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Research Article

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Chitosan Films Prepared With Low Nanometal Content for Developing Protective Denim Fabrics

Koruyucu Denim Kumaşlar Geliştirmek için Düşük Nanometal İçerikle Hazırlanan Kitosan Filmler

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ABSTRACT

At this study; Chitosan was obtained from crayfish and identified by X-ray diffraction (XRD), molecular weight and elemental analyses. Later, it was coated on fabrics alone and in combination with nano-metal. The protective properties of the coated fabrics was investigated by UV protection and antibacterial analyses. Crayfish chitosan had low crystallinity (72%), low molecular weight (Mw) (11.2 kDa) and low degree of deacetylation (DD) (16%). When used together, nano TiO_2 reduced the UV protection of the crayfish chitosan in both dyed and undyed denim fabrics. Chitosan+nano Ag coated fabric had the highest antibacterial activity (Antibacterial activity value (A): 4.27) against Staphylococcus aureus while chitosan+nano TiO2 coated fabric did not show any antibacterial efficiency (A: 1.89). After washed, the chitosan coated and the chitosan+nano Ag coated fabrics retained their antibacterial efficiency.

Key Words

Crayfish chitosan, denim, color, TiO_2, ZnO, Ag.

ÖZ

Bu çalışmada; Kitosan, kerevitten elde edildi ve X-ışını kırınımı (XRD), moleküler ağırlık ve elementel analizlerle tanımlandı. Daha sonra kumaşlara tek başına ve nano-metal ile kombinasyon halinde kaplandı. Kaplanan kumaşların koruyucu özellikleri UV koruma ve antibakteriyel analizlerle araştırıldı. Kerevit kitosan düşük kristalliğe (%72), düşük moleküler ağırlığa (Mw) (11.2 kDa) ve düşük deasetilasyon derecesine (DD) (%16) sahipti. Nano-TiO2 birlikte kullanıldığında, hem boyalı hem de boyasız denim kumaşlarda kerevit kitosanının UV korumasını azalttı. Chitosan+nano Ag kaplı kumaş, Staphylococcus aureus'a karşı en yüksek antibakteriyel aktiviteye (Antibakteriyel aktivite değeri (A): 4.27) sahipken, kitosan+nano TiO_2 kaplı kumaş herhangi bir antibakteriyel etkinlik göstermedi (A: 1.89). Yıkanan kitosan kaplı ve kitosan+nano Ag kaplı kumaşlar antibakteriyel etkinliklerini korudu.

Anahtar Kelimeler

Kerevit kitosan, denim, renk, TiO_2, ZnO, Ag.

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INTRODUCTION

Due to clothing comfort, functionality and long lifetimes, demand for denim fabrics in daily and workwear has increased [1]. Denim fabrics produced from 100% cotton are moisture-absorbent, soft, organic, breathable and environmentally friendly and it provides comfort for the user with their structural features. In addition to these advantages, the demand for denim fabric with functional and innovative features has increased in recent years. In this study, surface modification was made with crayfish chitosan and nano metals to increase the functional properties of denim fabric, without changing fabric weight and color. In addition, this study aimed to reveal whether there is a difference in the UV protection effect of chitosan on dyed and undyed denim fabrics.

It is among the expectations of users that denim fabric has functional features such as water, weather and UV protection against sudden changes in weather conditions, as well as windy, sunny and rainy atmospheric conditions. In order to prevent the harmful effects of sun rays, the UV protection feature is an expected feature of protective clothing. UV-A (in therange of 320 and 400 nm) and UV-B (in the range of 290 and 320 nm) radiation from sun rays cause various negative effects including sun burn on the skin, skin degeneration, immuno suppression, photo toxicity, photosensitivity, photoaging and skin cancer [2]. In order to reduce these harmful effects of the sun, clothes with high UV-protective properties and wearing comfort have become a necessity in recent years. Covering fabrics with organic and inorganic coating materials is one of the ways to change textile products into protective materials. Chitosan, which is one of the green biomass materials, has been getting more and more attention as protective coating material in recent years [3].

One of the features expected from protective clothing is antibacterial protection. However, cotton clothes, one of the most preferred clothing types by people, offer suitable living environments for bacteria with their porous and hydrophilic structures [4]. On one hand, this situation causes deterioration of the fabric structure and causes odor, on the other hand it causes to skin diseases and allergic effects in humans [5]. *Staphylococcus aureus* is a bacterium that is commonly found on the skin and mucous membranes of humans. *S. aureus,* which is an important pathogenic bacterium, causes a wide variety of community-acquired infections and hospital-acquired infections globally and exhibits multidrug resistance [6]. *S. aureus* is transferred from human skin to clothing or other shared textile products, including sheets, blankets, towels, and bedspreads, through air and direct contact [7]. It has also the potential to transmit and spread infection from contaminated clothing to other textile products or humans. Therefore, it is necessary to develop new antibacterial agents and protective textile coating that can be effective against this pathogenic bacterium.

Chitosan is the N-deacetylated glucosamine obtained from chitin, which is stored in the cell wall of fungi, or in the exoskeleton of arthropods such as crayfish, crab and insects. It consists of units linearly bounded by the β-1,4-glycosidic bond. Chitosan has superior chemical and biological properties including biocompatibility, nontoxicity, antimicrobial and anticancer activity [8]. According to Cervellon et al. [9], if a product has some basic properties such as natural, organic, biodegradable, biodynamic and ecological, the product can be classified as "green." Green polymers stand out as organic compounds that are nonallergic and nontoxic in terms of environment and human health and that can be decomposed by micro organisms [10]. The chitosan used in this research was produced from the waste shells of crayfish *Astacus leptodactylus*, and the waste shells of this species were transformed into a biotechnological product.

Recently, various nanoparticles with antimicrobial properties have been combined with chitosan to improve the antibacterial feature of chitosan coatings or nanocomposite films. Among these, Ag, TiO_2 and ZnO nanoparticles are of great interest. Because of their wide range antimicrobial effect, silver nanoparticles and silver colloids have attracted great interest as antibacterial agents to prevent wound infection or to product antibacterial textiles [11]. Ag nanoparticles have great antibacterial efficiency as they have an exceedingly large surface area that provides good interaction with bacteria. ZnO nanoparticles are widely used as antibacterial agents in fabric finishing due to their non-toxic, low cost and good biocompatible characters [12,13]. TiO_2 nanoparticles are used in textile applications to provide fabrics with UV protection, antibacteriality and self-cleaning [14]. In this study, unlike other studies nanoparticle concentrations were kept low by considering human health. Nanoparticles were coated on the denim fabric by means of crayfish chitosan and the coated denim fabrics tested for their bacterial and UV preservation.

This research aimed to develop bio-based, environmentally friendly, sustainable, ecological and functional textile coatings by evaluating crayfish waste shells as biomaterials and to determine the functional potential of the crayfish chitosan in terms of textile coatings.

MATERIALS and METHODS

Materials

Dyed and undyed denim weaving produced from 100% cotton and having weaving type with 3/1 twill were used in this study. For better interpretation of the results, fabrics with different frequencies and grams were also used in some analyses. In addition; undyed calico fabric produced from 100% cotton yarn was used for antibacterial activity analyses.

Chitosan covered on the fabric surfaces was produced from waste shells of the crayfish *A. leptodactylus* caught from the Altınyazı Dam lake (N 41° 4´ 45.4800” and E 26° 35´ 16.0764”) (Edirne, Turkey) on 10 July 2019.

Nano-TiO_2 particles (> 21 nm, white powder) were bought from Merc Company, while nano Ag particles (≤ 100 nm, purity 99.5%) and nano ZnO particles (particle size: <100 nm, nanopowder) were bought from Sigma Aldrich company. Before use, nanoparticles were homogenized by vortexing for 10 seconds.

Chitin and Chitosan Production from Crayfish

Twenty grams of the powdered crayfish shells sample were put into a baloon containing 2M, 250 mL of HCl solution. Then it was refluxed on the magnetic stirrer for 5 hours at 65–70 °C. After this, it was filtered by adding distilled water. The filtration was continued until the pH 7. With this process, the minerals in the shells of the crayfish were removed. In the next step, the samples were refluxed in 2M 250 mL of NaOH solution by stirring at 150 °C for 24 hours and rinsed with deionized water until pH 7. Thus, proteins in the structure were removed. Next, the precipitate was decolorated in a mixture containing pure water, ethyl alcohol and chloroform in a 4:2:1 ratio for 2 hours at 25 °C. Thus, the lipids and pigments remaining in the structure were removed. The chitin, which was isolated at the end of these processes, was dehydrated in the oven for 48 hours at 50 °C. Dry chitin samples were treated with a 70% NaOH at 140 °C, 800 rpm for 4 hours and then filtered by washing with deionized water until reaching pH 7. After this process, the derived chitosan was dehydrated in the oven at 50 °C for 48 hours.

