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Development of Antibacterial Nano-cellulose – Chitosan Films Activated with Nisin for Food and Medicine Application

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ABSTRACT

In this research, biodegradable Chitosan–Nano-Cellulose–Nisin (CH-NC-N) film was synthesized and utilized for antibacterial application in medicine and food packaging. The antibacterial chitosan–nano-cellulose–nisin film was characterized using various techniques such as mechanical and physical properties analysis, Scanning Electron Microscopy (SEM), X-Ray Diffraction (XRD), and Fourier Transform Infrared Spectroscopy (FTIR) techniques. The film's ability to inhibit growth of pathogenic bacteria including *Escherichia coli*, *Escherichia coli XDR, Klebsiella pneumonia XDR, Listeria monocytogenes, and Staphylococcus aureus* was examined. Furthermore, the film was used for meat packaging at a temperature of 4°C for a duration of 26 days. Data analysis revealed an improvement in the mechanical properties and water absorption of the film following the addition of nano-cellulose and nisin. The presence of nisin in the CH-CN film was confirmed through analysis of FTIR, XRD, and SEM data. Antimicrobial analysis of film determined the high potential of nisin as an antimicrobial agent in CH-CN-N film. Compared to the control, the CH-CN-N film successfully inhibited the growth of spoilage bacteria in meat for 26 days. Additionally, the sensory properties of meat packaged with this film were minimally affected. These results indicate that the chitosan-nano-cellulose-nisin film is suitable for utilization in food systems and medical applications.

Keywords: Antibacterial activity, Biocomposite, Food packaging, Shelf life of food, Spoiled meat.

INTRODUCTION

Preventing contamination by microorganisms and increasing the shelf life of food have become an essential issue due to the increase in world population and the globalization of the food market (Pirsa and Asadi, 2021). The utilization of spoiled meat or canned food endangers human health and can lead to hospitalization or even death. Antimicrobial packaging has attracted much interest among scientists and industry because of its capacity to prevent the growth of microorganisms on the surface of food products. However, directly applying antimicrobials to the surface of food could help limit the effects caused by the movement of effective ingredients into the food tissue. A severe lack of antimicrobial action might also appear because of the reaction or deactivation of the effective ingredients by food ingredients (Huq *et al.*, 2014; Khan *et al.*, 2016). Bioactive technology offers another resolution to limit bacterial growth in food products. In this type of packaging, inhibition of bacterial growth is possible by the targeted release of bioactive ingredients from the film substance to the food product overlays. The controlled released packaging is a novel group of effective ingredients that develop food safety and

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quality throughout storage (Salmieri *et al.*, 2014). The synthetic polymers are utilized in food packaging because of their exceptional thermal-mechanical, economic, and protective barrier properties. Today, it is known that synthetic polymers used in food packaging have compounds that are very dangerous for human health and the environment. Synthetic polymers cause serious damage to the environment due to their long decomposition time. Therefore, a serious problem in the world is the wide use of synthetic polymers in food packaging (Dehnad *et al.*, 2014a). However, the interest in using biodegradable biopolymers is expanding, especially in food packaging (Pirsa, 2021). However, application of natural polymers in food packaging is limited due to their inadequate mechanical and barrier properties. Therefore, degradable natural polymers are in high demand for fillers that enhance their mechanical and barrier properties. They also need bioactive ingredient that increase their antibacterial properties (Baysal *et al.*, 2023; Meydanju *et al.*, 2022; Pirsa and Mohammadi, 2021). Additionally, due to increasing consumer demand for the use of renewable materials in the production of biodegradable packaging films, many researchers in textile chemistry and fiber science have begun replacing petroleum-derived synthetic materials with environmentally friendly alternatives. (Meydanju *et al.*, 2022; Shabkhiz *et al.*, 2021). Bio-nanocomposites can be introduced as a new group of films containing a polymer matrix strengthened with nanofiber. Nano-cellulose reinforced films have attracted much interest today due to their great capacity in food packaging and their renewable nature (Lu *et al.*, 2021; Pirsa and Mohammadi, 2021). Chitosan, is the most abundant polymer after cellulose in nature. Chitosan, because of its biocompatibility, degradability, and harmless properties, has been used in food packaging (Baysal *et al.*, 2022; Khan *et al.*, 2016; Khan *et al.*, 2012). Nisin is a bacteriocin generated by *Lactococcus lactis* strains and is generally regarded as a safe substance by the USA FDA (Gedarawatte *et al.*, 2021; Huq *et al.*, 2014; Khan *et al.*, 2016). The present study aimed to provide the antimicrobial film of CH-NC-N for the first time. Loading CH-NC by nisin can prepare antimicrobial active CH-NC. The mechanical and wettability characteristics of the film were investigated. The film was characterized by FTIR, XRD, and SEM. The antimicrobial activity was evaluated against spoilage and pathogenic bacteria.

MATERIALS AND METHODS

Nano-Cellulose Synthesis

Nano-cellulose was prepared by acid hydrolysis from microcrystal cellulose (MCC, Sigma–Aldrich, USA). Quickly, sulfuric acid solution (95-97% Merck, Germany) (64 v/v%) was added to the aqueous MCC suspension (10 g 100 mL^{-1}) and kept at 44°C for two hours. The resulting suspension was centrifuged at 14,000 rpm for 15 minutes. The precipitate was then washed several times with distilled water to neutralize its acidity, and then sonicated (Chrom Thech Ultrasonic Processor, Korea) for 30 min in an ice bath and dried with a freeze dryer (Celebi and Kurt, 2015; García *et al.*, 2017). The nano-cellulose was characterized by XRD (Bruker, D8 Advance, Germany), FTIR (Bruker, Tensor27, Germany), and Dynamic light scattering (DLS) (Brookhaven, 90plus/BI-MAS USA) techniques.

