterial activity against *S. aureus* and *E. coli*. However, in the case of unmodified and TOCN-coated viscose fabric samples with immobilized chitosan (2, 5, respectively) and chitosan nanoparticles (3, 6, respectively), inhibition of both *S. aureus* and *E. coli* was observed in the contact zone with the sample (Fig. 4). Such results are expected considering that chitosan and chitosan nanoparticles are known for their antibacterial activity.[56,57] As previously explained, their antibacterial activity is based on several different mechanisms that occur simultaneously.[58] It is important to point out that inhibition in the contact zone, without a surrounding clear zone of

inhibition, is typical for large polymeric compounds such as chitosan. Large polymeric compounds have a high molecular weight that prevents their diffusing through agar. Because the agent cannot move through the agar, it can only inhibit those microorganisms that physically touch the application site or the exact contact zone where the sample and the inoculated agar meet.[59,60]

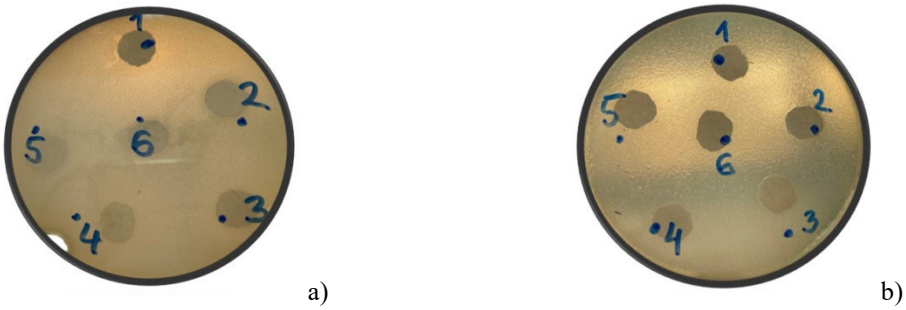


Figure 4: Antibacterial activity of fabrics before functionalization with BSC against a) *S. aureus* and b) *E. coli* (sample denotations V, V-CH, V-NCH, T-V, T-V-CH, T-V-NCH correspond to the numbers on Petri dishes 1, 2, 3, 4, 5, 6, respectively)

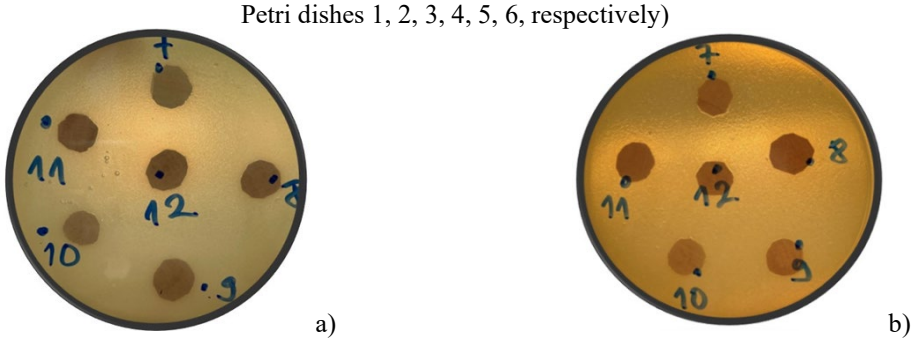


Figure 5: Antibacterial activity of fabrics functionalized with BSC against a) *S. aureus* and b) *E. coli* (sample denotations V-BSC, V-CH-BSC, V-NCH-BSC, T-V-BSC, T-V-CH-BSC, T-V-NCH-BSC correspond to the numbers on Petri dishes 7, 8, 9, 10, 11, 12, respectively)

Given that beech wood steaming condensate contains different bioactive compounds (Table 3), primarily phenols and flavonoids that have been found to have antibacterial activity,[61] it was expected that all tested fabrics would exhibit antibacterial activity. As in the case of chitosan, from Figure 5, it can be seen that after functionalization with BSC, all fabrics showed inhibition of *S. aureus* and *E. coli* in the contact zone with the sample. It is well known that plant extracts diffuse differently into the agar depending on their polarity. The plant extracts richer in compounds with better water solubility will diffuse more quickly and create larger inhibition zones, whereas the hydrophobic compounds would tend to remain on the agar surface and gradually evaporate during incubation. Thus, even if the zone of inhibition is not created, the plant extracts can actually be very effective if they show inhibition in the contact zone with the sample.[59,62] Inhibition of *S. aureus* and *E. coli* in the contact zone with samples of unmodified and TOCN-coated viscose fabric functionalized with BSC unequivocally (7, 10, respectively) indicates that BSC possesses antibacterial properties against *S. aureus* and *E. coli.* Therefore, its synergistic antibacterial action with chitosan (8, 11, respectively) and chitosan nanoparticles (9, 12, respectively) is expected. A more pronounced inhibition of *S. aureus* and *E. coli* in the contact zone with samples of unmodified and TOCN-coated viscose fabric with immobilized chitosan and chitosan nanoparticles functionalized with BSC compared to the inhibition of *S. aureus* and *E. coli* in the contact zone with the corresponding samples that were not functionalized with BSC was observed from Figure 4 and Figure 5. In other words, the inhibition of *S. aureus* and *E. coli* in the contact zone with samples V-CH-BSC (8), V-NCH-BSC (9), T-V-CH-BSC (11), T-V-NCH-BSC (12) seems to be more pronounced than with samples V-CH (2), V-NCH (3), T-V-CH (5), T-V-NCH (6), respectively.

