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Characterization and Antibacterial Activity of Nanochitosan - Nisin Biocomposite Film Prepared from Shrimp Shells

M. Mirhosseini[1*], M. Afzali[1], and S. Qasemabadi Ghotbuddini[1]

ABSTRACT

Nanochitosan composite film containing Nisin (NCH-N) was synthesized by solution cast method. Chitosan nanoparticles were prepared from shrimp shell.Characterizations of the prepared chitosan-nanoparticles were performed using Dynamic Light Scattering (DLS) and Fourier Transform Infrared Spectroscopy (FTIR) technique. The DLS analysis showed that the average size of chitosan- nanoparticles was 84.8 nm. Antimicrobial properties of edible (NCH-N) solution were also tested against pathogenic bacteria such as *E. coli, P. aeruginosa, S. aureus, B. cereus, L. monocytogenes, E . faecalis,* and eight clinical multidrug resistances *K. pneumonia* and *E. coli.* Addition of nisin to film significantly enhances the antimicrobial activity of the film against these tested pathogenic bacteria. This solution was also used as an antimicrobial coating on peaches. Based on the results, the peach coated with the film-forming NCH-N solutions presented a significantly lower amount of microorganisms growth than the uncoated peach, and significantly increased the shelf life of peaches. The color of the peach was not influenced by the films. The similarity of peaks in the spectrum of FTIR films confirms the absence of relevant interaction between the nisin and the polymer. The films were also analyzed by Scanning Electron Microscopy (SEM) to investigate the surface topography. Nanohitosan films were smooth and homogeneous. With the addition of nisin to nanochitosan films, the film became more uniform and homogeneous. The incorporation of nisin into edible nanochitosan films or coatings may be an attractive and convenient method for biopreservation of food.

Keywords: Bio-preservation, Dynamic light scattering, Edible film, Food protection.

INTRODUCTION

Food protection is an essential issue in food manufacturing since developing bacterial contaminations have been associated with foodborne illnesses. Pathogenic contamination of slightly handled ready-to-eat foods, fresh fruit, and vegetables are of increasing interest to human healthiness since consumers generally demand foods freshly and longer shelf life (Asadi and Pirsa, 2020 Pirsa and Asadi, 2021 Rezaei *et al.*, 2020 Sharifi and Pirsa, 2021). This highlights the prominence of the improvement of active packaging to inhibit the growing and distribution of pathogenic microorganisms in food (Asadi and Pirsa, 2020 Hosseini *et al.*, 2021 Pirsa and Asadi, 2021 Rezaei *et al.*, 2020 Sharifi and Pirsa, 2021). One of the major advances in active packaging is edible or biodegradable films. Edible films or biodegradable films have been considered for their capabilities to delay moisture, oxygen, aroma, and solute passage (Asadi and Pirsa, 2020). This is furthermore developed through film-carrying food additives such as antibacterial, antioxidants, flavors, and colorants (Cé *et al.*, 2012 Ozdemir and Floros, 2004 Sajad, 2020). Biodegradable films are biopolymeric materials are formed a thin layer on the surface of food compounds and act as a barrier against gases, moisture, and grease. These films protect food products

[1] Department of Biology, Payame Noor University, Tehran, Islamic Republic of Iran.
[*] Corresponding author; e-mail: m.mirhosseini@pnu.ac.ir

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from the mechanical stress and growth of microorganisms. These films also increase the quality, appearance, and shelf life of food (Asadi and Pirsa, 2020 Pirsa and Aghbolagh sharifi, 2020). Biodegradable films because of their eco-friendly characteristic such as, extensive diversity, and accessibility, non-toxicity, and low cost have fascinated the researchers' attention (Jabraili *et al.*, 2021; Pirsa, 2020). Biodegradable polymers are divided into four groups based on their chemical structure: polysaccharides, lipids, proteins, and polyesters (Asadi and Pirsa, 2020; Pirsa and Aghbolagh sharifi, 2020). Biodegradable polymers are easily converted into smaller units by microorganisms (Sani *et al.*, 2021). Carbohydrates have been widely used to produce biodegradable edible films due to film formation, renewability, and biodegradability (Sharifi and Pirsa, 2021). Chitosan is one of the carbohydrate polymers used to produce environmentally friendly films and composites (Pirsa and Aghbolagh Sharifi, 2020).