Antimicrobial Films Synthesis

Chitosan (medium molecular weight, deacetylation degree 75–85%, Sigma–Aldrich, USA), solution with different concentrations (1, 1.5, and 2%, w/v) in 1% acetic acid (100% Merck, Germany) was prepared for 24 h. Different concentrations of NCs (0.5, 0.65, 0.8, and 1 w/v%) were added to the chitosan solution and mixed for 30 min. Then, this solution was sonicated in an ice bath for 15 min. Finally, 0.6% (v/v) glycerol was added to the solutions and homogenized according to the above-mentioned conditions. Then, the solutions were poured into glass plates and

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dried at 30 °C for 20 h. Finally, after several times optimization- the film with 1.5% (w/v) chitosan, 0.65 (w/v) nano-cellulose, and 0.6% (v/v) glycerol (Merck, Germany) were selected for antimicrobial films synthesis (Celebi and Kurt, 2015; Dehnad *et al.*, 2014a; Dehnad *et al.*, 2014b). Nisin (Sigma–Aldrich, USA)–EDTA (Merck, Germany) mixture (60 or 30 μg mL^{-1} of nisin, and 30 mM of disodium ethylene diamine tetraacetate) was added to the CH-NC solution and homogenized according to the above-mentioned conditions. Then, the solutions were poured into glass plates and dried at 30 °C for 20 h (Celebi and Kurt, 2015; Dehnad *et al.*, 2014b).

Nanocomposite Film Characterization

SEM images of film surfaces were obtained with a MIRA III Tescan (Czechia) microscope. The samples were covered by a thin gold layer, then, their images were determined at an accelerating voltage of 10 to 20 kV at different magnifications. The X-Ray pattern extents were prepared with a Philips PW1730 diffractometer by Cu K_ radiation. The organic groups on the nanocomposite films were determined by FTIR-ATR (Fernandes *et al.*, 2010).

Air–Water Contact Angle Investigation

A drop of deionized water was placed on the surface of the films. Water droplet images on the surface of films were recorded by a digital camera (Sony, Model F707). The images were managed with Adobe Photoshop 6.0 software to gain contact angle information (Leceta *et al.*, 2013).

Mechanical Characteristics

The film mechanical characteristics were determined by a Sherli Micro350 (UK) under the ASTM manner D882. Primary grip departure and crosshead speed were adjusted at 50 mm and 5 mm/min, respectively. Young's Modulus (YM), and Tensile Strength (TS) were determined from curves of Stress-Strain (Szymańska-Chargot *et al.*, 2019).

Antibacterial Activity

The agar diffusion procedure was applied for defining antibacterial activity. One-centimeter squares (1 cm^2) were prepared from the films. Films were disposed on TSA (Tryptic Soy Agar, Merck, Germany), which was previously inoculated separately with 10^6 CFU mL^{-1} of *Escherichia coli PTCC1394*, *Staphylococcus aureus PTCC1431, Listeria monocytogenes PTCC1074, Escherichia coli XDR,* and *Klebsiella pneumonia XDR (from Milad laboratory, Yazd, Iran)*, respectively. Plates took place for 24 hours at 37˚C. The inhibition zone round samples were applied to determine the antimicrobial activity (Dehnad *et al.*, 2014a; Firouzabadi *et al.*, 2014). Also, CH-NC-N film was employed in a TSB (Tryptic Soy Broth, Merck, Germany) medium and inoculated separately with 10^7 CFU mL^{-1} of *L. monocytogenes*, *E. coli*, *S. aureus E. coli XDR,* and *K. pneumonia XDR*, respectively. The tubes were incubated at 25±1°C at a speed of 50 rpm. Samples (1 mL) were subsequently collected from the tested tubes over a period of 24 hours. Dilution series were prepared from these samples (10^{-1} to 10^{-9}) and cultured on TSA plates 37°C for 24 hours (Mirhosseini and Afzali, 2016).

Shelf-Life Investigation in Raw Meat

Freshly slaughtered meat was purchased from a butcher near Payam Noor University (Yazd, Iran). The meat sample was disinfected by immersing it in 70% ethanol and passing it through a flame. The 10 gr slabs of purified meat sample were wholly packaged with CH, CH-NC, CH-NC-N, or nylon films. The packaged meat samples were then placed on separate plates and

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stored for 26 days at 4°C. A physical and microbiological investigations were performed on days 1, 3, 5, 13, 20, and 26. Physical assessment of the meat was accomplished to evaluate its shelf-life operating a standardized method for color and odor. The meat samples were managed for bacterial counts using a standard method. One gram of sterile packaged meat was removed each time and placed in a 400 mL homogenization package containing 9 mL of 0.1% (w/v) bacto-peptone (Merck, Germany) and massaged with a Stomacher 400 laboratory blender (Seward, UK) at high speed for 120 sec. Suitable 10-fold dilutions of the homogenate were spread plated onto TSA and MRSA (DE MAN, ROGOSA, and SHARPE Agar, Mercke, Germany), for enumeration of the whole bacteria and Lactic Acid Bacteria (LAB). Then, plates were incubated at 25˚C for 72 hours before counting bacteria (Dehnad *et al.*, 2014b; Mirhosseini and Arjmand, 2014).