Antioxidant activity of fabrics

Free radicals are molecules that have one or more unpaired electrons in their structure. The unpaired electrons of free radicals easily react with other molecules, creating new free radicals. In contrast, antioxidants are molecules that 'neutralize' free radicals, whereby the antioxidants themselves become oxidized. In this way, antioxidants reduce the harmful effects of free radicals, their reactions with other molecules, and the creation of new free radicals.[15]

Excessive production of free radicals is a fundamental feature of inflammatory skin conditions such as atopic dermatitis, psoriasis, and vitiligo. For this reason, patients with inflammatory skin conditions need to use textile materials that possess antioxidant activity.[63]

Figures 6 and 7 show the results of the antioxidant activity of viscose fabric samples before and after functionalization with BSC. Due to the presence of hydroxy, carboxy, and aldehyde groups, V and T–V samples have the potential to donate hydrogen to reduce free radicals. It can be seen from Figure 6 that V and T–V samples possess low antioxidant activity: 1.05% in the case of V and 1.81% in the case of T-V sample. The antioxidant activity of V and T-V samples is correlated with the content of their carboxy groups: 0.063 mmol/g cellulose in the case of V, and 0.087 mmol/g cellulose in the case of T-V (Table 2). The statistically significant higher antioxidant activity of T-V, compared to V (Table 4), in addition to the generally higher content of carboxy groups, can be further explained by the fact that a significant portion of its carboxy groups are in the form of sodium carboxylate. Namely, there are assumptions that the presence of sodium ions changes the distribution of charges in the molecule, leading to better antioxidant properties.[64]

Chitosan and chitosan nanoparticles are known to reduce free radicals by donating hydrogen from their hydroxy and amino groups.[65,66] After the immobilization of chitosan and chitosan nanoparticles, the unmodified viscose fabric achieved a statistically significant change in antioxidant activity (by 0.19% in the case of V-CH and by 0.47% in the case of V-NCH) (Fig. 6, Table 4). However, after the immobilization of chitosan and chitosan nanoparticles, the TOCN-coated viscose fabric achieved a statistically significant lower antioxidant activity (by 0.38% in the case of T-V-CH and by 0.19% in the case of T-V-NCH) (Fig. 6, Table 4). The lower antioxidant activity can be explained by the established interactions between hydrogen/sodium donating groups of TOCN-coated viscose fabric and chitosan, as well as chitosan nanoparticles, during their immobilization on TOCN-coated viscose fabric.

If we compare the antioxidant activity of the samples with immobilized chitosan and chitosan nanoparticles, the samples with immobilized chitosan nanoparticles achieved a slightly higher antioxidant activity compared to their counterparts with immobilized chitosan (Fig. 6). In other words, the antioxidant activity of V-NCH and T-V-NCH samples is a significantly higher than the antioxidant activity of V-CH and T-V-CH samples, respectively (Table 4). This trend is in line with earlier research in which chitosan nanoparticles were shown to exhibit improved antioxidant properties compared to bulk chitosan.[15]

TOCN-coated viscose fabric showed a statistically significant higher antioxidant activity compared to unmodified viscose fabric, whether chitosan or chitosan nanoparticles were immobilized on it (T-V-CH > V-CH, T-V-NCH > V-NCH) (Fig. 6, Table 4). As already mentioned, the T-V sample has a higher content of carboxy groups, with a significant portion in the form of sodium carboxylate, compared to the V sample (Table 2), and consequently a significantly higher antioxidant activity. On the other hand, immobilization of chitosan and chitosan nanoparticles reduced their antioxidant activity. However, this reduction in antioxidant activity was carried out in such a way that the TOCN-coated viscose fabric with immobilized chitosan and chitosan nanoparticles still achieved higher antioxidant activity than the unmodified viscose fabric with immobilized chitosan and chitosan nanoparticles.