Preparation and Application of Chitosan Gel

Ten grams of the chitosan in powder form, obtained from shells of crayfish *A. leptodactylus* were dissolved in a 1L of solution containing distilled water and 1% acetic acid by stirring in a magnetic stirrer at 40 °C for 15 minutes. The chitosan solution containing nanoparticles was prepared by adding 0.05% nano TiO_2, 0.05% nano ZnO or 0.05% nano Ag into the prepared 1% chitosan solution. In denim fabrics, 0.5% by weight nano TiO_2 particles were used. The fabric samples were impregnated with the chitosan solution with a 60% compression pressure in a laboratory-type padding machine (ATAÇ F 350). Then, it was dehyrated (at 100°C) in a dryer (LABORTEKS 40 L216) and fixed at 110°C for 1 minute in the same machine. The amount of nanometals used was kept low considering the environmental and human health.

Identification of Chitosan

X-ray diffraction (XRD)

The crystal structure of the chitosan from crayfish *A. leptodactylus* was determined by examining the X-ray diffraction patterns. The X-ray diffraction graph of the crayfish chitosan was recorded with the Rigaku Miniflex 600 Cu X-Ray tube device at a scanning speed of 40 kV, 15 mA, 1.54 Angstroms wavelength and a scanning range of 2θ, 5°–45°. The % crystallinity of the crayfish chitosan was determined by the equation given below [15].

$$CrI_{110} = \left[\frac{(I_{110} - I_{am})}{I_{110}} \right] x100 \qquad (1)$$

$I_{110,}$ corresponds to the maximum intensity at 2θ≅20°; and I_{am}, corresponds to the intensity of amorphous diffraction at 2θ≅16°.

Elemental analysis

Elementel analysis of crayfish chitosan was performed by ThermoFinnigan elemental analyzer (Flash EA 1112 Series). Degree of deacetylation (DD) of chitosan was calculated according to the equation given below [15].

$$(DD = \left[\frac{\frac{C}{N} - 5.14}{1.72} \right] x100 \qquad (2)$$

In the formula, DD is the deacetylation degree of chitosan.

Molecular weight (Mw)

A dilution viscometer (Ubbelohde type) was used to measure viscosity-average molecular weights of crayfish chitosan. A solvent system consisting of 0.1 M of acetic acid + 0.2 M NaCl (1:1, v/v) was used to prepare chitosan solutions at different concentrations. Measurements were carried out at room temperature. The results were replaced by the Mark – Houwink equation and the molecular weight of chitosan obtained from crayfish was calculated [16].

$$\left[\eta\right] = kMv^{\alpha} \qquad (3)$$

[η]: intrinsic viscosity
Mv: viscosity-average molecular weight of the polymer
k and α: Mark – Houwink – Sakurada constants. k= 1.81x 10^{-3} and α = 0.93.

Analysis of Fabrics Coated with Crayfish Chitosan

Fourier transform infrared spectroscopy (FTIR) of coated fabrics

FTIR analysis was used to reveal the availability of chitosan and nanoparticles on the fabrics. The analysis was performed with the Perkin-Elmer FT-IR device in 32 scans with 4 cm^{-1} spectral resolution in the range of 4000–625 cm^{-1} wavelength.

Scanning electron microscopy (SEM) and Energy dispersive X-ray (EDX) analysis of the fabrics coated with crayfish chitosan

A scanning electron microscope (FEI QUANTA FEG 250) was used to observe the surface morphologies of the coatings made with crayfish chitosan and nanoparticles. The distribution of nano Ag, nano ZnO and nano TiO_2 on the calico fabric surface were also revealed by EDX analysis.

Color measurement of the crayfish chitosan-coated fabrics

Color measurement was performed with the X-RITE color spectrophotometer in accordance with the standard of TS EN ISO 105-J03, 2010 [17] and color measurement values were determined objectively according to the CIELAB system under D65 daylight [18]. Color measurements of the samples were made before and after coating, and then total color difference (ΔE) values were calculated by Real Color 1.3® software. In addition, the K/S color values of the chitosan coated and uncoated fabrics were measured using the same spectrophotometer according to the CIELAB D65-10 procedure.

UV permeability measurements of the crayfish chitosan-coated fabrics

UV measurement was performed by the Shimadzu 3600 Plus Spectrophotometer, with the Dry Assessment method according to the AATCC 183: 2014 standard [19], using Shimadzu UPF Calculation Software. For reliability, measurements were repeated 6 times in the spectral range of 280–400 nm (UPF), 315–400 nm (UVA) and 280–315 nm (UVB) in conditioned fabric samples, and average values were used.

Antibacterial efficiency assay of the crayfish chitosan and nanoparticle-coated fabrics

Antibacterial efficiency of fabrics covered with chitosan and Silver (Ag), Zincoxide (ZnO) and Titanium dioxide (TiO_2) nanoparticles has been tested against pathogenic bacteria *Staphylococcus aureus* (ATCC 6538 Gram (+)) by the method of JIS L 1902:2015 (Measurement of antibacterial effect and efficacy of textiles, Japanese Industry Standard) [20]. Antimicrobial assay was performed according to Erdogan [11].

Briefly, fabrics weigthing 0.4 g coated with chitosan and nanoparticles were sterilized at 121 °C for 15 min before the experiment. Then, the fabrics were inoculated with test microorganism in a volume of 0.2 mL and a concentration of $3x10^5$cfu/mL. Initial bacteria concentrations were measured at "time zero" immediately after elution, dilution and inoculation. Bacteria counting was done using the Plate Count method. Then, control and test specimens were inoculated with *S. aureus* and incubated in closed vials at 37±2 °C for approximately 18-24 h. After incubation, ultimate microbial concentrations were measured. The reduction in the number of micro organisms was calculated based on the primer concentrations and the control specimen.

After the coated fabric samples were washed once and subjected to tumble dry treatment at 40 °C according to ISO 6330:2012 method [21], their antibacterial activity was tested once again under the same conditions.

The bacteria growth rate was calculated for the control sample using this formula

$$F = C_t - C_0 \qquad (4)$$

The bacteria growth rate was calculated for the test sample using this formula

$$G = T_t - T_0 \qquad (5)$$

Antibacterial Efficiency Value was calculated using this formula

$$A = F - G \qquad (6)$$

Efficacy of antibacterial property 2≤A <3 (Efficacy), A≥3 (full efficacy).
A: Antibacterial activity
C_0: logarithm of the numbers of bacteria of control specimens right after inoculation at 0 h contact time
C_t: logarithm of the numbers of bacteria of control specimens after an 18 h to 24 h incubation
T_t: logarithm of the numbers of bacteria of antibacterial testing samples after an 18 h to 24 h incubation
T_0: logarithm of the numbers of bacteria of antibacterial testing specimen's right after inoculation (0 hours)

RESULTS and DISCUSSION

Characterization of the Crayfish Chitosan

XRD

In the XRD spectrum of the crayfish chitosan, two sharp crystalline peaks were seen at 10° and 20°, while three weak peaks at 22°, 29° and 35° were observed (Figure 1). A previous study showed that the X-ray diffractogram of the chitosan shows two diffraction peaks between about 10 and 10.5° (110) and between 20 and 20.5° [22]. Observed two crystalline peaks at 10° and 20° on the Chitosan-II diffractogram due to strong crystallizations. This has been reported as the result of moving the crystalline peaks of chitosan to a higher 2θ angle due to the deacetylation of the chitin [23]. These results are consistent with the findings in that study.

Crystallinity, which is an important structural feature affecting various properties of polymers was calculated as 72% for the crayfish chitosan. This value is quite high compared to those (40–46%) of chitosan from shrimp *Litopenaeus vannamei* [24]. Aranaz et al. [25]' emphasizes that the purity and crystallinity of the chitin differs according to the source of the chitin and that the crystallinity affects the catalytic properties of the chitin. The authors also reported that low crystalline chitin and chitosan are used very effectively in water treatment.