Statistical Analysis

All experiments were accomplished in triplicate. Data presented are the average of three replicates. The information were exposed to one-way Analysis Of Variant (ANOVA) by an SPSS software (SPSS Statistic 19.0). Post hoc multiple comparisons were defined by the Tukey's test with the significance level set at $P< 0.05$. SigmaPlot 12.3 and Excel 2017 software were used to draw curves and charts.

RESULTS

Nano-Cellulose Characteristics

The size of NCs was evaluated by DLS analysis. Data analysis showed that the particle size ranged from 16.1-101.2 nm, and the mean particle size was 40.76 nm (Figure 1). The XRD pattern of NC displayed peaks about nearby 2θ= 15, 16.5, and 22.5° designated as the crystal planes with Miller indications of -110, 110, 200, which signify the standard cellulose-I construction (Figure 2) (Celebi and Kurt, 2015). The FTIR spectra of C and NC presented an extensive band in the area "3,500–3,200" cm^{-1} that illustrated the free "O–H" stretching vibration of the "OH" groups in cellulose molecules. In addition, the spectra result of all samples indicated the specific "C–H" stretching vibration around "2,894" cm^{-1}

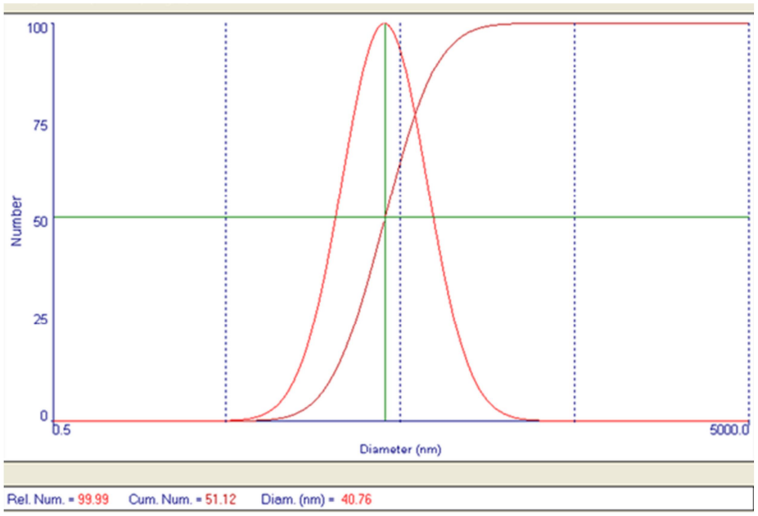

Figure 1. Light scattering diagram of nano-cellulose particles.

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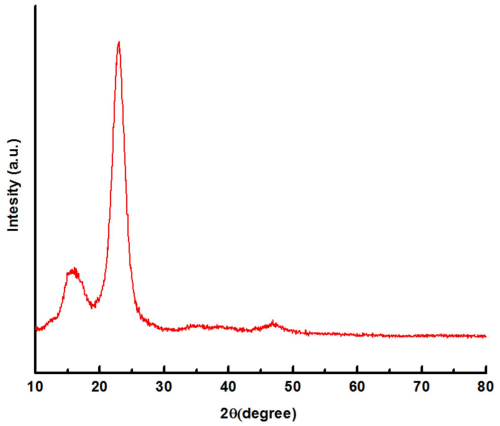

Figure 2. Investigation of X-Ray diffraction pattern of nano-celluloses.

(Celebi and Kurt, 2015; Khalil *et al.*, 2001). Moreover, the maximum vibration observed at "1,365" cm^{-1} in all samples originated from the "C–O" and "C–H" bond bending vibration in the polysaccharide aromatic rings (Celebi and Kurt, 2015; Le Troedec *et al.*, 2008). The peak studied in the spectra of total samples located at the 1,054 cm^{-1} point is because of the "C–O–C" stretching vibration in the pyranose ring. The greatest big absorption band that frequently enhances NC is that of "902" cm^{-1}, which withstands cellulose II, the matter of which gradually enhances from cellulose to nano-cellulose (Pappas *et al.*, 2002). The "C–O–C" glycosidic ether band at "1,105" cm^{-1} and "C–C" ring breathing band at ~"1,155" cm^{-1} of them result from the polysaccharide constituent is becoming progressively missing in nano-cellulose due to hydrolysis, and decreasing in the molecular weight (Figure 3) (Garside and Wyeth, 2003).

Chitosan/Nano-cellulose/Nisin Film Characterization

The XRD spectra of CH and CH-NC and CH-NC-N films presented typical sharp peaks at around 2θ= 8° and 2θ= 11° and a broad halo at 2θ= 20°. The two sharp peaks showed a hydrous crystalline construction,

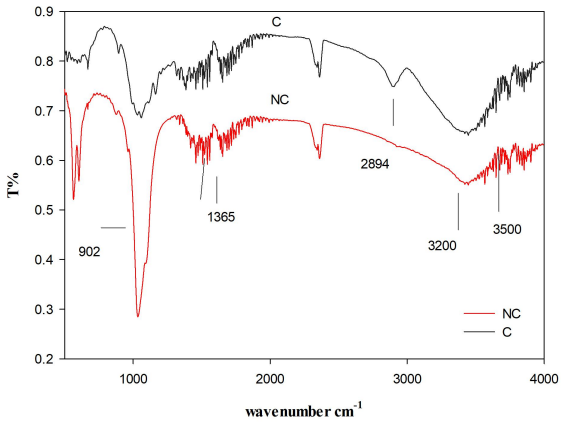

Figure 3. Infrared spectroscopy of cellulose and cellulose nanoparticles.