Chitosan is a linear polysaccharide made of accidentally dispersed β-(1-4)-bonded D-glucosamine and N-acetyl-D-glucosamine. It is made by treating shrimp and other crustacean shells with the alkali sodium hydroxide. The biopolymer is described as either chitin or chitosan, depending on the Degree of Deacetylation (DD), which is defined by the percentage of D- glucosamine, and N-acetyl-D-glucosamine. Fundamentally, chitosan is a straight-chain copolymer constituted of D-glucosamine and N-acetyl-D-glucosamine being obtained by the fractional deacetylation of chitin. Chitosan is the most abundant main biopolymer and is essentially similar to cellulose, which is made of only individual glucose monomer. Chitosan properties such as biodegradability, solubility, activity, and adsorption of various substrates depend on the amount of protonated amino groups in the polymeric chain, thus on the ratio of acetylated and non-acetylated D-glucosamine components. The amino groups (pKa from 6.2 to 7.0) entirely protonated in acids with pKa lesser than 6.2 constructing chitosan resolvable. Chitosan is unsolvable in water, aqueous bases, and organic solvents, and it is resolvable later disturbing in acids such as nitric, hydrochloric, acetic, phosphoric, and perchloric (Mohammadi *et al.*, 2019; Rinaudo, 2006). The usual making of chitosan from shrimp shells commonly contains three significant stages: demineralization, deproteinization, and deacetylation (Hossain and Iqbal, 2014). Because of its easiness, qualified tool accessibility, and independence of sample solubility, IR spectroscopy is one of the most considered techniques for descriptions of chitin and chitosan(Islam *et al.*, 2011). Shrimps are conventionally marketed headless and frequently barked of the external shells and tail. About 30-40% by weight, raw shrimp substance is removed as waste when treated shrimp is headless, shell-on yields (Samad *et al.*, 2015).

Bacteriocins and bacteriocin-like ingredients have been investigated for their potential use in controlling spoilage and pathogenic microorganisms in foodstuff (Cé *et al.*, 2012). The most common bacteriocin applied in the bioactive film is nisin because of its Generally Recognized As Safe (GRAS) agreement by the United States Food and Drug Administration. Nisin is a small polypeptide (34 amino acids) engendered by the *Lactococcus lactis* subsp. It displays antibacterial activity against a variety of gram-positive bacteria, such as the foodborne pathogens *Listeria monocytogenes*, *Staphylococcus aureus*, *Clostridium botulinum*, and its spore (Liu *et al.*, 2007). It is an amphiphilic peptide that is cationic at neutral pH, having an isoelectric point above 8.5. It is an efficient inhibitor of gram-positive bacteria and has been exhibited to adsorb to surfaces, maintain activity, and remove cells that have attached in vitro. Nisin prevents sensitive bacteria throughout a multi-step process that disrupts the phospholipid bilayer of the cell and produces transient pores. In recent years, the application of films as nisin delivery systems to decrease negative bacteria in food has

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attracted a lot of interest.(Liu *et al.*, 2007; Salmieri *et al.*, 2014). The purpose of this study was to produce chitosan nanoparticles from shrimp by-products that are harmful to the environment. The edible nanochitosan films containing nisin were prepared and properties of the edible films were analyzed. Also, antimicrobial properties of the films were studied in vitro to evaluate effects of the active film on peach fruit in increasing shelf life and reducing microbial load, which has not been reported in any study.

MATERIALS AND METHODS

Sodium hydroxide 95% purity, hydrochloric acid 37% purity, and sodium triphosphate 92% purity (TTP) were purchased from Mojalali company (Laboratory-grade, Tehran, Iran). Glacial acetic acid 100% purity was purchased from Merck company (Laboratory -grade, Darmstadt, Germany). Crab shells were purchased from the fish marketplace (Yazd, Iran).

Synthesis of Chitosan

Coarse Purification: 219 g of shrimp shells were washed with water to remove sand, and other soils, then, the shells were dried.

Protein Removal: The dried shells were milled and transferred to an Arlene Meyer. Then, 2% NaOH was added to the shells and heated at 60–70°C for one hour under agitation. The shells were strained off with a filter, and the process repeated. The residue must be approximately clear and colorless. Then, the shells were washed with distilled water until a neutral reaction. This stage is recognized as deproteinization.

Calcium Carbonate Removal: 7% Hydrochloric acid gradually enhanced the shells, and the mix was agitated at room temperature until no gas leaked any longer. As a test, 10 mL of hydrochloric acid was enhanced. If no additional gas production happened, the mixture was strained off and washed with water. The product was dried overnight in an oven at 60°C. As a result, 104.22 g of chitin was obtained from crab shells. This stage is recognized as demineralization.

Production of Chitosan from Chitin *via* Deacetylation: In a round-bottomed flask, 50% sodium hydroxide was enhanced to 20 g of the crop chitin. The mixture was heated at 125°C for two hours. The mixture became cold, and then 100 mL of water was added. The next day, the mixture was strained off, and the filtrate was rinsed with water to a neutral reaction and dried in the oven at 60°C. As a result, 14.36 g of chitosan was obtained from crab shells. FTIR spectra of chitosan and Sigma Aldrich chitosan were studied with potassium bromide pellets on a Shimadzu IRPrestige-21 spectrometer (Sanuja *et al.*, 2014).

Preparation of Chitosan Nanoparticles

Chitosan nanoparticles were made according to an improved procedure described by Yoksan *et al.* (2010). Chitosan solution [1% (w/v)] was made by stirring chitosan in an aqueous acetic acid solution (1% (v/v)) at room temperature overnight. The suspension was then centrifuged for 30 min at 14,000 rpm. TPP solution [0.25% (w/v), 200 mL] was then enhanced gradually into 200 mL of chitosan solution under magnetic agitating. Then, stirring was constantly done for 40 minutes. The produced particles were gathered by centrifugation at 14,000 rpm for 30 minutes at 4°C and washed several times with distilled water. Ultimately, ultrasonication was done by a sonicator (Chrom Tech—CT, Taiwan) in an ice bath for 30 minutes with a sequence of 0.7 seconds of sonication and 0.7 seconds of rest. The suspensions were dried at 60°C for 72 hours by Aven (MM group, Germany)(Hosseini *et al.*, 2013; Yoksan *et al.*, 2010).