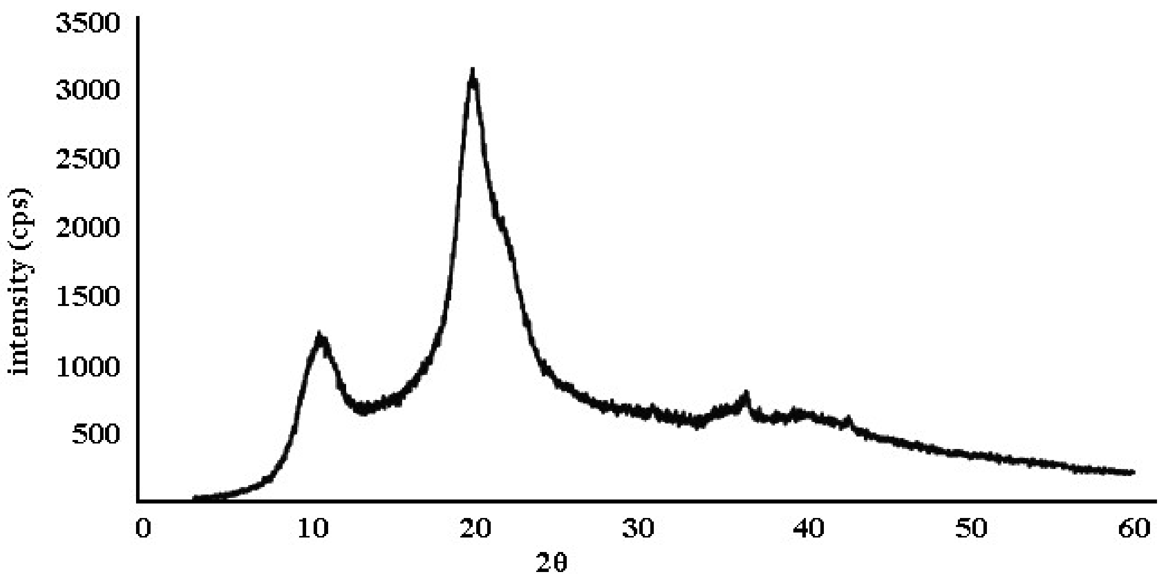

Figure 1. X-ray diffraction graph of the chitosan from A. *leptodactylus*..

Elemental analysis

Nitrogen (N), Carbon (C), and Hydrogen (H) contents of the crayfish chitosan were measured as 7.24%, 39.32% and 6.61%, respectively. The degree of deacetylation (DD) of the crayfish chitosan was calculated as 16.9%. Deacetylation degrees of chitosan obtained from various organisms were reported as 92.19% for shrimp *Metapenaeus stebbingi* [26],<20% for squid *Loligo chenisis* [27], and 22% for adults and nymphs of grasshopper *Dociostaurus maroccanus* [15], respectively. Hajji et al. [28] stated that the degree of acetylation affects the solubility, chemical reactivity and biodegradability of chitosan and therefore it affects the industrial applications of these polymers. It has been reported that a high degree of deacetylation gives chitosan a stronger antimicrobial efficiency [29].

Molecular weight

The molecular weight of the crayfish chitosan was calculated as 11.2 kDa. Molecular weights of chitosan obtained from different species were reported as 2.20 kDa for shrimp *M. stebbingi* [26], as 7.2 and 5.6 kDa for the adults and the nymphs grasshoppers, respectively [16] and as 8454 kDa for squid *L. chenisis* [27]. Considering the molecular weights of these chitosan samples, the crayfish chitosan can be defined as low molecular weight chitosan. Molecular weight affects the chemical and biological activities of chitosan such as crystallinity, solubility, viscosity and antioxidant, antibacterial, and anticancer properties [30].

Analysis Results of the Fabric Covered with the Crayfish Chitosan and TiO_2

Color properties of the crayfish chitosan-coated fabrics

In order to determine the effect of the chitosan coating on the fabric color, the color measurement results of dyed and chitosan-coated denim fabrics were examined, and it was evident that the total color difference values (ΔE) were quite close to each other (Table 1). Acceptable ΔE values for the textile industry are in the range of 0–1 [31]. The ΔE values determined were among the acceptable values. In addition, the absence of significant changes in the a* (red-green axis) and b* (yellow-blue axis) values indicate that the color coordinates did not change. In addition, K/S color values of chitosan-coated and uncoated denim fabrics were measured between 370 and 750 nm wavelength (Figure 2). The K/S values of the chitosan-coated and uncoated denim fabrics were almost the same. Only between 490-730 nm wavelengths, there is an acceptable, very slight color difference. A previous study reported that chitosan application did not have a negative effect on color fastness due to washing [32]. Suitcharit et al. [33] stated that in fabrics treated with chitosan, chitosan increased the color fastness of the fabric. The results indicate that chitosan coatings did not change the color characteristics of the denim fabrics. For this reason, the coating process can be applied with crayfish chitosan to cotton fabrics such as denim after dyeing or as a finishing process.

Table 1. Color Measurement Values of the Denim Fabrics Coated with the Crayfish Chitosan

	Uncoated blue denim fabric			Coated blue denim fabric			Color difference
	L*	a*	b*	L*	a*	b*	ΔE
1. measurement	31.49	-1.94	-19.32	30.15	-1.52	-18.78	1.14
2. measurement	30.87	-1.95	-18.99	29.19	-1.41	-18.45	0.75
3. measurement	31.30	-1.98	-19.33	29.15	-1.23	-18.25	0.89

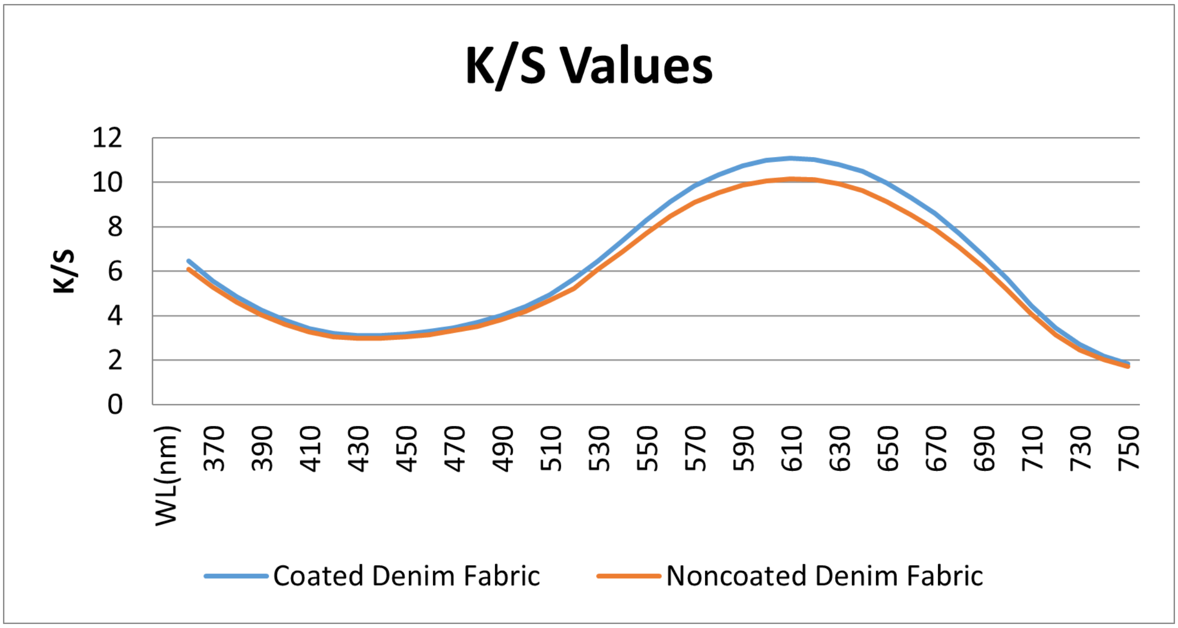

Figure 2. The graph showing the K/S values between 370-750 nm wavelength of chitosan-coated and uncoated denim fabrics.

FTIR analysis results of the coated fabrics

FTIR analysis results of the undyed calico fabrics were presented in Figure 3. The FTIR peaks of uncoated cotton fabric were recorded by Uğur et al. [14] at 3300 cm^{-1} (OH functional groups in cellulose), 2800–3000 cm^{-1} (C-H stretching), 1640 cm^{-1} (water adsorbed in the fabric structure), and at 1430 and 1368 cm^{-1} (C-H bending). The FTIR peaks of the uncoated calico fabric (Figure 3a) were almost identical to the ones announced by Uğur et al. [14]. Chen et al. [34] stated that the characteristic peaks for chitosan are I. carbonyl (C = O) band at around 1650 cm^{-1} and II. amide (NH_2) band at 1590 cm-1. In this study, these peaks were observed at around 1641 cm^{-1} and at 1560 cm^{-1} in the FTIR spectra of undyed calico fabric coated with crayfish chitosan (Figure 3b).The observation of chitosan-specific peaks in the FTIR spectrum of the crayfish chitosan-coated fabric and the peak shifts compared to the uncoated fabric indicated that chitosan was coated on the undyed calico fabric.

