**Advances in Materials Research**, *Vol. 9, No. 3 (2020) 233-249*
DOI: https://doi.org/10.12989/amr.2020.9.3.233


# Enhancement of antimicrobial properties of shoe lining leather using chitosan in leather finishing

Yead Mahmud [1], Md. Minhaz Uddin [1], Nizam Uddin [2] and Sayed Md. Shamsuddin*[1,3]

[1] *Institute of Leather Engineering & Technology, University of Dhaka, Dhaka-1209, Bangladesh*
[2] *Nutrition and Food Engineering, Faculty of Allied Health Science, Daffodil International University, 102, Shukrabad, Mirpur Road, Dhanmondi, Dhaka-1207, Bangladesh*
[3] *Department of Applied Chemistry and Chemical Engineering, Faculty of Engineering, University of Dhaka, Dhaka-1000, Bangladesh*

(*Received December 21, 2019, Revised June 18, 2020, Accepted October 6, 2020*)

**Abstract.** In this study, a chitosan based coating method was developed and applied on the shoe lining leather surface for evaluating its inhibition to bacterial and fungal attacks. At first, chitosan was prepared from raw prawn shells and then the prepared chitosan solution was applied onto the leather surface. Secondly, the characterization of the prepared chitosan and chitosan treated leather was performed by solubility test, ATR-FTIR, XRD pattern, SEM and TGA. Evaluation of antimicrobial efficacy of chitosan was assessed against two gram positive, two gram negative bacteria and a reputed fungi by agar diffusion test. The results of this study demonstrated that chitosan took place in both the surface of collagen fibres and inside the collagen matrix of crust leather. The chitosan showed strong antimicrobial activities against all the tested microorganisms and the inhibition increased with increasing percentage of chitosan. Therefore, the prepared chitosan in this study can be an environment friendly biocide, which functions simultaneously against different spoilage bacteria and fungi on the finished leather surface. Thus by using the prepared chitosan in shoe lining leather, the possibility of microbial attack during shoe wearing can be minimized which is one of the important hygienic requirements of footwear.

**Keywords:** chitosan; leather; antimicrobial property; leather coating; finishing

## 1. Introduction

Leather was considered to be the original materials for making footwear from the early stages of footwear industries and about half of all leather produced today is used to make shoes (Bieńkiewicz 1981).

In footwear industries, the first priority is to produce hygienic, highly qualified, safe footwear as well as eliminating microorganisms' growth in the footwear in order to meet the consumer's demand. However, the footwear's provide suitable environment for microbial growth like bacteria, fungi as there is available moisture, warmth conditions, nutrients from feet sweat and greases/olis from insoles inside the shoes during wearing (Orlita 2003). Also, leather having a network of collagen

*Corresponding author, Ph.D., E-mail: drnizam.nfe0041.c@diu.edu.bd
**Co-corresponding author, Ph.D., Professor, E-mail: sdin@du.ac.bd


http://www.techno-press.org/?journal=amr&subpage=5 ISSN: 2234-0912 (Print), 2234-179X (Online)

easily acts as a good source for quick growth of microorganisms (Fernandes *et al*. 2013). Specially, foot odor is mainly derived from isovaleric acid produced from staphylococcus epidermidis, degrades leucine present in sweat. Also, Bacillus subtilis was shown to be closely associated with increased foot odor (Widrow *et al*. 1991). Boahin *et al*. examined thin films of flesh left on the grained side of the leather, start to decay when they come in contact with moisture (Boahin *et al*. 2013).

In extreme cases, high growth and large proliferation of *Aspergillus niger* microbes on the leather surface of footwear will occur prospective health complications for the customers (Orlita 2003), i.e., foot ulceration and amputation (Jennings *et al*. 1999, Ara *et al*. 2006, Johannesson *et al*. 2009, Sánchez-Navarro *et al*. 2011).

To overcome these problems, bacterial and fungal eradication of the leather products can be achieved by fabricating leather with antimicrobial properties (Aksoy and Kaplan 2013). For example, antibacterial properties of the leather products can be obtained by applying antiperspirants, spray formulations and hygroscopic insoles in the foot or inside the shoe (Aksoy and Kaplan 2013). Besides, various natural and synthetic biocides have been used recently to preserve hides during processing (Orlita 2003, Stockman *et al*. 2007). However, the biocides have adverse effects on human health and environment (Sirvaityte *et al*. 2011). Kaygusuz *et al*. indicated that the use of $TiO_2$-$SiO_2$ nanocomposite improved certain performance characteristics of leather (Kaygusuz *et al*. 2017). Although the metal nanoparticles like silver nanoparticles (AgNPs) coatings are biocompatible, non-toxic and long-lasting products that does not create any toxicity problems, the process of their preparation is expensive (Liu *et al*. 2012, 2015, Velmurugan *et al*. 2014). On the other hand, marine chitin isolated chitosan is considered to be cheap, non-toxic, biocompatible, biodegradable for functional coatings development due to its strong inhibitory effect, a broad spectrum of antimicrobial activities and film forming capability (Muzzarelli 1977, Muzzarelli *et al*. 1990, 2012, Xu *et al*. 1996, Kumar *et al*. 2004, Rinaudo 2006, Santos *et al*. 2017, Furtado *et al*. 2018, Sousa *et al*. 2018). In the textile industry, eco-friendly chitosan has already been used to develop fabrics (El-Tahlawy *et al*. 2005, Dev *et al*. 2009, Tseng *et al*. 2009). In the leather industry, chitosan and chitosan derivatives, i.e., methacrylic acid–acrylamide–chitosan copolymer are found to be used as auxiliary agents in the chrome-tanned leather dyeing process (Burkinshaw and Jarvis 1996, Lv *et al*. 2011, Plavan 2012, Aslan 2013). For example, the synthesized PEGylated chitosan copolymers can also be efficiently used for antimicrobial coating of leather product (Luo *et al*. 2016). Fernandes *et al*. developed and applied chitosan based antimicrobial leather coatings for coating of footwear insoles (Fernandes *et al*. 2013). To the best of our knowledge, there is no application of chitosan-based coatings along with a binding agent for shoe lining leather in the literature. Therefore, the development and application of an antimicrobial chitosan coating for shoe lining leather would be highly demandable at present time in order to protect the foot from microbial attack.

A typical leather or textile coating formulation mainly contains polymeric binder and several additives like biocide, adhesion promoter, colorants etc, which are applicable through brushing, spraying, roll coating etc. (Billah 2018). Poly vinyl acetate (PVAc) is mainly used as a binder for different types of materials such as fibre, leather etc. (Mark 2014). The main advantages of PVAc are its easier and wider application, elasticity, resistance to ageing, low cost, availability, resistance to microbial attack and non-toxicity (Rebsamen 1983, Paris 2000, Kim *et al*. 2007, Šedivka 2015). It was reported that mixing PVAc with zinc sulphate and copper sulphate enhanced the antimicrobial characteristics of material (De Jesus *et al*. 2017, Marasigan *et al*. 2019). Besides, PVAc containing bioactive fillers showed effective antimicrobial activity against oral bacteria (Nagai *et al*. 2017). In addition, modified PVAc substrates can also be used in packaging. (Padmanabhan *et al*. 2015). In

case of prepared leather, the addition of PVAc will not only prolong the binding capability but also enhance the antimicrobial performance. Therefore, due to both antimicrobial and binding properties, PVAc was chosen to mix with chitosan solution before applying onto leather surface.

In this study, chitosan coating in addition to PVAc as a binding agent is applied on crust leather surface which is mainly used as lining leather in different types of footwear to resist bacterial and fungal attack when comes in contact with the foot. First, raw prawn shells were processed into chitosan solution and then the prepared chitosan solution was sprayed onto the leather surface of both grain and flesh sides. The prepared chitosan and chitosan treated shoe lining leather was evaluated by a number of analytical techniques and biological test.

## 2. Materials and methods

### *2.1 Raw materials*

#### *2.1.1 Leather*

Chrome tanned crust leather manufactured from goatskin was collected from Apex tannery, Savar leather industrial park, Hemayetpur, Dhaka, Bangladesh.