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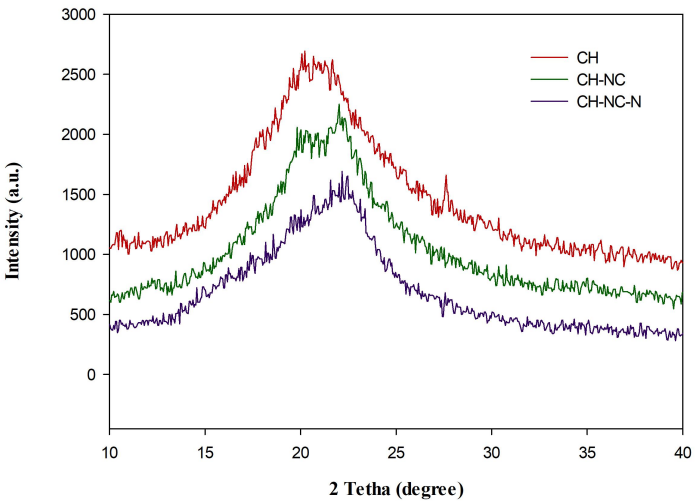

Figure 4. XRD photographs for chitosan–nano-cellulose- nisin biocomposite.

while the broad halo peak designated an amorphous construction of chitosan (Dehnad *et al.*, 2014a). The diffraction diagram for NC displayed three significant peaks at 2θ= 15, 16.5, and 22.5 degrees, which were assigned to [101], [101], and [002] atomic planes of cellulose I, respectively. The typical peak of NC at 2θ= 22.5° showed on the XRD pattern of CH-NC and CH-NC-N nanocomposite films. Also, the peak strength amplifies with the addition of NC in the film as a result of the trans-crystallization effect, which is described as the orientation of crystals of a semicrystalline matrix perpendicularly to the nano-celluloses (Celebi and Kurt, 2015; Dehnad *et al.*, 2014b). When, the chitosan peak is compared with the nanocomposite film peak, it could be observed that adding NCs and nisin replaced the situation of the chitosan peak to the more significant positions (nearby to the bounds of the NC peak), but reduced the peak strength and the spaces among the sheets (Figure 4) (Dehnad *et al.*, 2014b).

The FTIR pattern of the CH films is essentially transferrable to the stretching of intra- and intermolecular "OH" and "CH_2OH" vibrations at "3,500–3,250 cm^{-1}", overlayed with stretching "NH_2" (3,500–3,400 cm^{-1}) and "NH" secondary amides vibrations (3,300–3,280 cm^{-1}). Also, "2,960–2,870" cm^{-1} agrees to asymmetric and symmetric "CH" vibrations (Khan *et al.*, 2012). Amide I's vibrational peak at "1633" cm^{-1} and, moreover, amide II at "1,538" cm^{-1} have been detected. Due to the incorporation of NC into the chitosan, particular alterations can be noticed in the FTIR pattern of CH films. An intense peak showed at "3,342" cm^{-1}, not displayed in the control CH films. Moreover, the strength of the peak "3,342" cm^{-1} raised hydrogen bonding between CH and NC. Other bands at "1,538" and "1,340" cm^{-1} had their intensity increased after NC addition. Moreover, there was an extreme amplification in the strength of the absorption peaks at "1,054" and "1,032" cm^{-1} because of NC integration. Nevertheless, other variations created by the supplement of NC are minimal, as guessed from the small quantity of NC combined to formulate the CH-NC films (Khan *et al.*, 2012). Several changes can be identified in the full FTIR spectrum after adding nisin to the CH-NC film. The absorption peak was observed at "3,150–3,450" cm^{-1}, following the addition of nisin. This peak is due to the "OH" band of the peptides. Other bands seen with the addition of

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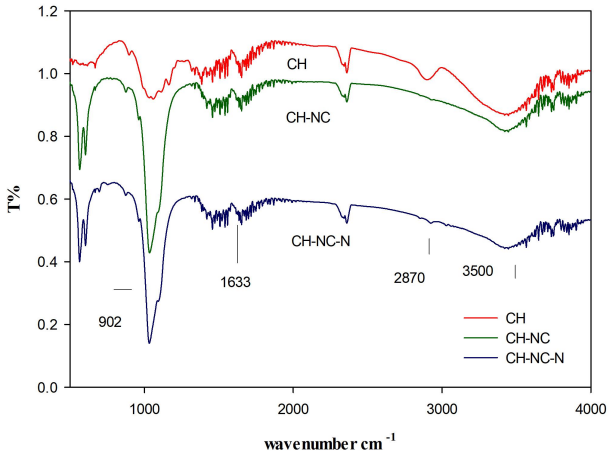
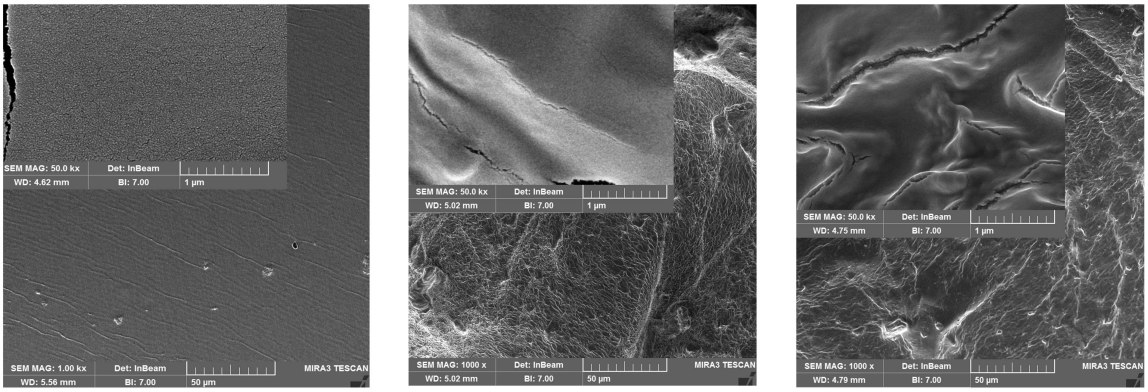