In the FTIR spectrum of the chitosan and nano ZnO coated calico fabric, peak shifts of 1 or 2 cm^{-1} were observed unlike the chitosan coated fabric (Figure 3c). In a previous study, the shift of the intensity of the peak in the FTIR spectra of ZnO nanoparticles reinforced bioplastic composite from 1045 cm^{-1} to 1025 cm^{-1} was attributed to the formation of the O-Zn coordination bond [35]. Zhong et al. [36] suggested that the peak observed at 1539 cm^{-1} ν(N\\H) in the FTIR spectra of nano ZnO/chitosan micro spheres shifted to 1560 cm^{-1} due to the formation of N-Zn bonding. However, Zhang et al. [13] reported that the addition of nano ZnO did not change the infrared spectrum of the chitosan film much and the composite film did not produce an obvious new peak when compared with the chitosan film. Similarly, the peak shifts observed in this study were not much due to the small amount of nano ZnO particles used.

The absorption band observed at 2893 cm^{-1} in the FTIR spectra of chitosan-coated fabric changed to give two peaks at 2896 cm^{-1} and 2864 cm^{-1} in the chitosan and nano Ag coated fabric (Figure 3d). Similarly, the intensity of the peaks observed at 1313 cm^{-1} and 995 cm^{-1} in the FTIR spectra of chitosan-coated fabric increased to 1314 cm^{-1} and 1002 cm^{-1}, respectively in the chitosan and nano Ag coated fabrics. The peak at 894 cm^{-1} decreased and disappeared. In addition, the peak of chitosan assigned to the tensile vibrations of the Amit C–O bonds, which was observed around 1641 cm^{-1} in the chitosan-coated fabric, shifted to 1639 cm^{-1} in the chitosan+nano Ag coated fabric. This result is also supported by Erdogan [11]. These peak shifts may be indicative of bond formation between the chitosan and nano Ag particles [37] or the chelation of Ag by chitosan [38,39].

The intensity of the peaks observed at 1641 cm^{-1} and 1560 cm^{-1} in FTIR spectra of the chitosan-coated fabric decreased to 1639 cm^{-1} and 1552 cm^{-1} in chitosan+nano TiO_2-coated fabric (Figure 3e). In other absorption bands, shifts of 1 cm^{-1} were observed. Zhang et al. [40] reported that in the FTIR spectrum of Ti–Chitosan, the band at 1636 cm^{-1} attributed to the $–NH_2$ bending underwent a shift of 21 cm^{-1} compared to chitosan, and this confirms that Ti^{4+} ions interacted with the $–NH_2$ group present in chitosan. Xing et al. [41] observed absorption peaks at 1639 cm^{-1} and 1563 cm^{-1}, 1412 cm^{-1}, 1035 cm^{-1} and 1152 cm^{-1} in the FTIR spectrum of chitosan films containing TiO_2 nanoparticles. The results of this research was consistent with the literature data [11,13,34,37,38,41].

In many studies, peak shifts at various rates have been reported as a result of the interaction of chitosan with nanometals [13,36,39,41]. Peak shift in the chitosan and nanometal-coated calico fabrics did not cause any notable changes in the FTIR spectra, but only minor changes were observed in the peak densities similar to the reported by [14,41,42]. Factors such as the presence of free amino groups and the size and concentration of TiO_2 nanoparticles affect the interaction between TiO_2 and chitosan [43,44]. This may be due to the very small amount of nanometals used in the coatings in this study.

The crayfish chitosan-coated dyed denim fabric exhibited some different absorption peaks at 3329 cm^{-1} and 1636 cm^{-1} in comparison to the crayfish chitosan-coated undyed calico fabric (Figure 4a). Similarly, shifts of several degrees were observed in other peaks. The peak at 1560 cm^{-1} observed in the undyed calico fabric disappeared in the dyed denim fabric. Two new peaks at 2289 cm^{-1} and 1734 cm^{-1} were observed in the FTIR spectra of crayfish chitosan coated dyed denim fabric. These shifts in the peaks are probably due to the presence of dyes and auxiliary chemicals on the dyed denim fabric.

The absorption peak observed at 1639 cm^{-1} in calico fabric coated with crayfish chitosan and nano TiO_2 was reduced to 1615 cm^{-1} in crayfish chitosan+TiO_2 coated raw denim fabric (Figure 4b). The peak observed at 1552 cm^{-1} in the FTIR spectrum of the undyed calico fabric disappeared in the spectrum of the raw denim fabric. Instead, a new peak formation was observed at 1105 cm^{-1} in the crayfish chitosan+TiO_2 coated raw denim fabric. In addition, the intensity of peak at 1022 cm^{-1} in the calico fabric increased to 1027 cm^{-1} in raw denim fabric coated with crayfish chitosan and nano TiO_2. A significant difference was observed at the absorption bands between 436–889 cm^{-1} in the FTIR spectra of raw denim fabric. This difference may be due to the fact that the amount of nano TiO_2 used in denim fabric (0.5%) is higher than that used in calico fabric (0. 05%). Zhang et al. [40] reported that a shift of 21 cm^{-1} in the FTIR spectrum of chitosan-TiO_2 composite compared to the band (at 1631 cm^{-1}) in the FTIR spectrum of chitosan showed the interaction between chitosan and TiO_2 nanoparticles. The peaks between 1637 and 1440 cm^{-1} observed in chitosan/TiO_2 nanocomposite coatings were indicative of a possible hydrogen bond between titanium and amino groups (NH_2) of chitosan [45]. The bands in the wavelength range of 385–950 cm^{-1} have been reported to characterize the O–Ti–O bonds between TiO_2 and chitosan[42,43].Considering the previous studies, results of that study confirm that crayfish chitosan+TiO_2 was successfully coated on the raw denim fabric.

SEM and EDX results of the coated fabrics with crayfish chitosan and nanoparticles

Surface morphology of the fabrics coated with crayfish chitosan and TiO_2 nanoparticles was examined by SEM and EDX analysis (Figure 5), and these fabrics were used for UV analysis. Figures 5b and 5c show that the crayfish chitosan, which forms a fluid solution, wraps the cotton denim fabric surface like a film. Previous studies reported the formation of a chitosan film layer on the cotton fibers, the nanoparticles trapped by chitosan and fixed on the fabric surface [11,46]. EDX analysis results showed that TiO_2 nanoparticles were distributed homogeneously on the denim fabric surface in general, despite the partial agglomeration (Figure 5a and 5b).

SEM and EDX images of undyed calico fabrics confirmed that chitosan was coated on the fabric surface in the form of a thin film by engulfing ZnO, TiO_2, and Ag nanoparticles (Figure 6 and Figure 7). While some studies reported that ZnO, Ag and TiO_2 nanoparticles distributed relatively homogeneous in chitosan display a smooth and flat appearance [11,40], other studies reported that these nanoparticles agglomerated in the chitosan nanocomposites and on the surface of cotton weaving and exhibited a rough and uneven appearance [12,42,46,47]. In this research, no aggregation was found on the fabric surface. This is due to the small amount of nanoparticles used and the nanoparticles were coated on the fabric after they were homogeneously dispersed in chitosan.

Crayfish chitosan coated undyed calico fabric contains 44.61% Carbon (C), 2.00% Nitrogen (N) and 53.39% Oxygen (O) by weight. Undyed calico fabric coated with crayfish chitosan+nano ZnO contains 44.51% C, 0.95% N, 52.93% O, and 1.61% Zinc (Zn) by weight. Crayfish