#### *2.1.2 Raw prawn shell*

Raw prawn shells were collected from prawn hatchery of Bagerhat district, Khulna division, Bangladesh.

#### *2.1.3 Standards*

Besides chitosan, Polyvinyl acetate (PVAc) and Acetic acid were purchased from Sigma-Aldrich Authorized Bangladesh Distributors, Kuri & Company (Pvt.) Ltd., 78, Motijheel Commercial Area Dhaka-1000, Bangladesh. Deionized water was used for the preparation of chitosan and acetic acid solutions.

#### *2.1.4 Preparation of chitosan*

Chitosan produced from raw shrimp shells according to the previous study (Dey *et al*. 2016, Islam *et al*. 2019). Three chitosan solutions (0.5%, 1% and 2% w/v) were prepared in 1% (v/v) acetic acid with continuous stirring at room temperature until complete dissolution (at least for 1 h). The prepared chitosan solutions were labeled as 0.5% CH, 1% CH and 2% CH.

### *2.2 Application of chitosan solution on crust leather*

#### *2.2.1 Stock solution of PVAc*

1 gm of PVAc was dissolved in 500 mL de-ionized water and heated under 70°C until dissolved.

#### *2.2.2 Modification of chitosan solution for application*

25 mL of each chitosan solution (CH 0.5%, CH 1%, and CH 2%) was taken in a beaker and mixed properly with 5 mL of PVAc solution which made a thin film on the leather sample.

#### *2.2.3 Surface modification of leather*

Modified chitosan solution was applied on leather by hand spraying. 30 mL of each prepared sample solution (CH 0.5%, CH 1%, and CH 2%) was applied twice on $1ft^2$ leather. The prepared

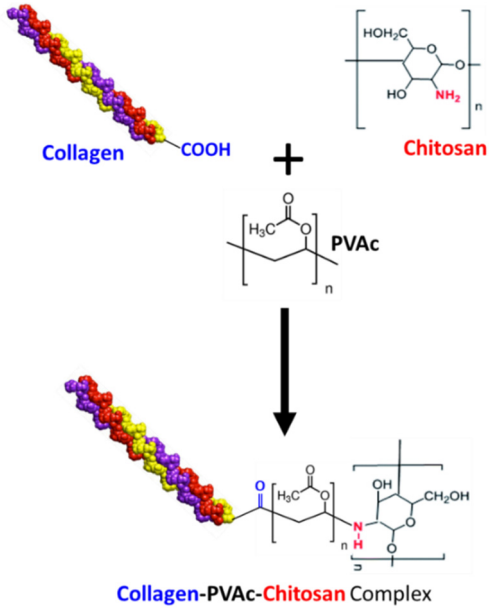



Fig. 1 Schematics of chemical interactions between leather (collagen), chitosan & PVAc

chitosan solutions 0.5% CH, 1% CH and 2% CH were applied on leather surfaces and the chitosan treated leathers were named as L-CH 0.5%, L- CH 1% and L-CH 2% respectively. The untreated leather was named as controlled and denoted by L.

### *2.3 Characterization of chitosan, chitosan treated and untreated leather*

#### *2.3.1 Attenuated total reflectance fourier transform infrared spectroscopy (ATR-FTIR)*

The ATR-FTIR analysis of chitosan and leather samples was performed by Perkin Elmer universal ATR sampling accessory in the range of 700-1000 $cm^{-1}$.

#### *2.3.2 Scanning electron microscopy (SEM)*

The surface morphology of chitosan and leather samples was studied by SEM machine (JSM-6490LA, JEOL, Japan).

Table 1 The physical tests carried out with standard method

| Sl. No. | Test name | Standard norm |
|---|---|---|
| 1 | Tensile Strength | IUP-6 |
| 2 | Stitch Tear Strength | DIN 53331 |
| 3 | Simple Tear Strength | SATRA PM 162 |
| 4 | Dry & Wet Rub Fastness | DIN 54021/ SLF 6 |
| 5 | Perspiration Fastness | BSEN-ISO-105 |
| 6 | Water Vapor Permeability | SATRA PM 172 |
| 7 | Shrinkage Temperature | SATRA PM 17 |

#### *2.3.3 Thermogravimetric analysis* (*TGA*)

Thermal characterization of chitosan and leather samples was carried out by thermo gravimetric analysis (TG/DTA 7200 EXSTAR, Hitachi, Japan) technique.

#### *2.3.4 X-ray diffraction* (*XRD*)

X-ray diffraction pattern of chitosan flakes was recorded through an x-ray diffractometer (Ultima IV, Rigaku Corporation, Japan) at room temperature.

### *2.4 Antimicrobial activity assay*

#### *2.4.1 Antibacterial assessment*

Antibacterial activity of prepared chitosan solutions and chitosan treated leather samples were accessed by checking zone of inhibition against *S. aureus*, *E. coli*, *B. subtilis* and *P. aeruginosa* in TSA media through agar well diffusion method.

#### *2.4.2 Antifungal assessment*

Antifungal activity of prepared chitosan solutions and chitosan treated leather samples were accessed by checking zone of inhibition against *Aspergillus niger* in TSB media through agar well diffusion method. The test was carried for 10 days.

### *2.5 Physical and mechanical characterization of leather*

Physical tests were carried out to determine the physical properties of chitosan coated leather and the name of physical tests were tabulated in Table 1.

## 3. Results and discussion

### *3.1 Mechanism of collagen, chitosan and PVAc*

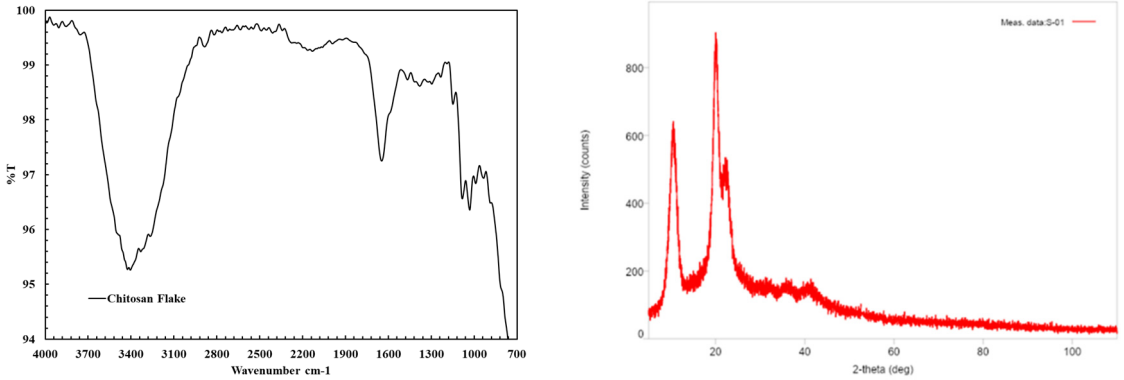
Fig. 1 represents the provable mechanism of chemical interactions between collagen of leather, chitosan & PVAc.

### *3.2 Characterization of chitosan*

The prepared chitosan solution was evaluated for its functional group, crystallinity and morphological features by ATR-FTIR, XRD and SEM analysis.

Fig. 2(a) showed the ATR-FTIR spectrum of chitosan with various characteristic peaks. In the spectrum, the detected absorption band at 1381.03 $cm^{-1}$ for C-O stretching vibration, 2885.51 $cm^{-1}$ for aliphatic C-H stretching and 3404 $cm^{-1}$ was assigned for O-H stretching coincided with N-H stretching (Monvisade and Siriphannon 2009, Zhang *et al*. 2011). A very strong peak at 1645.28 $cm^{-1}$ was assigned to the in-plane N-H bending vibration, which is the characteristic peak of chitosan polysaccharide (Mohanasrinivasan *et al*. 2014).

The XRD pattern of prepared chitosan identifying its crystallinity was represented in Fig. 2(b). Two characteristic broad diffraction peaks at 2θ around 10.42 and 19.92 were related to crystal-1 and crystal-2 respectively in the chitosan structure. According to the previous study (Bangyekan *et al*. 2006), the observed peaks are suggested to be the fingerprints of semi-crystalline chitosan with a high degree of crystallinity (Günister *et al*. 2007, Julkapli and Md Akil 2008).