Characterization of Chitosan Nanoparticles

The following analysis describes chitosan nanoparticles. The particle size distribution

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of chitosan nanoparticles was determined by Particle Size Analyser (Brookhaven 90 plus). The analysis was performed at a scattering angle of 90°C at a temperature of 25°C using samples diluted to different concentrations with distilled water. FTIR spectra of chitosan nanoparticles was taken with potassium bromide pellets on a Shimadzu IRPrestige-21 spectrometer.

Nanochitosan Film Preparation

One and a half g of chitosan nanoparticles were dissolved in 100 mL of 1% acetic acid. The suspension was strained across a silkscreen to eliminate undissolved material (Cé *et al.*, 2012). The nisin was incorporated into the nanochitosan solution to reach final concentrations of 0.046 mg/mL based on previous research (Mirhosseini and Afzali, 2016). The solutions were then cast in 10 cm polyacrylic plates and dried at 40°C for 24 hours.

Characterization of Nanochitosan Films

Spectroscopic Analysis

FTIR examination of nanochitosan/nisin film was performed by a Shimadzu IRPrestige-21 spectrometer. Infrared spectra were recorded in the 500–4000 cm^1 range.

Scanning Electron Microscopy

Gold platinum was added to the samples by a sputter cater (Balzers SCD050). The samples were then examined via a Phenom ProX scanning electron microscope (Phenom, Nederland).

Antibacterial Activity

The antibacterial activity of chitosan nanoparticles was studied by *Escherichia coli* PTCC1394, *Pseudomonas aeruginosa* PTCC 1074 (as gram-negative), and *Staphylococcus aureus* PTCC1431, *Bacillus cereus* PTCC1015, *Listeria monocytogenes PTCC*19112, *Enterococcus faecalis* PTCC1237 *(*as gram-positive), and eight clinical multidrug resistance bacteria including: *K. pneumonia,* and *E. coli* strains (Isolated urinary tract, Milad Laboratory, Yazd, Iran) through the spot on the lawn procedure (Mirhosseini and Firouzabadi, 2013). Edible coating solutions (20 µL) were used on Tryptic Soy Agar (TSA) (TSA, Merck, Germany) plates previously seeded with a swab submerged in indicator strain suspension, which corresponded to approximately 10^6 CFU/mL. Plates were incubated at 37°C for 24 hours, and the diameter (mm) of inhibition zones was measured. Antibiotic disk, including Vancomycin (VA), was applied as a positive control for comparing the inhibition of the growth of bacteria with chitosan nanoparticles. For negative control, acetic acid solution [1% (v/v)] was applied (Firouzabadi *et al.*, 2014).

In Situ Experiment

Peach was purchased from a local marketplace, chosen for the equal degree of ripeness, and free from defects and presence of pathogens. Fruits were washed and immersed for 1 min in each corresponding solution containing 15 g L^{-1} nanochitosan/0.046 mg mL^{-1} nisin, and 15 g L^{-1} nanochitosan. Untreated peach was applied as a control. The treating steps were performed at room temperature inside a downflow booth, and they were managed in suitable producer methods. Then, the peaches were drained and stored at refrigeration temperature (4+2°C) in plastic pots covered with Polyvinyl Chloride (PVC) films.

For microbiological analyses, 10 g samples were homogenized in 90 mL of 1 g L^{-1} peptone water. Decimal dilutions were made from the initial homogenate. The total aerobic counts were examined on plate count agar plates incubated at 35°C for 48 hours.

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Yeast and molds were determined on potato dextrose agar, after incubation at 28°C for four days.

Eosin methylene blue (EMB) agar incubated at 43 and 37°C for 24 hours was applied to identify *E. coli* and total coliforms, respectively. Three samples of each storage time and treatment were analyzed (Cé *et al.*, 2012).

Fungal decay was visually studied in peaches per treatment. A fruit was considered infected when observable corruption was detected (growth of mycelium on the fruit surface, brown spots, and a softening of the injured zone). The results stated the percentage of fruits infected. Two peaches were accidentally chosen from every treatment on days 0, 5, 10, and 14 to photo the growth of spoilage throughout the storage time applying a digital camera (Sony DSC-HX1, Japan).

Weight Loss

Peaches from every treatment were weighed immediately after air-drying at the beginning and throughout storage time. The results were expressed as the percentage of loss compared to the initial weight (Valenzuela *et al.*, 2015).

Statistical Analysis

All experiments were repeated three times. Data points were expressed as the mean±SD. Comparison was performed at the 95% probability level. SPSS (v. 19) was applied for all of the statistical analyses. Analysis Of Variance (ANOVA) and the significance of differences between the means of "Tukey's" tests ($P< 0.05$) was applied. Excel 2013 software was used to draw curves and charts.

RESULTS

Characterization of Chitosan Nanoparticles

Chitosan was prepared from shrimp shell, as written in the previous section. The most important differentiation between chitin and chitosan is that chitosan is soluble in 1% acetic acid. The description of chitosan was proved by FTIR analysis.