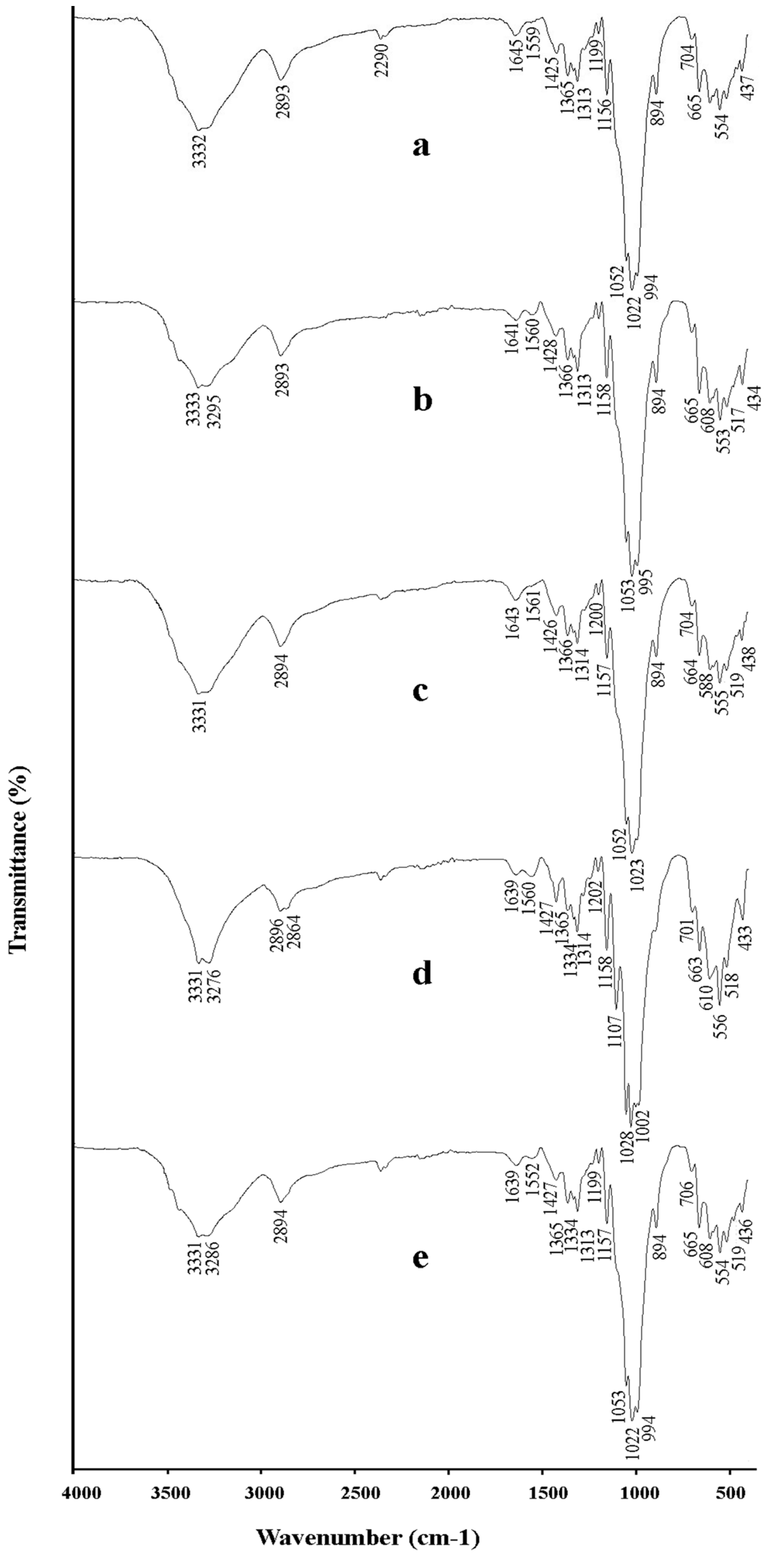

Figure 3. 3 FTIR graphs of undyed calico fabrics. a) uncoated fabric, b) Crayfish chitosan coated fabric, c) Crayfish chitosan+ nano ZnO coated fabric, d) Crayfish chitosan+nano Ag coated fabric, e) Crayfish chitosan+nano TiO_2 coated fabric.

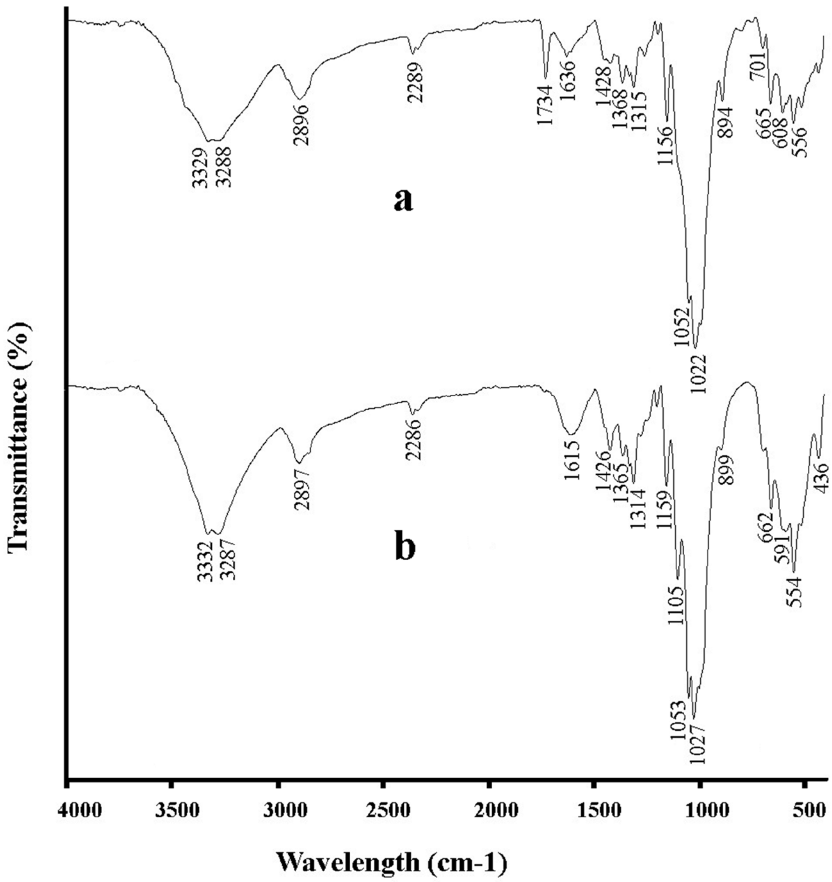

Figure 4. FTIR spectra of the chitosan and nano TiO_2-coated denim fabrics a) crayfish chitosan-coated dyed denim fabric, b) crayfish chitosan + nano TiO_2-coated raw denim fabric.

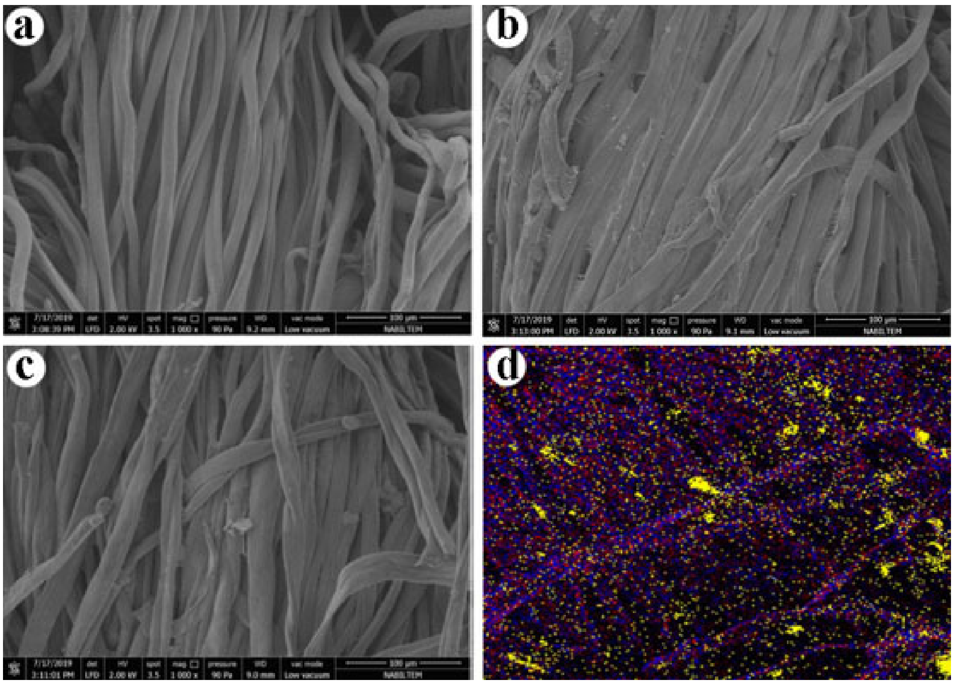

Figure 5. SEM and EDX images of the fabrics. a) uncoated and undyed calico fabric; b) blue dyed denim fabric covered with crayfish chitosan; and c) and d) undyed raw denim fabric covered with crayfish chitosan+nano TiO_2.