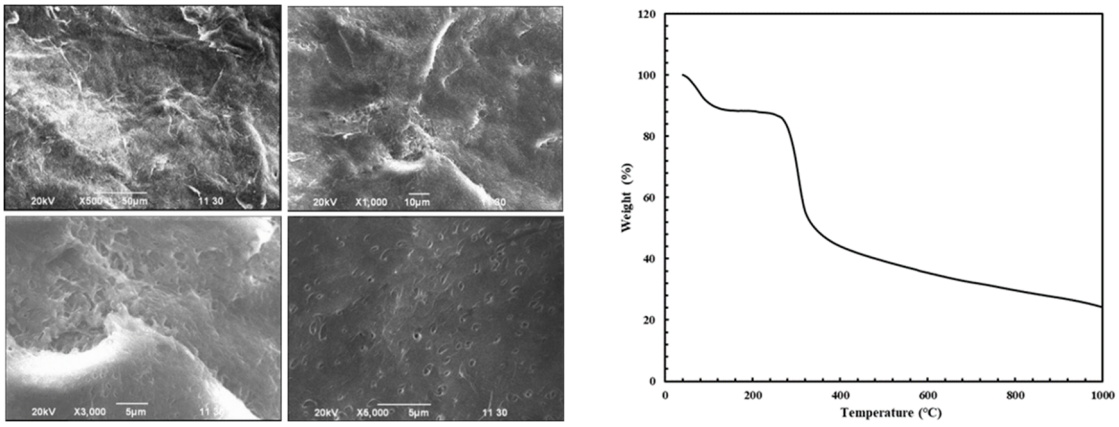



(a) ATR-FTIR spectra of chitosan flake (b) XRD pattern of chitosan

Fig. 2 ATR-FTIR spectra and XRD pattern of chitosan

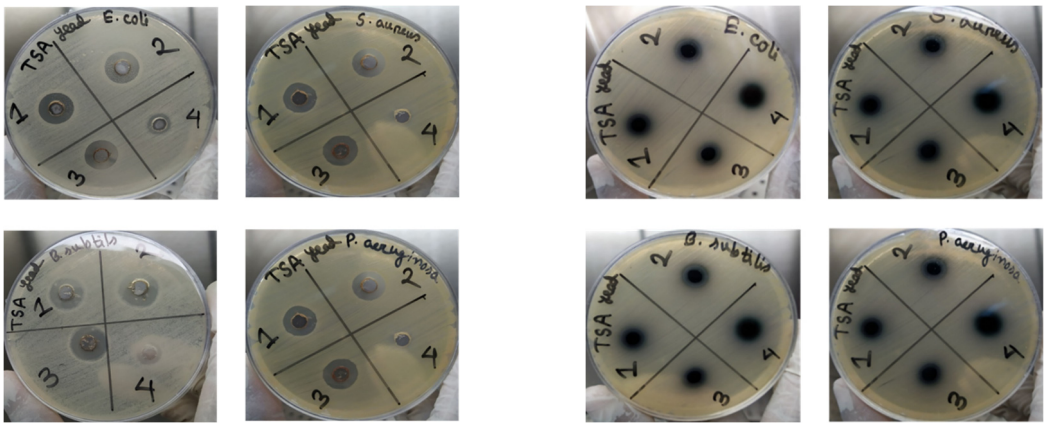
Fig. 3(a) illustrates the morphology of chitosan analyzed by Scanning Electron Microscope (SEM) micrographs, taken under four magnifications (500 µm, 1000 µm, 3000 µm and 5000 µm). As observed, the lower magnification micrographs showed non-smooth and non-homogeneous surface with straps and shrinkage, whereas higher magnification showed homogeneous surface (Islam *et al*. 2011).

Thermal stability of prepared chitosan was retrieved by thermogravimetric analysis (TGA) and illustrated in Fig. 3(b). In the temperature range of 22-100°C, the first weight loss step was observed, which corresponds to the loss of moisture (around 10%). In the temperature range of 248-600°C, a non-oxidative thermal degradation was observed to be occur in chitosan under nitrogen flow, which indicates the deacetylation, vaporization and elimination of volatile products (Wang *et al*. 2005). According to the previous study (Qu *et al*. 2000), the degradation of chitosan starts with amino groups forming unsaturated structures. During the pyrolysis of polysaccharides, the glyosidic bonds split randomly, which further decomposes by forming acetic acids, butyric acids and a series of lower fatty acids, where $C_2$, $C_3$ and $C_6$ dominated (Nieto *et al*. 1991).




(a) SEM images of chitosan at various magnifications (b) TGA thermogram of chitosan

Fig. 3 SEM images and TGA thermogram of chitosan



(a) Chitosan samples CH O.5% (1), CH 1% (2), CH 2% (3) and acetic acid 1% (v/v) (4)

(b) Leather samples L (4), L-CH O.5% (1), L-CH 1% (2) and L-CH 2% (3)

Fig. 4 The antibacterial activity of chitosan samples and leather samples

### *3.3 Antibacterial assessment of chitosan and chitosan coated leather*

The antimicrobial efficacy of chitosan and chitosan coated shoe lining leather was evaluated against two gram positive (*S. aureus*, *B. subtilis*), two gram negative bacteria (*E. coli*, *P. aeruginosa*) and a reputed fungi *A. niger* by means of agar diffusion test. Fig. 4(a) disclosed the zone of inhibition of the different chitosan solutions CH 0.5%, CH 1%, CH 2% along with 1% (v/v) acetic acid. In the Fig. 4(a), the clear zone of the petri dish was considered as inhibitory area. All selected bacteria were found to be very sensitive to the prepared chitosan solutions. No antibacterial activity was found for 1% (v/v) acetic acid against *E. coli*, *P. aeruginosa*, *B. subtilis* and *S. aureus*., which indicates the non-antibacterial activity of acetic acid. The remaining three samples inhibited the selected four bacteria. Among all the samples, the CH 2% sample was very active to inhibit the bacteria. The observed inhibition zones against all discussed strains were depicted in Table 2.

Table 2 Bacterial zone of inhibition (mm) of chitosan solutions, 1% (v/v) acetic acid, blank leather and chitosan coated leather

| Sample name | Zone of inhibition in mm (diameter) | | | |
|---|---|---|---|---|
| | *E. coli* | *S. aureus* | *B. subtilis* | *P. aeruginosa* |
| CH 0.5% | 14.5 ± 0.62 | 19.5 ± 0.43 | 17 ± 1.21 | 20.7 ± 1.05 |
| CH 1% | 15.7 ± 0.98 | 20.5 ± 0.73 | 18.5 ± 1.03 | 20.4 ± 0.89 |
| CH 2% | 19.3 ± 1.58 | 21 ± 0.53 | 18.8 ± 0.63 | 20.81 ± 1.29 |
| 1% (v/v) acetic acid | 0 | 0 | 0 | 0 |
| L | 0 | 0 | 0 | 0 |
| L-CH 0.5% | 9 ± 1.2 | 10 ± 0.34 | 9 ± 0.71 | 8 |
| L-CH 1% | 9.3 ± 0.52 | 10.5 ± .92 | 10 ± 0.34 | 9 |
| L-CH 2% | 9.6 ± 0.78 | 11 ± 0.23 | 11.8 ± 0.78 | 9.4 |

The results of the agar overlay test for sample leathers (L, L- CH 0.5%, L-CH 1% and L-CH 2%) against the specific four bacteria are presented in Fig. 4(b). As observed in Fig. 4, the non-treated chitosan solution sample (L) didn't show any zone of inhibition. On the other hand, the leather samples treated with chitosan solutions, showed significant inhibition zone (Fig. 4). The mean inhibition zones of treated leather with different concentrations of chitosan solutions against *E. coli*, *B. subtilis*, *P. aeruginosa* and *S. aureus*. were listed in Table 2. It was observed that the antibacterial property increased with the concentration of chitosan and thus the sample L-CH 2% exhibited excellent inhibitory effect against all the four bacteria. Ahmed *et al*. observed that the zone of inhibition of 5% chitosan was 49.7 ± 0.31, 33.4 ± 0.53, 50.4 ± 0.71 against *E. coli*, *S. aureus* and *P. aeruginosa* respectively (Ahmed *et al*. 2017). In our study, 0.5% chitosan showed the zone of inhibition of 14.5 ± 0.62, 19.5 ± 0.43, 17 ± 1.21 and 20.7 ± 1.05 against *E. coli*, *S. aureus*, *B. subtilis* and *P. aeruginosa* respectively. On the contrary, 1% chitosan showed the zone of inhibition of 15.7 ± 0.98, 20.5 ± 0.73, 18.5 ± 1.03 and 20.4 ± 0.89 against *E. coli*, *S. aureus*, *B. subtilis* and *P. aeruginosa* respectively. In addition, 2% chitosan showed inhibition zone of 19.3 ± 1.58, 21 ± 0.53, 18.8 ± 0.63 and 20.81 ± 1.29 against *E. coli*, *S. aureus*, *B. subtilis* and *P. aeruginosa* respectively. Therefore, it was assumed that all the prepared chitosan solutions and chitosan treated leather samples can inhibit the growth of *E. coli*, *B. subtilis*, *P. aeruginosa* and *S. Aureus* successfully.