It was established that phenols and flavonoids, which are found in the wood steaming condensates, reduce free radicals by donating hydrogen from their hydroxy groups and thus achieve exceptional antioxidant activity.[58,67] After functionalization with BSC, all samples of viscose fabrics achieved a drastically higher antioxidant activity, reaching 67.74-92.58% (Figs. 6 and 7).

It is important to point out that the general trend of the antioxidant activity of fabric samples remained the same as before their functionalization with BSC (Figs. 6 and 7), except for the V sample, which, after functionalization with BSC, has a higher antioxidant activity than the V-CH-BSC and V-NCH-BSC samples. Namely, in contrast to the assumed more intense binding of components of BSC, which contribute to the coloring of

cellulose/chitosan composite textile materials, as can be clearly seen in Figure 1, the lower antioxidant activity of these samples (Fig. 7) shows that these components are not the most favorable for antioxidant activity.

The results of statistical analysis of the antioxidant activity of viscose fabrics before and after functionalization with BSC using the *t*-test are shown in Table 4. Statistical analysis (Table 4) revealed a statistically significant difference in antioxidant activity between the viscose fabrics before functionalization with BSC, although the absolute magnitude of the change in antioxidant activity was relatively small.

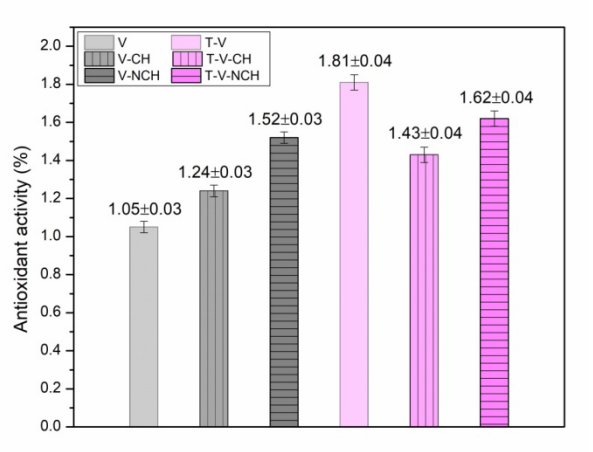


Figure 6: Antioxidant activity of fabrics before functionalization with BSC

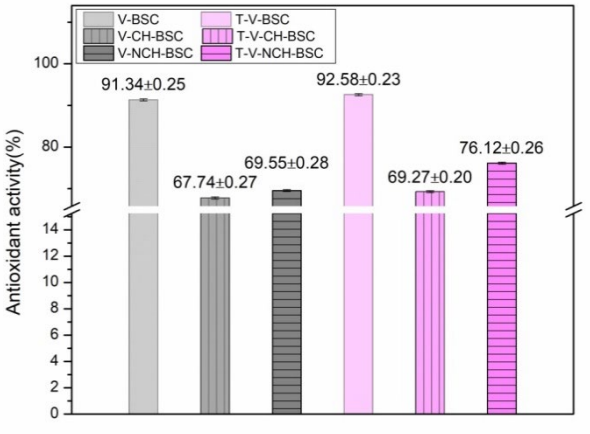


Figure 7: Antioxidant activity of fabrics functionalized with BSC

Table 4
Statistical results of the antioxidant activity of viscose fabrics before and after functionalization with BSC using the *t*-test

Values of parameter *t* for antioxidant activity of fabrics before functionalization with BSC			Values of parameter *t* for antioxidant activity of fabrics functionalized with BSC		
Dependent fabrics ($df=n_1$-1=2)					
Determined parameter *t*	Values of parameter *t*	Level of significance	Determined parameter *t*	Values of parameter *t*	Level of significance
$t_{V/V\text{-}CH}$	-7.18	0.05	$t_{V\text{-}BSC/V\text{-}CH\text{-}BSC}$	628.43	0.001
$t_{V/V\text{-}NCH}$	-140.00	0.001	$t_{V\text{-}BSC/V\text{-}NCH\text{-}BSC}$	97.91	0.001
$t_{V/T\text{-}V}$	-56.75	0.001	$t_{V\text{-}BSC/T\text{-}V\text{-}BSC}$	-10.56	0.01
$t_{V/T\text{-}V\text{-}CH}$	-18.91	0.01	$t_{V\text{-}BSC/T\text{-}V\text{-}CH\text{-}BSC}$	94.77	0.001
$t_{V/T\text{-}V\text{-}NCH}$	-28.28	0.01	$t_{V\text{-}BSC/T\text{-}V\text{-}NCH\text{-}BSC}$	89.07	0.001