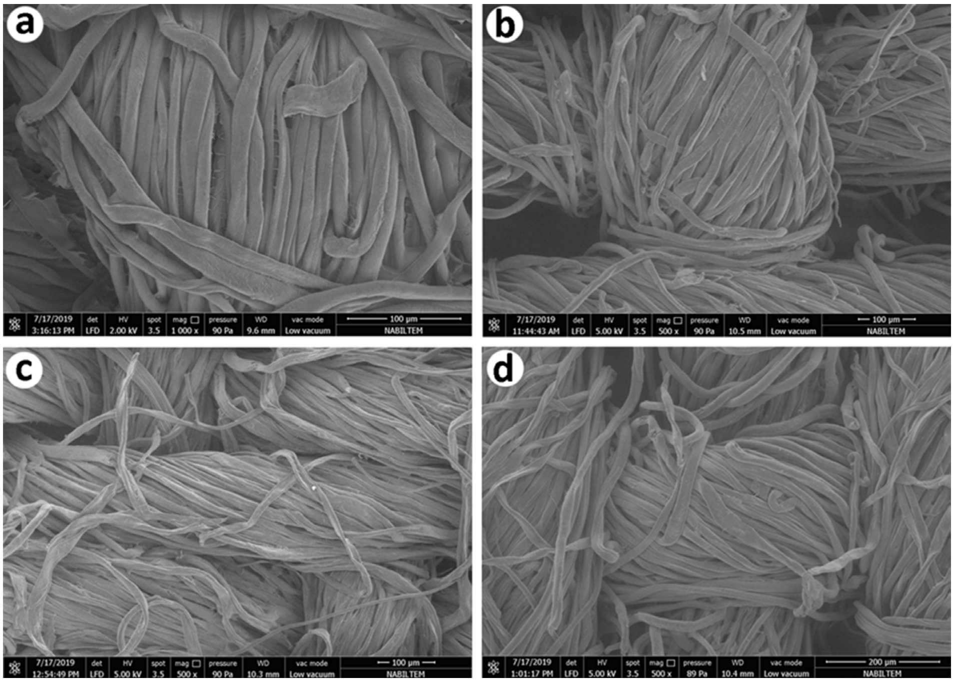

Figure 6. SEM images of undyed calico fabric. a) Crayfish chitosan coated fabric, b) Crayfish chitosan+nano ZnO coated fabric, c) Crayfish chitosan+nano Ag coated fabric, d) Crayfish chitosan+nano TiO_2 coated fabric.

chitosan+nano TiO_2 coated undyed calico fabric contains 44.93% C, 1.25% N, 53.75% O, and 0.06% Titanium (Ti) by weight. Finally, crayfish chitosan+nano Ag coated white calico fabric contains 45.79% C, 0.93% N, 53.25% O, and 0.03% silver (Ag) by weight. Lin et al.[39]measured 5.32% Ti and 5.74% Ag by weight in the EDS analysis of silver/titanium dioxide/chitosan adipate nanocomposites. Li et al. [48] reported that EDS diagram of chitosan composite films revealed the presence of 37.3% TiO_2 and 11.9% Ag particles by weight. The amount of nanoparticles used in this study was kept lower than in other studies considering health conditions. However, the results of this study are compatible with other studies and show that Ag, ZnO and TiO_2 nanoparticles are coated on cotton fabric with chitosan.

UV permeability results of the coated fabrics

UV permeability, blockade of UV rays and UPF values of the denim fabrics with and without chitosan coating were determined by the dry evaluation method according to the AATCC 183 test method (Table 2) [19]. In undyed denim fabrics, the UV protection factor (UPF) before coating varied between 12.83 and 13.87, depending on the degree of measurement. In chitosan-coated fabrics, UPF (39,17-43,83) increased, depending on the degree of measurement. Interestingly, the UPF values (22.63-26.46) in the chitosan+nano TiO_2-coated fabrics decreased compared to the chitosan-coated fabrics. In dyed denim fabrics, the UV protection factor (UPF) values before coating were between 16.44 and 18.81 and varied depending on the degree of measurement. UPF values (35.96 to 53.04) increased, depending on the degree of measurement in the chitosan-coated dyed fabrics. In chitosan+nano TiO_{2}- coated fabrics, UPF values (28.62-32.44) decreased compared to the chitosan-coated fabrics. In UV protection, according to the AATCC 183:2014 standard [19], UPF protection categories are defined as “good protection” between 15 and 24, “very good protection” between 25 and 39, “excellent protection” between 40 and 50 and above [49]. When the results of this study are compared with the standard values, the UPF values which were between 12 and 13 before coating increased to 39–43 after coating in chitosan-coated fabrics and reached an excellent level. By creating a thin layer of chitosan film on denim-fabric surfaces, a much higher UV protection can be achieved than in uncoated fabrics.

The UV radiation band consists of 3 zones: UV-A (from 315 to 400 nm), UV-B (from 280 to 315 nm) and UV-C (from 100 to 280 nm) [50,51]. Since UV-C is retained by ozone, it cannot reach the earth. However, it is known that UV-A, which can reach the earth, lowers the immune response of skin cells, while UV-B causes the for-

Table 2. UV Permeability Measurement Results of the Fabrics.

Fabric samples	Degrees of measurement	UVA Permeability T (UV-A)	UVB Permeability T (UV-B)	UV Protection Factor (UPF)	UVA Blocking(%)	UVB Blocking (%)
Uncoated and undyed denim fabric	0°	10.69%	7.13%	12.83	89.70%	93.20%
	45°	10.35%	6.85%	13.03		
	90°	9.84%	6.42%	13.87		
Uncoated and dyed denim fabric	0°	0.01%	0.01%	16.44	99.99%	99.99%
	45°	0.01%	0.01%	18.70		
	90°	0.01%	0.01%	18.81		
Crayfish chitosan coated undyed denim fabric	0°	4.53%	2.11%	39.17	95.61%	97.95%
	45°	4.54%	2.16%	38.44		
	90°	4.08%	1.88%	43.83		
Crayfish chitosan coated dyed denim fabric	0°	0.01%	0%	48.52	99.99%	100%
	0°	0.01%	0%	48.52		
	0°	0.01%	0%	48.52		
Crayfish chitosan+nano TiO_2 coated undyed denim fabric	0°	5.49%	3.63%	24.93	93.81%	96.39%
	45°	6.55%	3.88%	22.63		
	90°	5.78%	3.30%	26.46		
Crayfish chitosan+nano TiO_2 coated dyed denim fabric	0°	0.01%	0%	32.39	99.99%	100%
	45°	0.01%	0%	32.44		
	90°	0.01%	0%	28.62		

mation of skin cancers [50]. Therefore, UV-A and UV-B are crucial in testing the UV protection performance of fabrics. When the UV-A and UV-B blockage values of all denim fabrics are compared, it is observed that the UV blockage in dyed fabrics was at the highest values (99.99% to 100%). However, the UPF values differs according to the coating type. Although UV blocking values were high in dyed fabrics, nano TiO_2 reduced the UPF value of chitosan. When the nano TiO_2 was coated on the fabric surface with crayfish chitosan, the UV protection effect of the fabric was reduced. This may be due to the partial agglomeration of nano TiO_2 bonding to the chitosan. Yang et al. [52] stated that TiO_2 acts as a UV blocking additive by way of UV absorption. Perhaps aggregation and coating by chitosan prevents the UV absorption of TiO_2.

When UV light transmittance values (T%) of nondyed denim fabrics are evaluated according to wavelength, it is seen that the least permeability was in the fabric coated with crayfish chitosan between 280 and 400 nm wavelengths (between 1.7% and 7%) (Figure 8). At the same wavelengths, the UV permeability value (3.1% to 9%) of the chitosan+nano TiO_2 coated denim fabric increased compared with the chitosan-coated fabric. However, both coatings increased protection by reducing the permeability of the uncoated fabric at the same wavelength. The ultraviolet keeping properties of fabrics are determined by the fiber content and knitting, dyes used and coatings made [51]. Sarkar [50] stated that the fabrics dyed with dark colors and thick and heavy fabrics provided effective protection against UV rays and these fabrics have higher UPF values. The raw calico fabric, which is sparse and highly porous, provided less protection than the densely woven denim fabric [52]. The results of that study also support these findings.