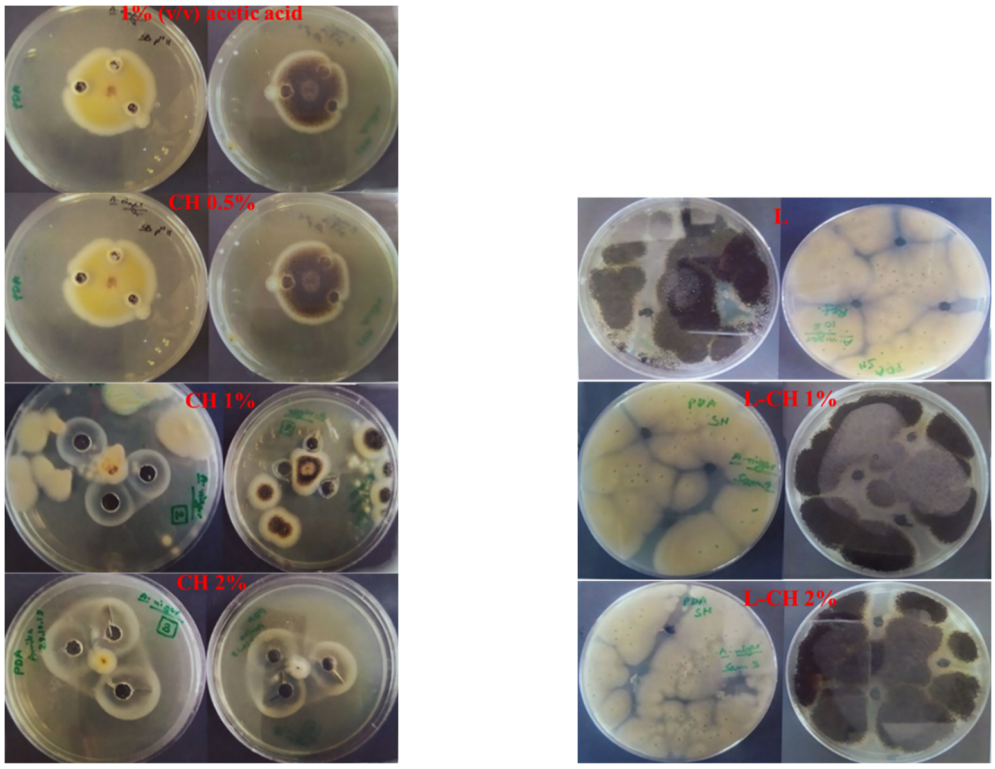
### *3.4 Antifungal assessment of chitosan and chitosan coated leather*

The antifungal activity of chitosan solutions like CH 0.5%, CH 1%, CH 2% along with 1% (v/v) acetic acid was assessed by clear zone of inhibition against *A. niger* and shown in Fig. 5(a). It was observed that 0.5% CH and 1% (v/v) acetic acid didn't show inhibition zone. On the other hand, CH 1% and CH 2% showed inhibition zone even after 10 days. The mean inhibition zone was also shown in Table 3, which declares that 2% chitosan solution had the highest zone of inhibition against *A. niger*.

Fig. 5(b) shows the development of inhibition zone of the leather samples, i.e., L, L-CH 1% and L-CH 2% after 10-days. The leather samples L-CH 1% and L-CH 2% had the ability of inhibition whereas blank leather L exhibited no zone of inhibition. The zone of inhibition was observed after 48 h. Even after 10 days L-CH 1% and L-CH 2% have shown inhibition zone. The mean inhibition zone is also shown in Table 3, which indicates the excellent inhibitory effect of L-CH 2% against *A. niger*. Besides, the inhibition of L-CH 1% is better than the blank leather L. It was observed from Table 3 that the antifungal properties increased with the concentration of chitosan. According to Ahmed *et al*., the zone of inhibition of 5% chitosan was 53 ± 0.94 and a drug standard antifungal [Amphotericin B (100 μg)] was 23.7 ± 0.46 against *A. niger* (Ahmed *et al*. 2017). In this study, the

Table 3 Fungal zone of inhibition (mm) of chitosan solutions and chitosan coated leather

| Sample name | Zone of inhibition against *A. niger* in mm (diameter) |
|---|---|
| CH 0.5% | 0 |
| CH 1% | 21.7 ± 0.65 |
| CH 2% | 24 ± 0.89 |
| L | 0 |
| L-CH 1% | 8 ± 0.43 |
| L-CH 2% | 10.7 ± 0.57 |




(a) 1% acetic acid solution, chitosan samples CH 0.5%, CH 1% and CH 2%

(b) Blank leather L, chitosan coated leather L-CH 1% and L-CH 2%

Fig. 5 The antifungal activity of prepared chitosan solutions, blank leather and chitosan coated leather

zone of inhibition showed by 1% and 2% chitosan was 21.7 ± 0.65, 24 ± 0.89 respectfully against *A. niger*. Therefore, it was assumed that the prepared chitosan solutions like CH 1%, CH 2% and thus leather samples treated with these samples have antifungal effect against *A. niger*.

### *3.5 Characterization of chitosan coated leather*

Usually, the treatment of leather with prepared chitosan solutions produces a translucent thin film on the surface. The pH of the leather L and chitosan coated leather surface was 3.7 and 3.5 respectively. Fig. 6 shows the ATR-FTIR spectrum of blank leather L, chitosan coated leather L-CH 1% and L-CH 2%. In case of blank leather L, the broad peak at 3782 $cm^{-1}$ and strong peak around 1450.47 $cm^{-1}$ indicating alcohol (-OH) and methylene groups respectively on the leather surface. There are also peaks at 2862 $cm^{-1}$ and 2929$cm^{-1}$ due to single bond C-H stretching. The absorption at 1649 $cm^{-1}$ for stretching of amide I group (Doyle *et al*. 1975) and 1591 $cm^{-1}$ for amide II group (Vichi *et al*. 2018). Furthermore, the presence of fat and the hydrolysable tannin was confirmed by the absorption at 2862 $cm^{-1}$ (Doyle *et al*. 1975) and bending C-O-C at 1286.52 $cm^{-1}$ respectively (Vichi *et al*. 2018). Yakimets *et al*. labelled the region of 1800-1500 $cm^{-1}$ as complex IR bands due to their correlations to the secondary structure of collagen and its interaction with the environment (Payne and Veis 1988, Yakimets *et al*. 2005). Therefore, the blank leather sample L had amide I and II group of collagens and hydrolysable tannins. Besides, the absorption peak at 2305 $cm^{-1}$ was