FTIR Analysis of Chitosan and Chitosan Nanoparticles

Figure 1 shows the FTIR spectra of chitosan, chitosan nanoparticle. Generally, chitosan exhibits typical peaks at 3433 (OH and NH_2 stretching), 2920 (CH stretching), 1647 (amide I), 1088 (C-O-C stretching), and 591 cm^{-1} (pyranose ring stretching vibration) (Figure 1). For chitosan nanoparticles, the peak of amide I (NH_2 bending) shifted from 1,647 to 1,651 cm^{-1}, and new peaks appeared at 1238 (C-O-C stretch) and 1,555 cm^{-1} (amide II), indicating the intricate pattern via the electrostatic interface between NH^{3+} groups of chitosan and phosphoric groups of TPP in the nanoparticle. This result is consistent with other researchers (Hosseini *et al.*, 2013; Jingou *et al.*, 2011; Yoksan *et al.*, 2010).

Particle Size Distribution of Chitosan Nanoparticles

Particle distributions of chitosan nanoparticles are shown in Figure 2, which shows that most of the chitosan nanoparticles were distributed between the ranges of 33.7 and 168.2 nm, and the mean size of chitosan nanoparticles was 84.8 nm.

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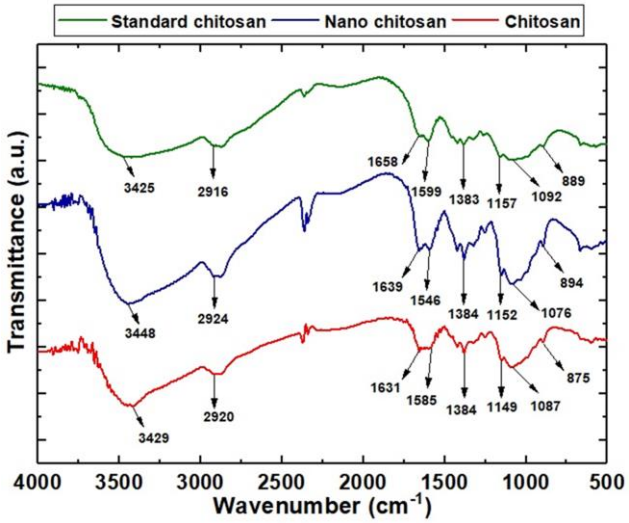

Figure 1. FTIR spectra of standard chitosan, prepared chitosan and chitosan nanoparticles.

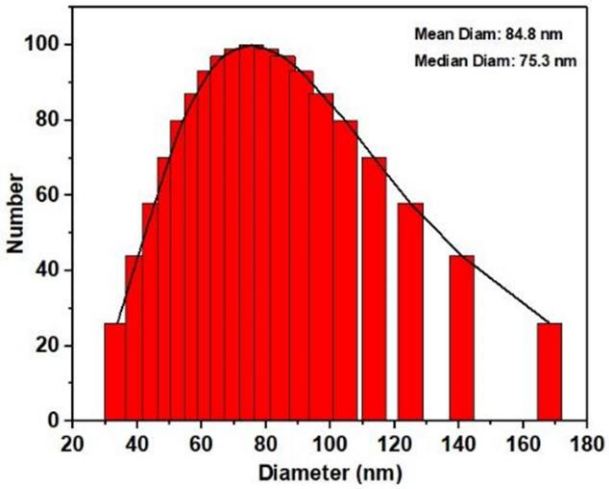

Figure 2. Particle size distributions of chitosan nanoparticles.

Characterization of Nanochitosan Films

FITR Analysis

In order to study the interaction between components of films integrating the antimicrobial agent's nisin, the primary analysis was obtained over polyelectrolytes interactions. It has been determine, that the carboxyl group of the anionic polymer may interact with the amino group of chitosan and produces an ionic complex (Vargas *et al.*, 2006). It was revealed by shifts in the absorption bands of amino groups, carboxylic groups, and amide bonds in the FTIR spectra (Figure 3). The more intensity detected at about 3,437 cm^{-1} resulting from N–H and O–H stretching vibrations for the nisin-loaded nanoparticles than that of the nanochitosan films is because of the existence and integration of nisin inside the nanoparticle mixture by possibly physicochemical interactions. A similar behavior for the nisin integrated nanoparticles, i.e., the more intensity in the absorption band at 1,639 cm^{-1} (Figure 3) because of C=O stretching vibration, with a slight shift about 4 cm^{-1} from the 1,635 to 1,639 cm^{-1} was furthermore detected compared with the chitosan nanoparticle

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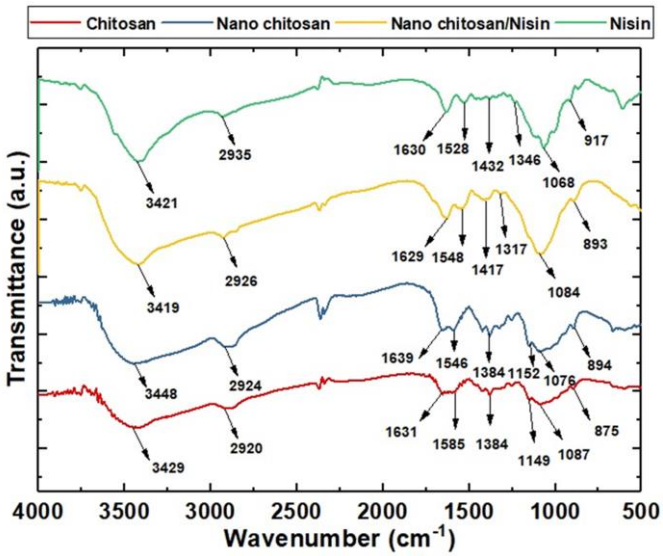