Figure 5. Infrared spectroscopy of Chitosan (CH), Chitosan-Nano-Cellulose (CH-NC), and Chitosan-Nano-Cellulose –Nisin (CH-NC-N) films.

nisin are: at "1,656", and "1,537" cm^{-1}, which are mainly related to the amide I and amide II bands, respectively, and both are related to the peptide bond (Figure 5) (Salmieri *et al.*, 2014).

The chitosan SEM image (Figure 6-a) indicates a relatively smooth surface without phase separation, signifying complete chitosan dissolution in the acidic solution. By adding nano-cellulose to the chitosan film, NCs shows as white areas in the CH film. The nanocomposite film has shown a dense and uniform structure, which is indicative of proper distribution and uniform distribution of nano-celluloses. It also shows strong adhesion between nano-celluloses and chitosan in the nanocomposite film (Figure 6-b) (Celebi and Kurt, 2015). With the addition of nisin to nanocomposite films, the structure of the film has become more uniform and homogeneous (Figure 6-c).

Data analysis showed that the contact angle was around 86.16 for chitosan film. However, with the addition of NC, the value of the contact angle (81.71) was reduced. The addition of nisin to the nanocomposite reduced the contact angle (76.88) (Figure 7).



A: CH B: CH-NC C: CH-NC-N

Figure 6. SEM of Chitosan (CH), Chitosan-Nano-Cellulose (CH-NC), and Chitosan-Nano-Cellulose–Nisin (CH-NC-N) films.

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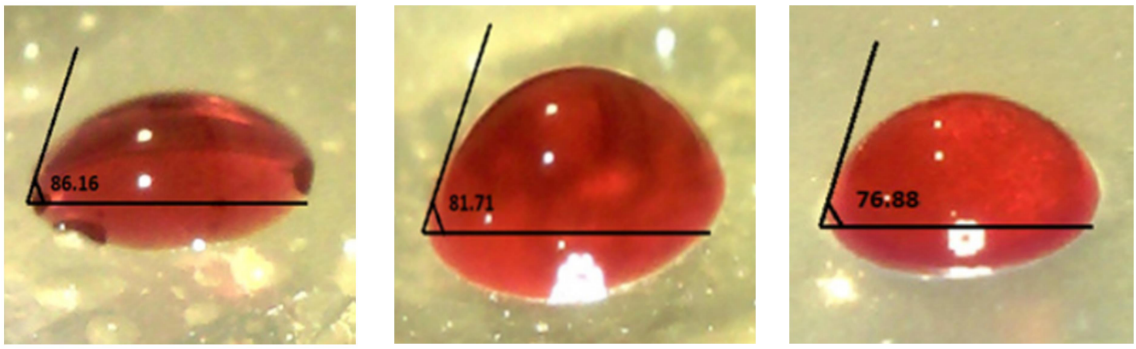

A: CH B: CH-NC C: CH- NC-N

Figure 7. Contact angle values of Chitosan (CH), Chitosan-Nano-Cellulose (CH-NC), and Chitosan-Nano-Cellulose–Nisin (CH-NC-N) films.

Mechanical properties of films show that by applying a force of up to 56.67 N, the elongation to breakpoint reached 14.78 mm, and with more force, the film tore. Adding nano-cellulose and nisin to the chitosan film increases the tensile strength and length to a rupture point of 1.9 to 14.78 mm. The YM also increases from 2.178 to 39.172 MPa, indicating that the tensile strength of the CH-NC-N film is higher than that of the control film (Figure 8).

Antibacterial Assay in Medium

The results of the investigation of the antimicrobial property in the solid medium are presented in Table 1. Data analysis showed that the films prepared with 30 µg mL^{-1} of nisin-nano-cellulose-chitosan had no antimicrobial effect on any of the tested bacteria. By increasing the nisin concentration to 60 µg mL^{-1}, the nanocomposite film had an antimicrobial effect on all the tested bacteria.

In this research, CH-NC-N film had inhibitory activity on *S. aureus*, *L. monocytogenes*, *E. coli*, *E. coli XDR,* and *K. pneumonia XDR* in a liquid medium. The number of colonies of *S. aureus*, *L. monocytogenes, E. coli*, and *K. pneumonia XDR* in the medium containing CH-NC-N film are drastically reduced ($P \leq 0.05$). The results also showed that adding nisin and nano-cellulose to the chitosan film reduced the growth of *E. coli XDR* ($P \geq 0.05$) (Figure 9).

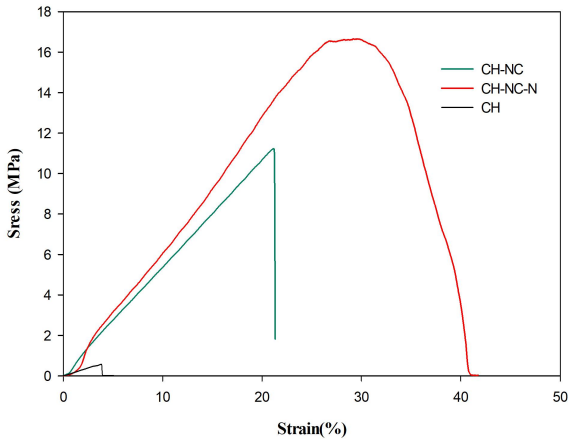

Figure 8. Mechanical properties of Chitosan (CH), Chitosan-Nano-Cellulose (CH-NC), and Chitosan-Nano-Cellulose–Nisin (CH-NC-N) films.