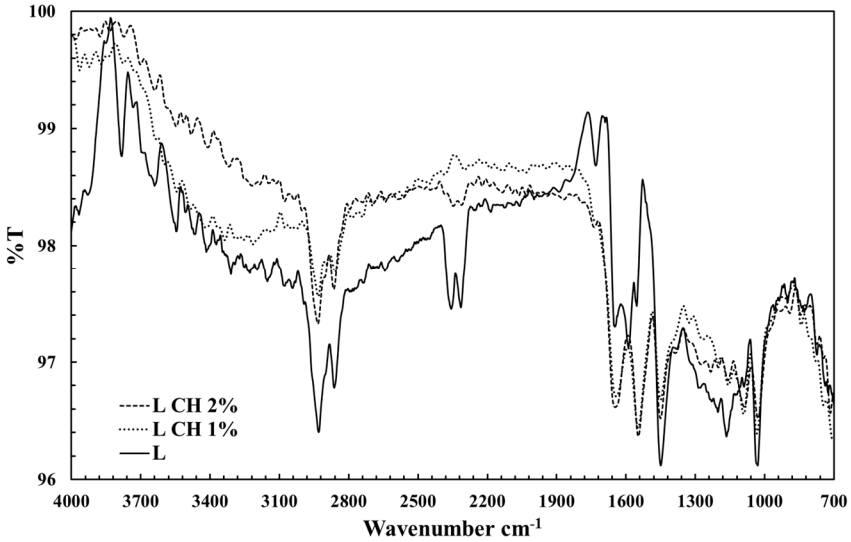



Fig. 6 ATR-FTIR spectra of untreated and chitosan coated leather samples

appeared on the spectra (Fig. 6), which may be for animal fat used in fat liquoring operation. In addition, the peak at 839 $cm^{-1}$ may be for bending aromatic ring and stretching of C-X bond which indicated the dye used spread on leather in excess amount. In the spectra of L-CH 2% (Fig. 6), all the absorption peaks as in the spectra of L were also found. The additional peaks were the absorption band at 1325 $cm^{-1}$ for C-O stretching vibration of primary alcoholic groups, 2866 $cm^{-1}$ for aliphatic C-H stretching and 3547 $cm^{-1}$ for O-H stretching overlapped with N-H stretching. The strong peak at 1653 $cm^{-1}$ was assigned to the in-plane N-H bending vibration, which is a characteristic peak of chitosan polysaccharide. It should be noted that some peaks were found to be shifted due to the addition of chitosan in the treated leather sample. The spectra of L-CH 1% were almost identical to the spectra of L-CH 2% (refer to Fig. 6). In this way, the presence of thin film of chitosan on treated leather was confirmed.

The blank leather sample, both 1% and 2% chitosan treated leather samples were evaluated by using SEM in order to verify the characteristics of thin film and the distribution of chitosan onto the leather sample surface and shown in Fig. 7. The SEM micrograph of L-CH 1% reveals that a thin, bright, transparent film was obtained on the leather substrate as well as the chitosan coating was evenly distributed throughout the grain surface and homogeneously diffused through the pores. Additionally, the pore structure of the treated leather sample was tighter than the untreated leather sample and did not completely fill the leather pores. For this reason, the water vapor permeability of L-CH 1% treated leather was almost similar as untreated leather. Although the microgram of L-CH 2% leather sample looked similar as L-CH 1% surface but due to little high concentration of chitosan, it filled the pores of the leather surface more. As a result, the water vapor permeability of L-CH 2% was decreased compared with untreated leather.

The way to determine the processing has been carried correctly is to measure the ‘hydrothermal stability’–its resistance to wet heat–more commonly referred to as the ‘shrinkage temperature’. In order to understand the thermal stability, the leather sample L, treated leather samples, L-CH 1% and L-CH 2% were decomposed in the range of room temperature to about 600°C at a heating rate of 20°C/min. The results of TGA and shrinkage temperature tests of treated and untreated leather

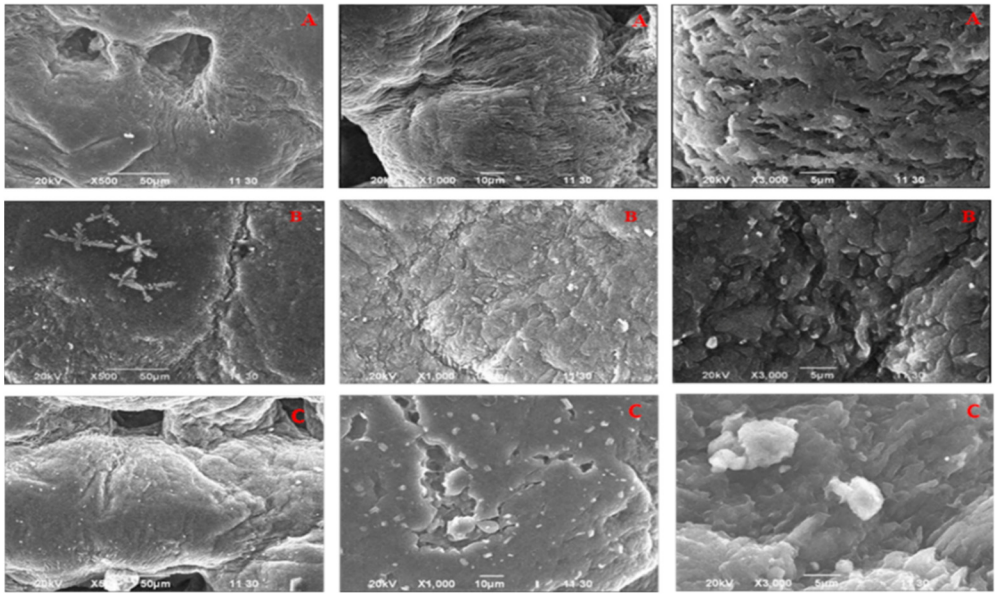


Fig. 7 SEM image of untreated leather sample, L(A); 1% chitosan treated leather sample, L-CH 1% (B) and 2% chitosan treated leather sample, L-CH 2% (C)

samples were presented in Fig. 8. For untreated leather sample, a very smooth loss of weight at around 300°C was observed followed by the main decomposition step between 370°C and 450°C.

A first degradation stage at around 64°C was attributed to the release of water whereas a second stage at about 220°C to 400°C is related to cleavage of collagen bonds from leather waste involving the decomposition into $CO_2$ as well as some $NO_x$ species. A third step occurred between 400-680°C was attributed to the degradation of the polymer chains of higher molecular weight and leather tanning agents. Banon *et al*. also studied the leather decomposition in the range of 25-800°C at four heating rates (5, 10, 15 and 20°C/min) (Bañón *et al*. 2016). For treated leather samples, L-CH 1% and L-CH 2%, there is a first weight loss (about 20%) at around 56-140°C and a second weight loss (about 63%) at around 260-440°C. As the final temperature of the experiments was 600°C, the constant loss of weight at high temperature for untreated and treated leather sample showed similar trend. Therefore, it was found that chitosan incorporation doesn't degrade the thermal stability of treated leather.

### *3.6 Evaluation of physical and mechanical properties of leather samples*

In order to check whether the strength of the modified leather samples were changed significantly or not resulting in improved antimicrobial property, the leather samples L, L-CH 1% and L-CH 2% were subjected to different tests, i.e., tensile strength, stitch tear strength, simple tear strength, tear strength, water vapor permeability, perspiration fastness and dry/wet rub fastness.

#### *3.6.1 Tensile strength test*

The tensile strength test and percentage of elongation of leather samples L, L-CH 1% and L-CH 2% were shown in Table 4. The obtained results showed that incorporating chitosan increase the tensile strength test and percent of elongation.

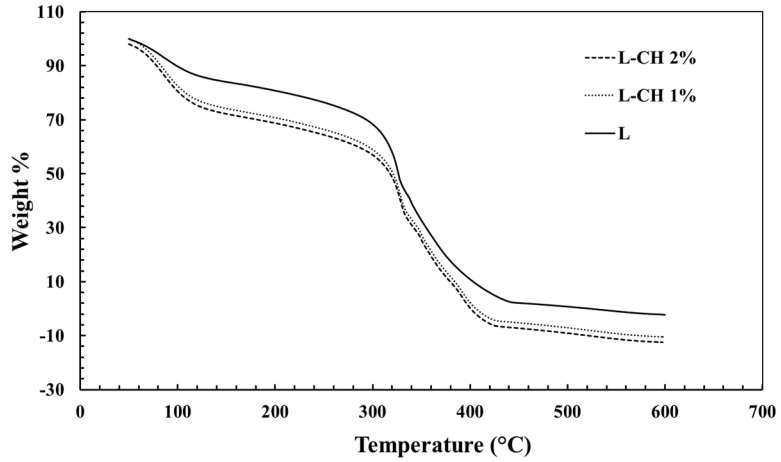



Fig. 8 TGA thermogram of chitosan treated and untreated leather samples

### *3.6.2 Stitch tear strength and simple tear strength*

The stich tear strength and simple tear strength tests were carried out to know the fiber strength of prepared leather samples. Table 4 revealed the higher stich tear strength and simple tear strength of chitosan coated leather than untreated leather.