Figure 3. FTIR spectra of chitosan film, nanochitosan film, Nanochitosan containing Nisin film (NCH/N) and nisin.

film alone. It could be another confirmatory proof for the existence of nisin inside the nanoparticle films during the physicochemical interactions (Qi *et al.*, 2011; Zohri *et al.*, 2010).

SEM Analysis Nanochitosan Films

The films were studied by SEM. The surface area structure of the chitosan film was irregular and heterogeneous. The surface exhibited holes and wrinkles. Nanochitosan film has a homogeneous and dense structure that indicates proper dispersion, and distribution uniformity of nanochitosan in the acidic solution. With the addition of nisin to nanochitosan films, the film became more uniform and homogeneous (Figure 4).

Antimicrobial Activity Chitosan Nanoparticles Films

Edible chitosan nanoparticles solution prepared with 15 g L^{-1} chitosan nanoparticles were evaluated for their antibacterial activity against *E. coli, P. aeruginosa, S. aureus, B. cereus, L. monocytogenes, E .Faecalis*, and eight clinical multidrug resistance bacteria including: *K. pneumonia,* and *E. coli* strains. The chitosan nanoparticles exhibited antimicrobial activity against all strains (Table 1). When solution, including nisin, were investigated, an increase in inhibitory activity was often detected (Table 1).

The importance of this research lies in the quantification of the log CFU/g of the uncoated and coated peaches because comparable experiments observed the antimicrobial effect of the coating on fungal decay by qualitative determination by simple observation, and microbiological counts were only done in a small number of reports (Campaniello *et al.*, 2008; Ribeiro *et al.*, 2007). Microbiological analyses on minimally processed peach samples covered with different chitosan nanoparticle films showed the absence of aerobic counts total coliforms, and *E. coli* (incubated at 37°C) at time zero and low counts after 14 days ($P< 0.05$). Total coliforms, and *E. coli* (incubated at 43°C) were not revealed at this time ($P< 0.05$). The existence of antimicrobial nisin in the chitosan films caused considerable decrease in total counts (Figure 5). The decrease in yeast and molds was also detected in samples treated with chitosan nanoparticle films containing the antimicrobial nisin ($P< 0.05$). The number of

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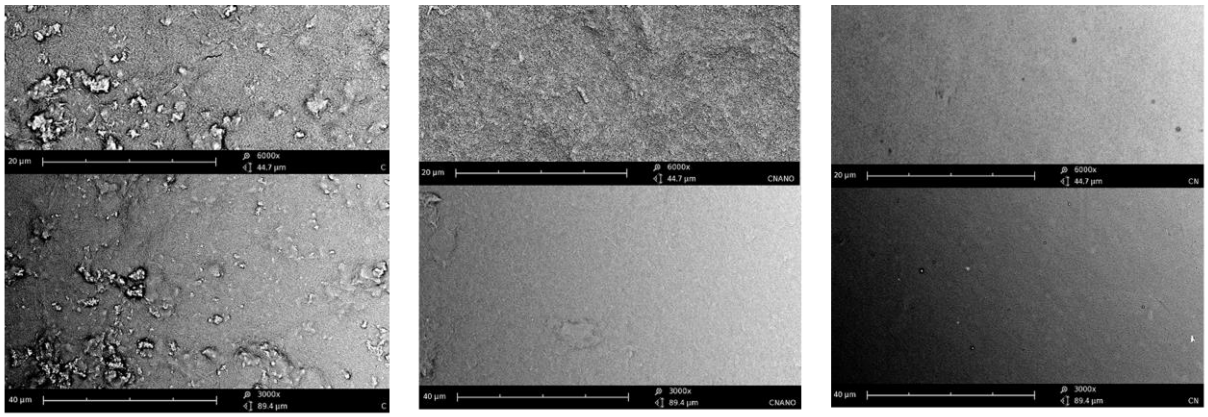
E. coli, yeast, and mold on the fourteenth day compared to the seventh day also decreased in control, probably due to competition and antagonism between microorganisms on the surface of peaches (Figure 5).

On day 5, the uncoated peaches exhibited the progress of mycelium on the tip of the fruit. In the peaches coated with "nanochitosan films", the detected fungal infection showed no lesions. At the end of the storage period, all peach samples had fungal contamination, though the infection in the coated fruits was more focused than in the uncoated fruits, which had infections that covered most of the surface of the fruit. The only slight focalized fungal growth was detected in peaches coated with "nanochitosan containing nisin films". The



A: CH B:NCH C: NCH-N

Figure 4. SEM of the surface of: (a) Chitosan film (CH), (b) Nanochitosan film (NCH) and (c) Nanochitosan containing .isin film (NCH-N).