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Table 1. Inhibition zone of agar disc diffusion tests for chitosan-nano-cellulose-nisin biocomposites.

Strains	Treatment				
	Control (mm)	Chitosan (mm)	Chitosan - Nano-cellulose (mm)	Chitosan-Nano-cellulose-Nisin (30 µg mL^{-1} of nisin) (mm)	Chitosan-Nano-cellulose-Nisin (60 µg mL^{-1} of nisin) (mm)
Escherichia coli	-[a]	-	-	-	20±0
Staphylococcus aureus	-	-	-	-	20±0
Escherichia coli XDR	-	-	-	-	20±0
Klebsiella pneumonia XDR	-	-	-	-	30±0.1
Listeria monocytogene	-	-	-	-	19.5±0.2

[a] -: No inhibition zone.

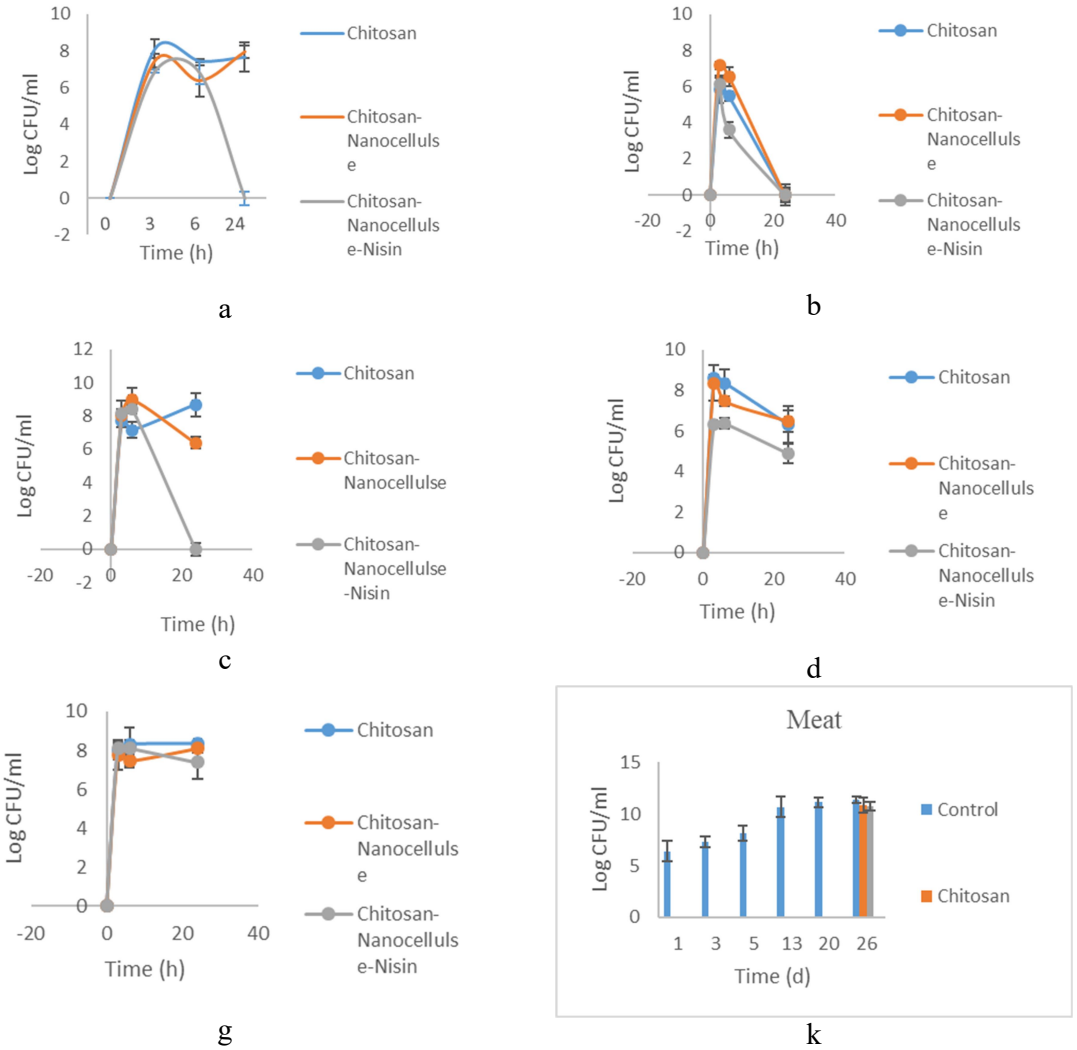

Figure 9. Inhibitory effect of chitosan-nano-cellulose nanocomposite against bacteria in liquid medium and bacteria initially present on meat samples. a: *S. aureus,* b: *L. monocytogenes*, c: *E. coli*, d: *E. coli XDR, g: Klebsiella pneumonia* XDR, and k: Meat.

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Table 2. Discoloration and putrid odor scores over time for packaged meat samples.[a]

Treatment	Days						
	0	1	3	5	13	20	26
Control	-	±	+	+	+	++	+++
Chitosan	-	-	-	-	-	±	+
Chitosan - Nano-cellulose	-	-	-	-	-	±	+
Chitosan-Nano-cellulose-Nisin	-	-	-	-	-	-	-

[a] -: No discoloration and no putrid odor, +: No discoloration and putrid odor.