### *3.6.3 Dry and wet rub fastness test*

The dry and wet rub fastness test was carried to evaluate the properties of the thin film produced due to coating of leather with chitosan and the results were shown in Table 5. The chitosan treated

Table 4 Physical strength of blank leather and chitosan coated leather

| Physical strength | L | L-CH 1% | L-CH 2% |
|---|---|---|---|
| Tensile strength(kg/cm$^2$) | 155 | 170 | 167 |
| % of elongation | 15 | 20 | 23 |
| Stich tear strength (kg/cm) | 80 | 100 | 115 |
| Simple tear strength (kg/cm) | 75 | 85 | 90 |

Table 5 Dry and wet rub fastness of blank and chitosan coated leather; gray scale rating (Cycle)

| Sample name | Test | Leather | | | Cotton felt | | |
|---|---|---|---|---|---|---|---|
| | | After 256 | After 512 | After 1024 | After 256 | After 512 | After 1024 |
| L | Dry rub fastness | - | 4/5 | 4/5 | - | 5 | 4 |
| L-CH 1% | | - | 4/5 | 4 | - | 4/5 | 4/5 |
| L-CH 2% | | - | 5 | 4 | - | 5 | 4 |
| L | Wet rub fastness | 4 | 3/4 | - | 3/4 | 3 | - |
| L-CH 1% | | 4/5 | 4 | - | 3/4 | 3/4 | - |
| L-CH 2% | | 4/5 | 4 | - | 3/4 | 3 | - |

Table 6 Water vapor permeability of blank and chitosan coated leather

| Sample name | Water vapor permeability (mg/cm$^2$-hr) |
|---|---|
| L | 2.1 |
| L-CH 1% | 2.1 |
| L-CH 2% | 2.08 |

leather showed good performance compared to blank leather. It appears that the color fastness property of the treated leather was not reduced for chitosan coating.

### *3.6.4 Water vapor permeability*

Water vapor permeability test was carried out to determine the water-vapor permeability of chitosan coated leather in order to pass through the vapor produced inside the shoe. Shoe lining leather should have better water vapor permeability for the comfortness of the user. The water vapor permeability of blank leather L, chitosan coated leather L-CH 1% and L-CH 2% compared in the Table 6. The results clearly indicate similar water vapor permeability of the chitosan coated leather as blank leather.

### *3.6.5 Perspiration fastness test*

This method determines the fastness to color change of leathers due to passing perspiration solution through it. The result of perspiration fastness test of the samples L, L-CH 1% and L-CH 2% were tabulated in Table 7. It indicates that the perspiration fastness properties of the leather were not reduced for coating with chitosan.

### *3.6.6 Shrinkage temperature*

One of the very important properties of the processed leather are resistant to shrinkage when subjected to moist heat. Raw hides or skins shrink very easily at temperatures of about 65°C, whereas chrome tanning increases its shrinkage temperatures up to a maximum of around 110°C. The shrinkage temperature determination for untreated leather (L) and chitosan treated leather L-CH 1% and L-CH 2% were tabulated in Table 8. From the results, it was found that the untreated leather shrinks at 106°C. After treating with chitosan solution, the treated leathers were shrinked at 108°C and 109°C. As a result, the shrinkage temperature of leather increased for treating with chitosan solution and it increases along with the increasing percentage of chitosan.

From the above described physical and mechanical test analysis, it was assumed that chitosan treated crust leather showed better physical and mechanical properties than blank leather.

Table 7 Perspiration fastness test of blank and chitosan coated leather (gray scale rating)

| | Sample | Cellulose | Cotton | Nylon | Polyester | Acrylic | Wool | Leather |
|---|---|---|---|---|---|---|---|---|
| L | Grain | 5 | 4 | 4 | 3/4 | 4 | 3/4 | 5 |
| | Flesh | 4 | 4/5 | 4 | 4/5 | 4 | 4 | 5 |
| L-CH 1% | Grain | 4 | 4/5 | 3 | 4/5 | 4/5 | 3/4 | 5 |
| | Flesh | 4/5 | 4/5 | 4 | 4/5 | 4/5 | 4 | 5 |
| L-CH 2% | Grain | 4/5 | 3/4 | 3/4 | 3/4 | 4 | 4/5 | 5 |
| | Flesh | 4 | 3 | 3/4 | 4 | 4 | 4 | 5 |

Table 8 Shrinkage temperature of blank and chitosan coated leather

| Sample name | Shrinkage temperature (°C) |
|---|---|
| L | 106 |
| L-CH 1% | 108 |
| L-CH 2% | 109 |

## 4. Conclusions

In this study, the antimicrobial activity of chitosan-coated crust leather was successfully achieved. Prepared chitosan was characterized by ATR-FTIR analysis, XRD pattern, SEM and thermogravimetric analysis. The leather surface was treated with chitosan solution using PVAc binder which helped to create a thin transparent film on the leather surface. The evaluation of antibacterial activity of chitosan revealed that the inhibition zone of chitosan solution (2% w/v, acetic acid) was 19.3 ± 1.58, 21 ± 0.53, 18.8 ± .63 and 20.81 ± 1.29 against *E. coli*, *S. aureus*, *B. subtilis and P. aeruginosa* respectively. The inhibition zone of leather treated with chitosan solution (2% w/v, acetic acid) was 9.6 ± 0.78, 11 ± 0.23, 11.8 ± 0.54 and 9.4 ± 0.78 against *E. coli*, *S. aureus*, *B. subtilis and P. aeruginosa* respectively. It is also found that chitosan-coated leather has antifungal activity against *A. niger*. The inhibition zone of chitosan solution (2% w/v, acetic acid) and leather treated with this solution against *A. niger* was 24 ± 0.89 and 10.7 ± 0.57 respectively. As higher concentration of chitosan has higher the inhibitory effect, the chitosan incorporation successfully inhibits both microbial and fungal growths on the finished leather surface. Since a very low concentration of chitosan is required to make the protective coating, this method can be employed industrially as a low-cost as well as environmentally friendly alternatives to inhibit bacterial and fungal attacks of finished leather product.

## Acknowledgments

The authors are grateful to the Center for Advance Research in Sciences (CARS) and Institute of Leather Engineering and Technology (ILET), University of Dhaka for the lab facility.

## Conflicts of interest

The authors declared that they have no conflict of interest.