Table 1. Inhibition zone diameters of nanochitosan, and nanochitosan/nisin in presence of *E. coli*, *P. aeruginosa*, *S. aureus*, *B. cereus*, *L. monocytogenes*, *E .Faecalis*, and 8 clinical multidrug resistance bacteria including: *K. pneumonia,* and *E. coli* strains (based on Table 1) culture.

Strains	1% Acetic acid (mm)	Vancomycin a (mm)	Nanochitosan (mm)	Nanochitosan/Nisin (mm)
E. coli	0	0	7.93±0.1	10.08±0.27
S. aureus	0	15.58±0.1	7.93±0.26	10.08±0.11
B. cereus	6.03±0.12	13.07±0.21	8.2±0.3	10.12±0.25
P. aeruginosa	8.63±0.23	16.73±0.1	8.63±0.11	11.82±0.13
L. monocytogenes	0	18.8±0.11	8.57±0.23	10.26±0.14
E. faecalis	0	19.04±0.23	7.59±0.14	10.24±0.1
NDR_ *E. coli*	0	0	7.93±0.1	8.81±.15
NDR_ *K. pneumoniae*	0	0	7.69±0.15	9.27±0.16
MDR_ *E. coli*	0	0	6.85±0.19	9.15±0.1
MDR_ *K. pneumoniae*	0	11.23±0.1	7.34±0.13	9.13±0.19
PDR_ *E. coli*	0	9.81±0.34	7.75±0.09	9.81±0.26
PDR_ *K. pneumoniae*	0	0	7.77±0.43	9.64±0.33
XDR_ *E. coli*	0	0	8.32±0.25	9.16±0.35
XDR_ *K. pneumoniae*	0	0	7.69±0.13	9.27±0.1

[a] Vancomycin (VA) antibiotic was used as positive control and 1% acetic acid was used as a negative control.

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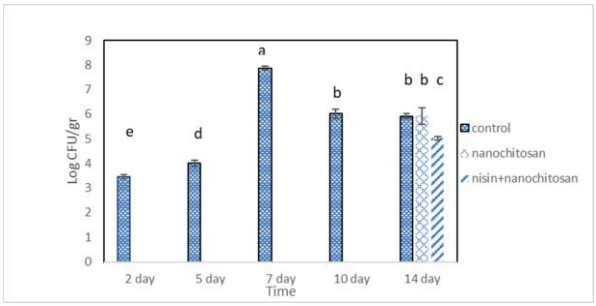
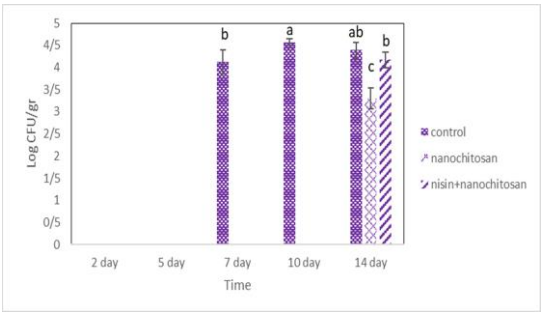


(a)



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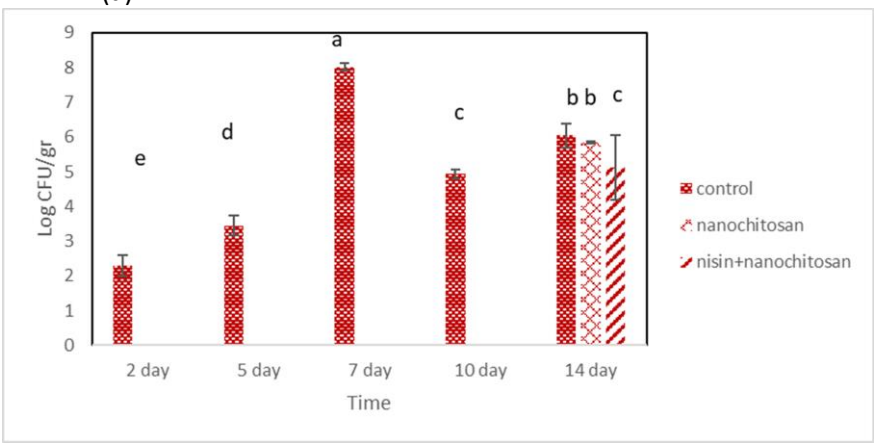