Shelf-Life Invistigatin in Raw Meat

The results showed that after 26 days, only the CH-NC-N film prevented the growth of bacteria and preserved the meat sample compared with the control ($P \leq 0.05$). Also, the results showed that no LABs were isolated from any of the samples during the 26 days (Figure 9).

Furthermore, on the 26th day, small discoloration and a slight off-odor were observed in the packaged meat samples, indicating the initiation of spoilage (Table 2).

DISCUSSION

Newly, nano-cellulose composite packaging materials with antimicrobial activity are an excitable subject in a recent investigation. A nanocomposite packaging material prepared from natural antimicrobials, chitosan, or nano-cellulose has the possibility to be widely operated in the food manufacturing (Dehnad *et al.*, 2014a; Dehnad *et al.*, 2014b; Lu *et al.*, 2021). However, we can investigate more into the combination of chitosan, and nano-cellulose with other antimicrobial agents.

In this research, the CH-NC-N biocomposite with 30 μg mL^{-1} of nisin had no inhibitory effect on the tested bacteria. By increasing the nisin concentration to 60 μg mL^{-1} due to the increase in the release rate of nisin in the culture medium, they showed an inhibitory effect on all tested bacteria. Ce *et al*. (2012) stated the antibacterial activity of chitosan films, including peptide p34, nisin, and natamycin, on *L. monocytosis, S. aureus, E. coli, B. cereus*, and *L. acidophilus*. These films showed excellent antimicrobial properties against all tested bacteria (Cé *et al.*, 2012). The nisin penetrates the bacterium's cytoplasm and creates a hole in this membrane, which causes essential substances to leak out of the cell, which finally leads to bacterial death (Gedarawatte *et al.*, 2021). Nisin displays an antibacterial effect versus a wide variety of Gram-positive bacteria, but has slight activity versus fungi, yeasts, and Gram-negative bacteria (Gedarawatte *et al.*, 2021; Khan *et al.*, 2016). It is also able to kill such microorganisms in combination with other antimicrobials. Therefore, in this study, EDTA was used together with nisin. The combination of nisin and EDTA causes the inhibitory effect of the produced nanocomposite on Gram-negative bacteria. When EDTA is added to the environment, 30-50% of lipopolysaccharide and other proteins of the outer membrane of Gram-negative bacteria are released immediately. Oxidation of magnesium and calcium ions in the lipopolysaccharide layer of the bacterial outer membrane also causes instability of the lipopolysaccharide layer and increases cell permeability. Therefore, the access of nisin to the cytoplasmic membrane in Gram-

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negative bacteria is facilitated and it causes the permeability and leakage of substances from the cytoplasmic membrane and, finally, cell death. Khan *et al*. (2016) described that a nisin and EDTA formulation had a synergistic influence versus *E. coli* and *L. monocytogenes*.

In the present study, nanocomposite containing nisin was used to evaluate the antimicrobial effect on the meat. The results indicated that only the nanocomposite containing nisin effectively inhibited bacterial growth and preserved the meat sample after 26 days.

Salmieri *et al*. (2014) conducted a study on the inhibition of *L. monocytogenes* in hams cooked with nanocomposites made from polylactic acid–nano-cellulose–nisin. Their results determined nisin as a robust antimicrobial agent in the produced nanocomposite.

Treatment of the ground meat by chitosan–nano-cellulose composite reduced lactic acid bacteria compared with nylon-packaged ground meat at 3 and 25°C afterward six days of storing, respectively (Dehnad *et al.*, 2014b). Pattanayaiying *et al*. (2015) incorporated lauric arginate and nisin Z in pullulan films. The findings from this report designate the LAE- and LAE–nisin Z-containing pullulan films exhibited outstanding inhibition versus foodborne bacteria on fresh and extra-managed muscle foods (Pattanayaiying *et al.*, 2015). Yang *et al.* (2020) produced sugarcane bagasse nano-cellulose/nisin hybrid films. Data analysis showed that the nisin concentration had considerable effect on the antibacterial activity, light transmission, gas barrier, and mechanical properties of these films. This film was employed as a lining on low-molecular-weight polyethylene plastics for hamburger packaging. The results showed that the film entirely prevented *L. monocytogenes* through 7 days of storing at 4°C (Yang *et al.*, 2020). Polyvinyl alcohol/chitosan/modified bacterial nano - cellulose (mBNC) films were produced by incorporating 4-hexyl resorcinol (4HR) for food packaging purposes. The results showed that the integration of 4-HR and mBNCs enhanced the film's yellowness and redness but reduced the film's transparency and brightness. The water vapor permeability, moisture content, and elongation at the break of the films were developed by adding 4-HR and mBNCs, while tensile strength and water solubility were decreased. The antioxidant properties of the films were significantly developed by adding 4-HR. The active films presented outstanding antimicrobial influence versus spoilage bacteria on vacuity-packed frozen raw beef (Choo *et al.*, 2021). Gedarawatte *et al.* (2021) conducted a study on the antibacterial activity of nisin-loaded bacterial cellulose nanocrystals versus certain meat spoilage lactic acid bacteria. The results showed that the nisin-loaded BCNs can be applied as antimicrobial ingredients in active food packaging (Gedarawatte *et al.*, 2021).