## References

Ahmed, A.A., Sofy, A.R. and Sharaf, A.M.A. (2017), "Effectiveness of chitosan as naturally-derived antimicrobial to fulfill the needs of today's consumers looking for food without hazards of chemical preservatives", *J. Microbiol. Res.*, **7**, 55-67. https://doi.org/10.5923/j.microbiology.20170703.02

Aksoy, A. and Kaplan, S. (2013), "Production and performance analysis of an antibacterial foot sweat pad", *Fibers Polym.*, **14**(2), 316-323. https://doi.org/10.1007/s12221-013-0316-z

Ara, K., Hama, M., Akiba, S., Koike, K., Okisaka, K., Hagura, T., Kamiya, T. and Tomita, F. (2006), "Foot odor due to microbial metabolism and its control", *Can. J. Microbiol.*, **52**(4), 357-364.

https://doi.org/10.1139/w05-130
Aslan, A. (2013), "Improving the dyeing properties of vegetable tanned leathers using chitosan formate", *Ekoloji*, **22**, 26-35. https://doi.org/10.5053/ekoloji.2013.864
Bangyekan, C., Aht-Ong, D. and Srikulkit, K. (2006), "Preparation and properties evaluation of chitosan-coated cassava starch films", *Carbohydr. Polym.*, **63**(1), 61-71. https://doi.org/10.1016/j.carbpol.2005.07.032
Bañón, E., Marcilla, A., García, A.N., Martínez, P. and León, M. (2016), "Kinetic model of the thermal pyrolysis of chrome tanned leather treated with NaOH under different conditions using thermogravimetric analysis", *Waste Manage.*, **48**, 285-299. https://doi.org/10.1016/j.wasman.2015.10.012
Bieńkiewicz, K. (1981), *Physical Chemistry of Leather Making*, Krieger Publishing Company, Huntington, N.Y., USA.
Billah, S.M.R. (2018), "Textile Coatings", *Funct. Polym.*, 1-58.
Boahin, J.O.B., Asubonteng, K. and Adu-Gyamfi, V.E. (2013), "Sanative measures against offensive odour that affect indigenous tanned leathers in Ghana", *J. Sci. Technol.*, **33**(1), 68-74. https://doi.org/10.4314/just.v33i1.7
Burkinshaw, S.M. and Jarvis, A.N. (1996), "The use of chitosan in the dyeing of full chrome leather with reactive dyes", *Dyes Pigm.*, **31**(1), 35-52. https://doi.org/10.1016/0143-7208(95)00094-1
De Jesus, A.P.O., Roxas-Villanueva, R.L. and Herrera, M.U. (2017), "Antimicrobial and water-triggered release characteristics of a copper sulfate-polyvinyl acetate adhesive composite", *Proceedings of IOP Conference Series*: *Materials Science and Engineering*, **201**, 012007.
Dev, V.G., Venugopal, J., Sudha, S., Deepika, G. and Ramakrishna, S. (2009), "Dyeing and antimicrobial characteristics of chitosan treated wool fabrics with henna dye", *Carbohydr. Polym.*, **75**(4), 646-650. https://doi.org/10.1016/j.carbpol.2008.09.003
Dey, S.C., Al-Amin, M., Rashid, T.U., Sultan, M.Z., Ashaduzzaman, M., Sarker, M. and Shamsuddin, S.M. (2016), "Preparation, characterization and performance evaluation of chitosan as an adsorbent for remazol red", *Int. J. Latest Res. Eng. Tech.*, **2**, 52-62.
Doyle, B.B., Bendit, E.G. and Blout, E.R. (1975), "Infrared spectroscopy of collagen and collagen-like polypeptides", *Biopolymers*, **14**(5), 937-957. https://doi.org/10.1002/bip.1975.360140505
El-Tahlawy, K.F., El-Bendary, M.A., Elhendawy, A.G. and Hudson, S.M. (2005), "The antimicrobial activity of cotton fabrics treated with different crosslinking agents and chitosan", *Carbohydr. Polym.*, **60**(4), 421-430. https://doi.org/10.1016/j.carbpol.2005.02.019
Fernandes, I.P., Amaral, J.S., Pinto, V., Ferreira, M.J. and Barreiro, M.F. (2013), "Development of chitosan-based antimicrobial leather coatings", *Carbohydr. Polym.*, **98**(1), 1229-1235. https://doi.org/10.1016/j.carbpol.2013.07.030
Furtado, G.T.F.D.S., Fideles, T.B., Cruz, R.D.C.A.L., Souza, J.W.D.L., Rodriguez Barbero, M.A. and Fook, M.V.L. (2018), "Chitosan/NaF Particles Prepared Via Ionotropic Gelation: Evaluation of Particles Size and Morphology", *Mater. Res.*, **21**. https://doi.org/10.1590/1980-5373-mr-2018-0101
Günister, E., Pestreli, D., Ünlü, C.H., Atıcı, O. and Güngör, N. (2007), "Synthesis and characterization of chitosan-MMT biocomposite systems", *Carbohydr. Polym.*, **67**(3), 358-365. https://doi.org/10.1016/j.carbpol.2006.06.004
Islam, M.M., Masum, S.M., Rahman, M.M., Molla, M.A.I., Shaikh, A.A. and Roy, S.K. (2011), "Preparation of chitosan from shrimp shell and investigation of its properties", *Int. J. Basic Appl. Sci.*, **11**, 77-80.
Islam, M.N., Khan, M.N., Mallik, A.K. and Rahman, M.M. (2019), "Preparation of bio-inspired trimethoxysilyl group terminated poly(1-vinylimidazole)-modified-chitosan composite for adsorption of chromium (VI) ions", *J. Hazard. Mater.*, **379**, 120792. https://doi.org/10.1016/j.jhazmat.2019.120792
Jennings, M.B., Alfieri, D., Kosinski, M. and Weinberg, J.M. (1999), "An investigator-blind study of the efficacy and safety of azithromycin versus cefadroxil in the treatment of skin and skin structure infections of the foot", *The Foot*, **9**(2), 68-72. https://doi.org/10.1054/foot.1999.0521
Johannesson, A., Larsson, G.U., Ramstrand, N., Turkiewicz, A., Wiréhn, A.B. and Atroshi, I. (2009), "Incidence of lower-limb amputation in the diabetic and nondiabetic general population", *Diabetes Care*, **32**(2), 275. https://doi.org/10.2337/dc08-1639

Julkapli, N.M. and Md Akil, H. (2008), *X-Ray Powder Diffraction* (*XRD*) *studies on kenaf dust filled chitosan bio-composites*, Neutron and X-ray Scattering 2007.

Kaygusuz, M.K., Meyer, M. and Aslan, A. (2017), "The Effect of $TiO_2$-$SiO_2$ Nanocomposite on the Performance Characteristics of Leather", *Mater. Res.*, **20**(4), 1103-1110. https://doi.org/10.1590/1980-5373-mr-2017-0180

Kim, S., Kim, H.J., Choi, Y.M. and Jang, S.W. (2007), "Characteristics of non-plasticizer PVAc resin for wood products", *J. Korean Wood Sci. Technol.*, **35**(2), 61-68.

Kumar, M.R., Muzzarelli, R.A.A., Muzzarelli, C., Sashiwa, H. and Domb, A.J. (2004), "Chitosan chemistry and pharmaceutical perspectives", *Chem. Rev.*, **104**(12), 6017-6084. https://doi.org/10.1021/cr030441b

Liu, H., Zhao, Y., Cheng, S., Huang, N. and Leng, Y. (2012), "Syntheses of novel chitosan derivative with excellent solubility, anticoagulation, and antibacterial property by chemical modification", *J. Appl. Polym. Sci.*, **124**(4), 2641-2648. https://doi.org/10.1002/app.34889

Liu, G., Luo, Q., Wang, H., Zhuang, W. and Wang, Y. (2015), "In situ synthesis of multidentate PEGylated chitosan modified gold nanoparticles with good stability and biocompatibility", *RSC Adv.*, **5**(86), 70109-70116. https://doi.org/10.1039/C5RA11600G

Luo, Q., Gao, H., Peng, L., Liu, G. and Zhang, Z. (2016), "Synthesis of PEGylated chitosan copolymers as efficiently antimicrobial coatings for leather", *J. Appl. Polym. Sci.*, **133**(22). https://doi.org/10.1002/app.43465

Lv, S.H., Yan, X.L. and Gao, R.J. (2011), "Preparation and properties of copolymer of methacrylic acid and acrylamide onto degraded chitosan initiated by HRP/H2O2/ACAC", *Appl. Mech. Mater.*, **80**, 396-399. https://doi.org/10.4028/www.scientific.net/AMM.80-81.396

Marasigan, E.J.B., Roxas-Villanueva, R.M.L. and Herrera, M.U. (2019), "Antimicrobial property and water-triggered release characteristics of zinc sulphate-polyvinyl acetate adhesive blend", *Journal of Physics: Conference Series*, **1191**, 012036. https://doi.org/10.1088/1742-6596/1191/1/012036

Mark, H.F. (2014), *Encyclopedia of Polymer Science and Technology*, Wiley & Sons.