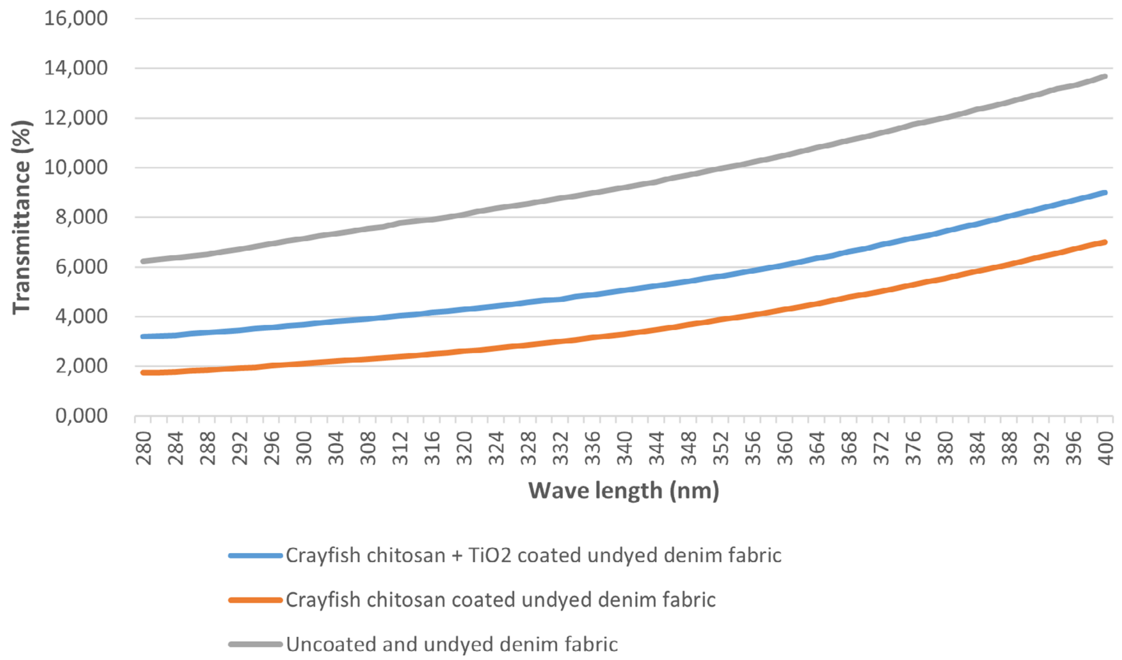

Figure 8. UV permeability of undyed raw denim fabrics.

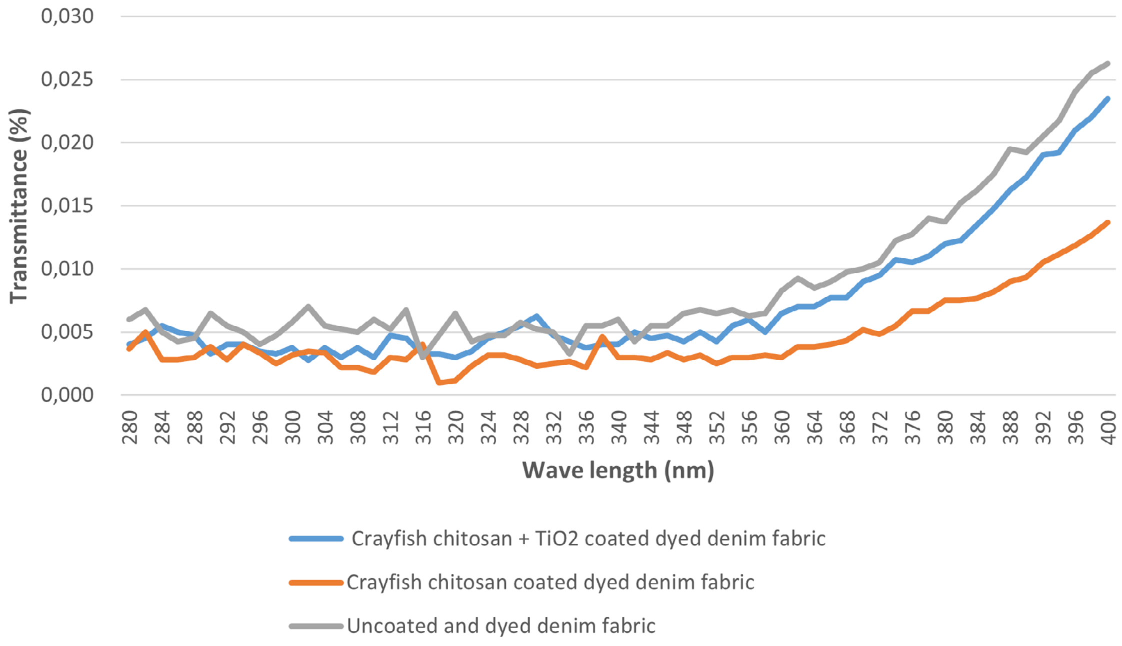

Figure 9. UV permeability of the dyed denim fabrics.

When the T% values of the blue-dyed denim fabrics coated with crayfish chitosan were evaluated according to the wavelength, it was evident that the least permeability (between 0.004% and 0.014%) was between 280 and 400 nm wavelengths (Figure 9). In chitosan+nano TiO_2 coated denim fabrics, the permeability values (0.004% to 0.024%) at the same wavelengths increased compared with those of the chitosan-coated fabric. However, both coatings increased protection by reducing the permeability (0.006% to 0.026%) of the uncoated dyed fabric at the same wavelength. Sarkar [50] found a direct correlation between the weight of the fabric and the UPF values, and noted that the thick fabrics provided more protection against ultraviolet rays. The author also noted that dyeing the cotton fabrics with natural colorants significantly enhanced the ultraviolet shielding capabilities of the fabrics and provided effective protection against ultraviolet rays. In this study, the difference between the dyed and the nondyed fabrics is clearly seen in Figure 8 and Figure 9. UV protection was higher in dyed fabrics.

Table 3. Antibacterial activity results of undyed calico fabrics according to JIS L 1902-2015 standard test method

Samples	**Number of reproducing microorganisms (CFU/sample)**		**Logaritmic reduction**		**Growth value**	**Antibacterial activity value**
	Contact time (0 hour)	*After incubation (24 hours)*	*Log (C_0) and Log (T_0)*	*Log (Ct) and Log (Tt)*	**F= Ct-C_0 and *G= Tt-T_0*	*A=F-G*
Untreated sample	$5.24x10^4$	$7.6x10^6$	4.72	6.88	2.16	-
Crayfish chitosa coated fabric	$5.22x10^4$	$4.04x10^4$	4.72	4.61	-0.11	A=2.27; A>2 %99 Efficacy
Chitosan+nano TiO_2 coated fabric	$4.2x10^4$	$7.8x10^4$	4.62	4.89	0.27	A=1.89; A<2 No efficacy
Chitosan+nano ZnO coated fabric	$3.66x10^4$	$1.6x10^3$	4.56	3.20	-1.36	A=3.52; A>3 99.9% Full efficacy
Chitosan+nano Ag coated fabric	$2.98x10^4$	$2x10^2$	4.41	2.30	-2.11	A=4.27; A>3 %99.9 Full efficacy
Untreated sample (after wash)	$6x10^4$	$4.4x10^7$	4.78	7.64	2.87	-
Crayfish chitosan coated fabric (after wash)	$5.41x10^4$	$2.36x10^5$	4.73	5.37	0.64	A=2.22; A>2 %99 Efficacy
Chitosan+nano TiO_2 coated (after wash)	$7.2x10^4$	$8.6x10^5$	4.86	5.93	1.08	A=1.79; A<2 No Efficiacy
Chitosan+nano ZnO coated fabric (after wash)	$7x10^4$	$4.44x10^6$	4.85	6.65	1.80	A=1.06; A<2 No Efficacy
Chitosan+nano Ag coated (after wash)	6x104	4x103	4.78	3.60	-1.18	A=4.04; A>3 %99.9 Full efficacy

Chen et al. [34] discovered that chitosan/TiO_2-coated cotton fabrics exhibited excellent UV protection. The authors also pointed out that this nanocomposite can be applied on the materials directly in contact with the human body. However, in this study, it was observed that TiO_2 nanoparticles reduced the UV-blocking function of crayfish chitosan. For this reason, it may be confirmed that using only chitosan coatings in the UV-protective fabric production can provide sufficient UV protection, and it will be safer for clothes in direct contact with the human body because chitosan is an organic substance.

Antibacterial activity results of coated fabrics

Antibacterial efficiency of chitosan and nanometal coated undyed calico fabric samples was measured after 24 hours of incubation with 2 mL of bacterial solution at the concentration of $3x10^5$ cfu/mL (Table 3). Antibacterial activity values (A) of undyed calico fabrics were calculated as 4.27, 3.52, 2.27 and 1.89 respectively, for chitosan+nano Ag, chitosan+nano ZnO, crayfish chitosan, and chitosan+nano TiO_2 coated fabrics. Chitosan+nano Ag coated fabric showed the highest antibacterial activity against *S. aureus*, followed by chitosan+nano ZnO coated fabric and chitosan coated undyed calico fabric, respectively. Chitosan+nano TiO_2 coated fabric, did not show antibacterial activity against *S. aureus* (Table 3).