FTIR and XRD analysis described the molecular interaction after adding nano-cellulose and nisin. Nisin, being a cationic peptide, exhibits a negative interaction with chitosan due to their positive charges in acidic conditions (Ibarguren *et al.*, 2017; Saini *et al.*, 2016). The SEM images also show that the nano-cellulose, and nisin are evenly distributed inside the nanocomposite.

Measuring the contact angle on a film is a suitable marker of the degree of hydrophilicity and hydrophobicity. The concluding shape of the water drop on the film surface can be captured as an indicator of the surface wettability (Leceta *et al.*, 2013). Chitosan, due to having the functional groups of amines, acetamides, or hydroxyl groups, are hydrophilic polymer. Though, with the addition of NC and nisin, the contact angle value was reduced, which could be ascribed to the hydrophilic properties of cellulose particles. Nano-celluloses have more hydrophilic properties than cellulose polymers. The NC hydrophilicity could be the consequence of the incorporation of polar sulfate bands, the revealing of hydroxyl bands, and the prohibition of non-polar constituents from

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the cellulose construction throughout the production procedure (Celebi and Kurt, 2015;(Bahar *et al.*, 2012). In all instances, films made by microfluidics had lower contact angle values, signifying modifications in the conformation of molecules, and the revealing of the hydrophilic bands to the outside (Celebi and Kurt, 2015; Leceta *et al.*, 2013). The addition of nisin to the nanocomposite reduced the water contact angle value. This indicates an increase in water absorption in the film due to the hydrophilicity of nisin.

Food coatings must resist scratches and brittleness and show flexibility. Therefore, the mechanical properties of the bio-nanocomposite were investigated. The results of the CH-NC-N film show that Young's Modulus (YM) , tensile strength (TS), and uniform elongation (UE) increase. The increase in breaking point after the addition of nano-cellulose may be due to the coordinated relationships between the polymer and nano-cellulose, the effective transfer of force through the polymer and nano-cellulose layers. Bonding between anionic sulfate groups of nano-cellulose, and cationic amide groups in chitosan could create better interaction between the matrix and the filler. This can conduct superior TS amounts of the nanocomposite films (Dehnad *et al.*, 2014a).

CONCLUSIONS

In this research, chitosan-based nanocomposite film was produced, including chitosan, nano-cellulose, and nisin. Nano-cellulose was synthesized from cellulose particles with acid hydrolysis. The value of TS and YM of bio−nanocomposite are considerably enhanced with the increase of nisin and nano-cellulose

The development of mechanical and physical properties of the CH-NC-N films was mainly because of the filling effect of nano-cellulose. The presence of nisin and nano-cellulose in the synthesized bio-nanocomposite has been proven by XRD and FTIR spectra. SEM imag of the CH-NC-N films showed a uniform construction, specifying appropriate spreading of nano-cellulose and nisin into the chitosan film. Furthermore, the chitosan-nano-cellulose-nisin bioactive composite considerably inhibited all the tested pathogenic bacteria. However, examination of all informations showed that the CH-NC-N film had an extraordinary possibility for utilization in the antimicrobial meat packaging. The evidence can assist the food industry in implementing the necessary processing requirements to ensure food safety while producing products with enhanced sensory properties and reduced energy costs.

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توسعه فیلم های ضد میکروبی نانو سلولز - کیتوزان فعال شده با نیزین برای کاربردهای غذایی و پزشکی

م. میرحسینی، و ر. دهقان

چکیده

در این تحقیق فیلم کیتوزان زیست تخریب پذیر - نانو سلولز - نیزین (CH-NC-N) سنتز شد و برای کاربرد آنتی باکتریال در پزشکی و بسته مواد غذایی مورد استفاده قرار گرفت. فیلم ضد باکتری کیتوسان-نانو-سلولز-نیزین با استفاده از تکنیک‌های مختلفی مانند آنالیز خواص مکانیکی و فیزیکی، میکروسکوپ الکترونی

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روبشی(SEM) ، پراش اشعه ایکس (XRD) و طیف‌سنجی مادون قرمز تبدیل فوریه (FTIR) بررسی شد. فعالیت ضد باکتریایی فیلم کیتوزان - نانو سلولز - نیزین علیه باکتری های عامل بیماری مثل: اشریشیا کلی، استافیلوکوکوس آرئوس، لیستریا مونوسیتوژنز، اشریشیا کلی XDR و کلبسیلا پنومونیه XDR مورد بررسی قرار گرفت. همچنین از این فیلم برای بسته بندی گوشت در دمای ٤ درجه سانتی گراد به مدت ٢٦ روز استفاده شد. تجزیه و تحلیل داده ها نشان داد که خواص مکانیکی و جذب آب فیلم پس از افزودن نانو سلولز و نیزین بهبود یافته است. تجزیه و تحلیل داده‌های FTIR، XRD و SEM امکان توصیف حضور نیزین در فیلم-CH CNرا فراهم کرد. تجزیه و تحلیل ضد میکروبی فیلم، پتانسیل بالای نیزین را به عنوان یک عامل ضد میکروبی در فیلم CH-CN-N در برابر همه باکتری‌های پاتوژن آزمایش شده مشخص کرد. فیلم CH-CN-N رشد کل باکتری های عامل فساد طبیعی را در گوشت به مدت ٢٦ روز در مقایسه با شاهد مهار کرد. همچنین نتایج نشان داد که خواص ظاهری و فیزیکی گوشت بسته بندی شده با این فیلم تغییر چندانی نداشته است. نتایج نشان می‌دهد که فیلم کیتوزان - نانو سلولز - نیزین برای استفاده در سیستم‌های غذایی و کاربرد پزشکی مناسب است.

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