Mohanasrinivasan, V., Mishra, M., Paliwal, J.S., Singh, S.K., Selvarajan, E., Suganthi, V. and Devi, C.S. (2014), "Studies on heavy metal removal efficiency and antibacterial activity of chitosan prepared from shrimp shell waste", *3 Biotech*, **4**(2), 167-175. https://doi.org/10.1007/s13205-013-0140-6

Monvisade, P. and Siriphannon, P. (2009), "Chitosan intercalated montmorillonite: Preparation, characterization and cationic dye adsorption", *Appl. Clay Sci.*, **42**(3), 427-431. https://doi.org/10.1016/j.clay.2008.04.013

Muzzarelli, R.A.A. (1977), Chapter 1 – Enzymic synthesis of chitin and chitosan, In: *Chitin*, (R.A.A. Muzzarelli), Pergamon, pp. 5-44.

Muzzarelli, R., Tarsi, R., Filippini, O., Giovanetti, E., Biagini, G. and Varaldo, P.E. (1990), "Antimicrobial properties of N-carboxybutyl chitosan", *Antimicrob. Agents Chemother.*, **34**(10), 2019-2023. https://doi.org/10.1128/AAC.34.10.2019

Muzzarelli, R.A., Boudrant, J., Meyer, D., Manno, N., DeMarchis, M. and Paoletti, M.G. (2012), "Current views on fungal chitin/chitosan, human chitinases, food preservation, glucans, pectins and inulin: A tribute to Henri Braconnot, precursor of the carbohydrate polymers science, on the chitin bicentennial", *Carbohydr. Polym.*, **87**(2), 995-1012. https://doi.org/10.1016/j.carbpol.2011.09.063

Nagai, K., Domon, H., Oda, M., Shirai, T., Ohsumi, T., Terao, Y. and Arai, Y. (2017), "Antimicrobial activity of ethylene-vinyl acetate containing bioactive filler against oral bacteria", *Dent. Mater. J.*, **36**(6), 1881-1361. https://doi.org/10.4012/dmj.2016-321

Nieto, J.M., Peniche-Covas, C. and Padro, G. (1991), "Characterization of chitosan by pyrolysis-mass spectrometry, thermal analysis and differential scanning calorimetry", *Thermochim. Acta*, **176**, 63-68. https://doi.org/10.1016/0040-6031(91)80260-P

Orlita, A. (2003), "Microbial biodeterioration of leather and its control: a review", *Int. Biodeterior. Biodegrad.*, **53**(3), 157-163. https://doi.org/10.1016/S0964-8305(03)00089-1

Padmanabhan, S.C., Hasim, S.A., Romero, M.C., Kerry, J. and Morris, M.A. (2015), "The modification of poly vinyl acetate (PVA) substrates and their improved antimicrobial activity for use in active packaging applications", *J. Food Process. Technol.*, **6**(9).

Paris, J. (2000), "Adhesives for paper, board and foils", *Int. J. Adhes. Adhes.* **20**(2): 89-90.

Payne, K.J. and Veis, A. (1988), "Fourier transform IR spectroscopy of collagen and gelatin solutions: Deconvolution of the amide I band for conformational studies", *Biopolymers*, **27**(11), 1749-1760. https://doi.org/10.1002/bip.360271105

Plavan, V. (2012), "Chrome tanning improvement by chitosan application", *J. Soc. Leather Tech. Chemi.*, **96**, 89-93.

Qu, X., Wirsen, A. and Albertsson, A.C. (2000), "Effect of lactic/glycolic acid side chains on the thermal degradation kinetics of chitosan derivatives", *Polym.*, **41**(13), 4841-4847. https://doi.org/10.1016/S0032-3861(99)00704-1

Rebsamen, W. (1983), *Adhesive Binding Library Books*, Mekatronics Inc., NY, USA.

Rinaudo, M. (2006), "Chitin and chitosan: Properties and applications", *Prog. Polym. Sci.*, **31**(7), 603-632. https://doi.org/10.1016/j.progpolymsci.2006.06.001

Sánchez-Navarro, M.M., Cuesta-Garrote, N., Arán-Aís, F. and Orgilés-Barceló, C. (2011), "Microencapsulation of Melaleuca alternifolia (Tea Tree) Oil as Biocide for Footwear Applications", *J. Dispersion Sci. Technol.*, **32**(12), 1722-1727. https://doi.org/10.1080/01932691.2011.616126

Santos, F.K.G.D., Silva, K.N.D.O., Xavier, T.D.N., Leite, R.H.D.L. and Aroucha, E.M.M. (2017), "Effect of the addition of carnauba wax on physicochemical properties of chitosan films", *Mater. Res.*, **20**, 479-484. https://doi.org/10.1590/1980-5373-mr-2016-1010

Šedivka, P., Bomba, J., Böhm, M., and Boška, P. (2015), "Influence of temperature on the strength of bonded joints", *BioResources*, **10**(3), 3999-4010.

Sirvaityte, J., Siugzdaite, J. and Valeika, V. (2011), "Application of commercial essential oils of eucalyptus and lavender as natural preservative for leather tanning industry", *Rev. Chim.* (*Bucharest*, *Rom.*), **62**, 884-893.

Sousa, L.L.D., Ricci, V.P., Prado, D.G., Apolinario, R.C., Vercik, L.C.D.O., Rigo, E.C.D.S., Fernandes, M.C.D.S. and Mariano, N.A. (2018), "Titanium coating with hydroxyapatite and chitosan doped with silver nitrate", *Mater. Res.*, **20**(suppl 2), 863-868. https://doi.org/10.1590/1980-5373-mr-2017-0021

Stockman, G., Didato, D. and Hurlow, E. (2007), "Antibiotics in hide preservation and bacterial control", *J. Am. Leather Chem. Assoc.* **102**, 62-67.

Tseng, H.J., Hsu, S.H., Wu, M.W., Hsueh, T.H. and Tu, P.C. (2009), "Nylon textiles grafted with chitosan by open air plasma and their antimicrobial effect", *Fibers Polym.*, **10**(1), 53-59. https://doi.org/10.1007/s12221-009-0053-5

Velmurugan, P., Cho, M., Lee, S.M., Park, J.H., Bae, S. and Oh, B.T. (2014), "Antimicrobial fabrication of cotton fabric and leather using green-synthesized nanosilver", *Carbohydr. Polym.*, **106**, 319-325. https://doi.org/10.1016/j.carbpol.2014.02.021

Vichi, A., Eliazyan, G. and Kazarian, S.G. (2018), "Study of the degradation and conservation of historical leather book covers with macro attenuated total reflection–Fourier transform infrared spectroscopic imaging", *ACS Omega*, **3**(7), 7150-7157. https://doi.org/10.1021/acsomega.8b00773

Wang, S.F., Shen, L., Tong, Y.J., Chen, L., Phang, I.Y., Lim, P.Q. and Liu, T.X. (2005), "Biopolymer chitosan/montmorillonite nanocomposites: Preparation and characterization", *Polym. Degrad. Stab.*, **90**(1), 123-131. https://doi.org/10.1016/j.polymdegradstab.2005.03.001

Widrow, C.A., Kellie, S.M., Saltzman, B.R. and Mathur-Wagh, U. (1991), "Pyomyositis in patients with the human immunodeficiency virus: An unusual form of disseminated bacterial infection", *Am. J. Med.*, **91**(2), 129-136. https://doi.org/10.1016/0002-9343(91)90004-H

Xu, J., McCarthy, S.P., Gross, R.A. and Kaplan, D.L. (1996), "Chitosan film acylation and effects on biodegradability", *Macromol.*, **29**(10), 3436-3440. https://doi.org/10.1021/ma951638b

Yakimets, I., Wellner, N., Smith, A.C., Wilson, R.H., Farhat, I. and Mitchell, J. (2005), "Mechanical properties with respect to water content of gelatin films in glassy state", *Polym.*, **46**(26), 12577-12585. https://doi.org/10.1016/j.polymer.2005.10.090

Zhang, A.J., Qin, Q.L., Zhang, H., WANg, H.T., Li, X., Miao, L. and Wu, Y.J. (2011), "Preparation and characterisation of food-grade chitosan from housefly larvae", *Czech J. Food Sci.*, **29**, 616-623.