$t_{T\text{-}V/T\text{-}V\text{-}CH}$	31.06	0.01	$t_{T\text{-}V\text{-}BSC/T\text{-}V\text{-}CH\text{-}BSC}$	153.40	0.001
$t_{T\text{-}V/T\text{-}V\text{-}NCH}$	15.25	0.01	$t_{T\text{-}V\text{-}BSC/T\text{-}V\text{-}NCH\text{-}BSC}$	301.51	0.001
Independent fabrics ($df=n_1+n_2-2=4$)					
$t_{V\text{-}CH/V\text{-}NCH}$	-11.857	0.001	$t_{V\text{-}CH\text{-}BSC/V\text{-}NCH\text{-}BSC}$	-8.17	0.01
$t_{V\text{-}CH/T\text{-}V}$	-20.31	0.001	$t_{V\text{-}CH\text{-}BSC/T\text{-}V\text{-}BSC}$	-121.76	0.001
$t_{V\text{-}CH/T\text{-}V\text{-}CH}$	-7.25	0.01	$t_{V\text{-}CH\text{-}BSC/T\text{-}V\text{-}CH\text{-}BSC}$	-7.92	0.01
$t_{V\text{-}CH/T\text{-}V\text{-}NCH}$	-14.37	0.001	$t_{V\text{-}CH\text{-}BSC/T\text{-}V\text{-}NCH\text{-}BSC}$	-38.73	0.001
$t_{V\text{-}NCH/T\text{-}V}$	-9.91	0.001	$t_{V\text{-}NCH\text{-}BSC/T\text{-}V\text{-}BSC}$	-110.82	0.001
$t_{V\text{-}NCH/T\text{-}V\text{-}CH}$	2.97	0.05	$t_{V\text{-}NCH\text{-}BSC/T\text{-}V\text{-}CH\text{-}BSC}$	1.40	/
$t_{V\text{-}NCH/T\text{-}V\text{-}NCH}$	-3.80	0.05	$t_{V\text{-}NCH\text{-}BSC/T\text{-}V\text{-}NCH\text{-}BSC}$	-29.87	0.001
$t_{T\text{-}V\text{-}CH/T\text{-}V\text{-}NCH}$	-6.15	0.01	$t_{T\text{-}V\text{-}CH\text{-}BSC/T\text{-}V\text{-}NCH\text{-}BSC}$	-35.73	0.001

df – degrees of freedom, *n* – sample size, / - no statistically significant difference

As observed before functionalization with BSC, a statistically significant difference in antioxidant activity was also found among the viscose fabrics after functionalization with BSC, except between V-NCH-BSC and T-V-CH-BSC samples, for which no statistically significant difference was observed (*t*V-NCH-BSC/T-V-CH-BSC=1.41, Table 4).

CONCLUSION

Cellulose/chitosan composite textile materials, with improved antibacterial and antioxidant properties, were successfully obtained by functionalization with BSC. Coating the unmodified viscose fabric with TOCN increased the carboxy group content by 0.024 mmol/g cellulose and aldehyde group content by 0.007 mmol/g cellulose, which contributed to improved immobilization of chitosan and chitosan nanoparticles on its surface.

Functionalization with BSC of unmodified and TOCN-coated viscose fabrics, with and without immobilized chitosan and chitosan nanoparticles, resulted in their brown coloration. A visually observed more intense coloration of viscose fabric samples with immobilized chitosan and chitosan nanoparticles indicates that the BSC components that contribute to the coloring show a greater affinity to positively charged groups. The more intense coloration of the samples with immobilized chitosan compared to that of the samples with immobilized chitosan nanoparticles can be explained by the greater amount of immobilized chitosan and more accessible positively charged groups.

Antibacterial testing demonstrated that functionalization with BSC contributed to the inhibition of both *S. aureus* and *E. coli*, and indicated that this effect was more pronounced when combined with chitosan or chitosan nanoparticles, probably due to the enhanced antibacterial response compared with the corresponding samples not functionalized with BSC.

Unmodified and TOCN-coated viscose fabrics, with and without immobilized chitosan and chitosan nanoparticles, exhibited very low antioxidant activity, ranging from 1.05% to 1.81%. Functionalization with BSC of unmodified and TOCN-coated viscose fabrics with and without immobilized chitosan and chitosan nanoparticles led to a drastic increase in their antioxidant activity, ranging from 67.74% to 92.58%.

Finally, it is important to point out that this study presents for the first time the concept of functionalizing textile materials with beech wood steaming condensate and encourages the tendency to improve the properties of textile materials while respecting the sustainability principles.

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