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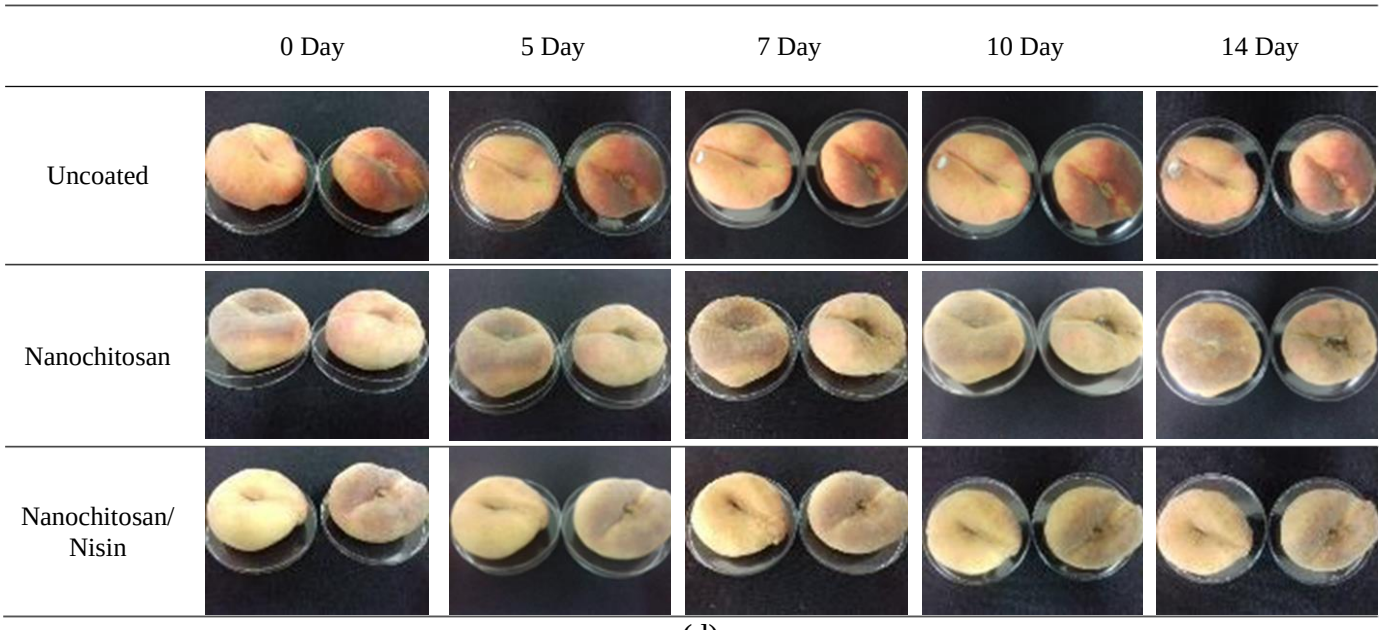

(d)

Figure 5. Evolution of total aerobic counts (a), *E. coli* and total coliforms (b), mold and yeast counts (c), and fungal decay and appearance (d) during the storage of uncoated and coated Peaches with chitosan nanoparticles, nisin/chitosan nanoparticle, and edible films. Different letters in each day show significant differences ($P \leq 0.05$).

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Table 2. Weight loss (respect control), uncoated and peaches coated with nanochitosan, and nanochitosan/nisin, edible films.

	Weight loss (%)		
Day	Uncoated	Nanochitosan	Nanochitosan/Nisin
5	4.04 ±0.35	2.63 ±0.28	2.03 ± 0.33
7	5.57 ±0.38	3.84 ± 0.47	3.06 ±0.23
10	7.52 ±0.49	5.6 ± 0.35	4.22 ±0.57
14	10.91 ±0.41	8.55 ±0.48	7.07±0.87

coatings (Figure 5-d) did not influence the color of the peaches ($P< 0.05$).

Physicochemical Properties

Table 2 displays the weight loss progress of uncoated and coated peaches during storage. On 5, 7, 10, and 14 days of storage, the uncoated peaches exhibited the highest weight loss.

DISCUSSION

Chitosan edible films have been used as a coating on meat, vegetables, and fruits (Devlieghere *et al.*, 2004; Ye *et al.*, 2008), but the edible film of nanochitosan has not been used as a fruit coating. In this work, peaches color was not affected by the coatings (Figure 5). These results were favorable because several reports have indicated that the use of film-forming solutions on strawberries can alter the color of the strawberries and enhance the opacity of the coated fruits (Valenzuela *et al.*, 2015; Vargas *et al.*, 2006).

In addition, "nanochitosan films", including either nisin, displayed inhibitory activity versus some bacteria. The efficiency of nanochitosan films integrating nisin was displayed on *E. coli*, *P. aeruginosa*, *S. aureus*, *B. cereus*, *L. monocytogenes*, *E . Faecalis*, and eight clinical multidrug resistances *K. pneumonia* and *E. coli*. The synergistic effect of chitosan with nisin increased their antimicrobial spectra to the bacteria. In this regard, bacteriocins have been provided as additional barriers to inhibiting the growth of microorganisms, and their blend with many chemicals, such as organic acids and chelating agents, has been successfully described (Cé *et al.*, 2012; Sobrino-López and Martín-Belloso, 2008).

The combination of chitosan nanoparticles with nisin has not been formally studied and appears interesting enough to control microbial growth in situ. Millette *et al*. (2007) measured the progressive inhibitory influence of nisin-containing developed alginate films/beads on *S. aureus*. Based on the results, it has been shown that hydrophobic and degradable sterile films or particles containing different concentrations of nisin can be used to regulate the growing of microorganisms sensible for decay at the surface of curved beef or other meat crops. In a study, Zohri *et al*. (2010) compared the antimicrobial effect of nisin and nisin-loaded chitosan/alginate nanoparticles on the growth of *S. aureus* in pasteurized and raw milk. A twofold increase in the antimicrobial effect identified for the nisin-loaded nanoparticles was observed compared to nisin alone at a concentration of 450 IU mL^{-1}. These results are due to the higher permeability and gradual release of nisin from chitosan/alginate nanoparticles throughout storage and its gradual action over longer periods compared to the nanoparticle-free samples (Zohri *et al.*, 2013).