The antibacterial effect of chitosan is determined by numerous factors including the Mw, DD, concentration, microorganism species, pH, presence of metal cations and temperature [53]. In this study, Mw and DD, which are the key factors controlling the anti bacterial efficiency of chitosan, were discussed. It has been reported that increasing in the number of cationic amino groups depending on the increase in the DD of chitosan greatly increase the antimicrobial effect of chitosan [29]. Chitosan with a high DD (97.5%) had a higher positive charge density and provides a stronger antibacterial effect against *S. aureus* than the chitosan having a moderate DD (83.7%) [54]. In our study, the DD of the crayfish chitosan was calculated as 16%. This value is quite low. However, the crayfish chitosan showed good antibacterial activity against *S. aureus*. Iqbal [55] stated that anionic bacterial cells are attracted to cationic materials and this leads to cell death in bacteria by causing cell membrane disruption and cell leakage.

The Mw of the crayfish chitosan is 11.2 kDa, and it is classified as low molecular weight chitosan. Chitosan with high molecular weight tends to accumulate more on the surface of fabrics, thus increasing the accessibility of amino groups by bacteria and leading to the better antibacterial activity of chitosan [32]. Shin and Yoo [32] observed that *S. aureus* was effectively inhibited (over 90% reduction) by high molecular weight chitosans (210 kDa and 100 kDa) at 0.5% concentration. On the contrary, another paper showed that low molecular weight chitosan has a stronger antibacterial effect against resistant strains like *S. aureus* [56]. Luo and Li [37] stated that chitosan with a Mw of 1050.5 Da and a DD of 90.20% had a significantly higher rmicrobial reducing rate on *S. aureus.* The authors emphasized that there is a strong parallel correlation between DD and Mw, however, when the two factors are compared, DD has a higher impact than Mw on determining the antibacterial activity [37]. Since the DD of the crayfish chitosan is quite low in our study, it reduces antibacterial activity.

Ag nanoparticles are known as effective antibacterial agents. Nano Ag particles in Ag/chitosan composites synthesized by Chen et al. [38] showed a synergistic effect with chitosan and nano Ag provided higher antibacterial effeciency than chitosan against both gram-positive bacteria and gram-negative bacteria. Wiegand et al. [57] measured the microbial proliferation reduction values of nano Ag reinforced composites for *S. aureus* and *Pseudomonas aeruginosa* in the range 3.3-7.0 log CFU using the same method given in this study. In this study, these values were measured as 2.30 log CFU before washing and 3.60 log CFU after washing for chitosan+nano Ag coated fabric. Another study revealed that nano Ag particles deposited cotton fabric had a very good antibacterial efficiency for *S. aureus* (95% reduction) and *E.coli* (92% reduction) due to the amount of nano Ag particles coated on the fabric [51]. In this research, the chitosan+nano Ag coated fabric also provided the highest antibacterial effect against *S. aureus*. Nano Ag particles, which have an extremely large interaction area, bind to the bacterial cell by providing better contact with microorganisms, then penetrate bacterial cell or cell membrane, disrupt cellular activities and kill bacteria [48, 58].

According to a study by Zhong et al. [36] chitosan and nano ZnO alone exhibited much weaker antibacterial efficiency, however, nano ZnO/chitosan composite showed around 89.5% and 94.0% bacteriostatic activity against *S. aureus* and *E. coli*, respectively. Belay et al. [12] stated that the differences in the shape and dimentions of ZnO nanoparticles deposited on cotton fabrics affected the antibacterial activities of the nanoparticles against *E. coli* and *S. aureus* bacteria. The authors explained that smaller-sized ZnO nanoparticles exhibit higher antibacterial activity by easily penetrating bacteria cell membrane because of their large interaction areas. According to Morones et al. [59], the interaction between bacteria and nano ZnO increase depending on the reduction in the size of nanoparticles. In addition, it is stated that the nanoparticle concentration is also effective on antibacterial effect [12,36]. In this research, ZnO nanoparticles combined with chitosan showed higher antibacterial efficiency for *S. aureus.* However, the antibacterial effect was lost in the washed chitosan+nano ZnO coated fabric due to the decrease in the amount of ZnO nanoparticles. It is stated that the bacteriostatic mechanism of nano ZnO is due to the small size effect, the release of Zn^{2+}, and the formation of reactive oxygen species (ROS) [12,36].

Although TiO_2 coated on the fabric surface for UV protection is quite effective, its antibacterial efficiency is low. Li et al. [48] investigated the antibacterial effect of TiO_2 and Ag nanoparticles coated cotton fabrics for *S. aureus* and *E. coli*. The authors reported that while even a small dose of Ag nanoparticles showed a significant effect in bacterial reduction (90% inhibition rate), a significant inhibition zone was not observed in cotton fabric coated with TiO_2 nanoparticles. Nguyen et al. [60] decorated TiO_2 and ZnO nanoparticles with Ag nanoparticles to increase their antibacterial activities. Pure Ti and Zn nanoparticles did not show bacteriostatic effects against *S. aureus* and ***E. coli***, however, Ag particle addition led to good antibacterial activity even at a low amount. Similarly, in this study, chitosan+nano TiO_2 coated undyed calico fabric did not show any antibacterial effect on *S. aureus*. However, Xing et al. [41] reported that the TiO_2 reinforced chitosan nanocomposite showed a bacteriostatic effect on the proliferation of *E. coli* and *S. aureus* with the increase of TiO_2 nanoparticle concentration added to the chitosan coating. The low antibacterial activity of the chitosan+nano TiO_2 coated undyed calico fabric in this study may be due to the very low amount of TiO_2 nanoparticles used. Also, Xing et al. [41] suggested that TiO_2 exhibits antimicrobial activity when exposed to sunlight or ultraviolet light due to its strong oxidizing properties.

Crayfish chitosan exhibited different antibacterial activities against *S. aureus* when used alone and combined with different nanoparticles. The antibacterial effect of chitosan and nanoparticle-coated undyed calico fabrics were affected by the low Mw and low DD of crayfish chitosan, very low nanoparticle concentrations (0.05%), and the sizes of Ag, ZnO, and TiO_2 nanoparticles.

Antibacterial activity results after washing

Antibacterial effect values of the undyed calico fabrics after washing were measured as 4.04, 1.06, 2.22, and 1.79, respectively for chitosan+nano Ag coating, chitosan+nano ZnO coating, crayfish chitosan coating, and chitosan+nano TiO_2 coating. Chitosan+nano ZnO coated fabric lost its antibacterial efficiency for *S. aureus* while the fabrics coated with chitosan and chitosan+nano Ag retained their antibacterial efficiency after washing. Li et al. [48] found that the *E. coli* inhibition rate of cotton fabrics coated with nanoparticles at different concentrations decreased by 8% after 50 cycles of commercial washing. Similar results were obtained in this study as well. Zhang and Neau [61] reported that molecules with low Mw and low DDA are more vulnerable to biodegradation and chemical degradation. Zhang et al. [62] stated that the use of glutaric dialdehyde as a crosslinking agent increased the chitosan uptake of the surface of cotton fabrics and increased the resistance of its antibacterial properties to washing. The accumulation of chitosan molecules on the fabric surface will further strengthen the Van der Waals forces between chitosan and cellulose molecules, and bacterial growth will decrease in direct proportion to the increasing amount of chitosan [62]. According to Belay et al. [12] nanoparticle size and shape greatly affect the adhesion of nanoparticles to fibers. The authors point out that particles with larger sizes and aggregated nanoparticles can be quickly detachable from the fabric, while smaller sized nanoparticles insert deeper and attach strongly to the fiber surface. The loss of antibacterial activity in the undyed calico fabric may have been due to the low Mw and DD of the crayfish chitosan, the decrease in the amount of chitosan on the fabric surface after the washing, and the separation of nanoparticles from the fabric.

CONCLUSION

The chitosan coating covered the denim fabric surface like a film layer without causing a significant increase in fabric weight and without changing the color of the fabric. Therefore, it can be applied to dyed fabrics as well as untreated fabrics. While the crayfish chitosan coating increased the UV protection performance of the fabric, the addition of nano TiO_2 decreased the UV blocking function of the crayfish chitosan. Fabric structural properties such as density, porosity and weight also affected the UV permeability of the denim fabric. When lower permeability is desired, fabric density values should be selected as high as the usage conditions allow. Despite its low degree of deacetylation, crayfish chitosan provided good antibacterial effect for *S. aureus* both alone and together with nanoparticles. Low concentrations of Ag and ZnO nanoparticles applied together with chitosan will be sufficient to impart antibacterial properties to fabrics. Since various textile auxiliary chemicals such as wetting agents and surfactants are not needed while coating chitosan onto fabrics, these coatings can be used safely on fabrics in direct contact with the human body. Protective fabrics with crayfish chitosan coating can be used in the production of clothing for sportsmen's garments that need multiple functional features, work clothes for agricultural workers and outside workers, and for people with skin sensitivity and skin cancer patients. In addition, this study will support denim manufacturers' efforts to create new markets by adding new functions to denim garments.

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