The success in reducing microbial counts in situ exposes that hybrid films can be

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appropriate to increase the shelf life of a treated pear or other vegetables. SEM detected the different appearances of the film surface. Nanochitosan film has a homogeneous and dense structure that indicates proper dispersion and distribution uniformity of nanochitosan in the acidic solution. With the addition of nisin to nanochitosan films, the film has become more uniform and homogeneous (Figure 4). This result conformed to that studied for composite chitosan-hydroxyl propyl methylcellulose films, which displayed a very complex surface area arrangement (Möller *et al.*, 2004). SEM and atomic force microscopy analyses showed a comparatively smooth and homogeneous character for composite starch–chitosan films, but stage separation was detected with a combination of ferulic acid at higher concentrations (Mathew and Abraham, 2008). A very smooth and homogeneous surface has been detected for iota-carrageenan films (Karbowiak *et al.*, 2006). However, combined films (with an acetic acid ester of mono and diglycerides) exhibited an enhancement of surface heterogeneity with the enhancement of fat inserted into the film stracture.

FTIR study specified no fundamental alterations in the chitosan polymer. The spectra of chitosan films integrating nisin exhibited a similar pattern on their major peaks as the control chitosan films. Nisin is a cationic peptide of 3,350 Da (Liu and Hansen, 1990), and a negative collaboration with chitosan nanoparticle would be supposed since both molecules are positively charged at acidic pH.

CONCLUSIONS

Chitosan nanoparticles were synthesized from shrimp shells. The production of chitosan nanoparticles was confirmed by DLS and FTIR techniques. The biodegradable/antifungal–antibacterial film based on nanochitosan–nisin was prepared by solution casting method. The structure of the film and the interaction between nanochitosan and nisin was confirmed by SEM, and FTIR pattern. According to the results, fresh peaches coated with NCH, and NCH/N had longer shelf lives than uncoated fruits. This effect is mainly due to the antifungal activity of NCH, that remains when the NCH is combined with nisin. Edible nanochitosan films are an attractive selection to preserve the quality and safety of slightly handled food. The integration of antimicrobials such as nisin, to edible nanochitosan films or covering may be a possible and desirable technique for food biopreservation.

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بررسی خصوصیات و فعالیت ضد باکتریایی فیلم بیوکامپوزیت نانوکیتوسان-نایسین تهیه شده از پوسته میگو

م. میرحسینی، م. افضلی، و ث. قاسم آبادی قطب الدینی

چکیده

فیلم کامپوزیت نانوکیتوسان حاوی نایسین (NCH-N) به روش ریخته گری محلول سنتز شد. ابتدا نانوذره کیتوسان از پوسته میگو تهیه شد. شناسایی نانوذرات کیتوزان تهیه شده با استفاده از روش پراکندگی نور پویا (DLS) و طیف‌سنجی فروسرخ تبدیل فوریه (FTIR) انجام شد. تجزیه و تحلیل DLS نشان داد که اندازه متوسط نانوذرات کیتوزان ۸٤.۸ نانومتر است. فعالیت ضدمیکروبی محلول NCH-N در مقابل باکتری های استافیلوکوکوس آرئوس، اشرشیاکلی، سودوموناس آئروینوزا، لیستریا منوسایتوژنز، با سیلوس سرئوس و هشت سویه مقاوم بیمارستانی اشرشیاکلی و کلبسیلا پنومونیه بررسی شد. اضافه شدن؛ نایسین به فیلم به طور قابل توجهی فعالیت ضد میکروبی فیلم را در برابر این باکتری های بیماریزای آزمایش شده افزایش داده بود. این محلول به عنوان پوشش ضدمیکروبی بر روی هلو همچنین استفاده گردید. بر اساس نتایج، هلوهای پوشش داده شده با محلول های تشکیل دهنده فیلم NCH-N، میزان رشد میکروارگانیسم ها را روی سطح هلوها به طور قابل توجهی نسبت به هلوهای بدون پوشش کاهش یافته بود و ماندگاری هلوه را افزایش یافته بود. رنگ؛ هلو ها تحت تأثیر فیلم ها قرار نگرفته بود. شباهت پیک ها در طیف FTIR فیلم های نانوذرات کیتوسان و نانودرات کیتوسان حاوی نیزین عدم وجود برهمکنش مرتبط بین نایسین و پلیمر را تایید می کند. فیلم ها همچنین با میکروسکوپ الکترونی روبشی (SEM) برای بررسی توپوگرافی سطح مورد تجزیه و تحلیل قرار گرفتند. نانوکیتوسان؛ فیلم ها صاف و همگن بودند. با افزودن نایسین به فیلم های نانوکیتوسان، فیلم یکنواخت تر و همگن تر شد. بنابراین ترکیب نایسین در لایه‌ها یا پوشش‌های خواراکی نانوکیتوسان ممکن است یک روش جذاب و راحتی برای محافظت زیستی مواد غذایی باشد.

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