



Original research

## Fabrication and characterization of nanochitin-reinforced carboxymethyl cellulose edible film

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### ABSTRACT

Nanochitin (NC) and carboxymethyl cellulose (CMC) are biodegradable polymers that are prepared from aqua cultural and natural resources. NC at three concentrations (0.1, 0.5 and 1% w/w) was added to the CMC film using casting methods. The optimum result was obtained through the nanocomposite film with 1% NC in terms of water solubility (WS) (17%), moisture content (MC) (14%), and moisture absorption (MA) (16.28%). Water vapor permeability (WVP) of composite film is reduced by increasing concentration of NC. The lowest water vapor permeability value belongs to CMC/NC 1% with  $0.30 \text{ g s}^{-1} \text{ m}^{-1} \text{ Pa}^{-1} \times 10^{-10}$ . Moreover, tensile strength (TS) and elongation at break (EB) improved in CMC/NC film by increasing the NC content. By adding NC, the tensile strength of the nanocomposite was considerably enhanced from 4.98 to 24.59 MP. The differential scanning calorimetry (DSC) revealed that the glass transition temperature of NC (204.81°C) was lower than CMC (206.31°C). X-ray analysis confirmed the emersion of crystalline peaks in CMC/NC. However, high concentration (1%) of NC led to the aggregation of NC in CMC film. Antibacterial activity was obtained against five pathogen bacteria and the result showed an effective inhibition on *E. coli* and *S. aureus*. The inhibition zones for CMC/ 1% NC were 3.66 mm and 3.00 mm against *E. coli* and *S. aureus* respectively. In conclusion, the results suggested that the quality of the CMC-based films can be improved through the addition of NC.

Keywords: Nanochitin; Carboxymethyl cellulose; Biopolymer; Edible film

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## 1. Introduction

In food industry, many plastic materials are used for packaging purpose, most common polyvinyl chloride, polyamide, polyethylene and polyethylene terephthalate. Since packages are made mainly of chemical polymers, they are not biodegradable so can get piled up and pollute environment. For this problem, people are demanding for modern easily-decomposable packages that are natural resourced and environmentally friendly. To fulfill this demand, researchers have focused their investigations on synthesizing biopolymers to produce green packaging materials. One of the global interests in food packaging is to find biodegradable polymers that can be used

as alternatives for synthetic ones; therefore, finding recyclable and sustainable alternatives to synthetic plastic for food packaging is urgently needed. (Rubentheren et al., 2015; Perez et al., 2021). Biopolymers known as environmentally friendly, are low-cost and biodegradable. Common biopolymers to use as natural polymers are polysaccharide, lipid and protein. Biopolymers have many benefits. They provide product protection by prohibiting moisture, water vapor and gas transfer, thus increasing shelf life of perishable foods. They can also act as an additive carrier to keep and release preservatives and other active compounds used in intelligent packaging (Shojaee-Aliabadi et al., 2013). One usual polysaccharide in production of edible film, is carboxymethyl cellulose (CMC).

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CMC edible films are anionic, nontoxic, high viscose, non-allergenic and water-soluble (Dashipour et al., 2015; Andres – Bello et al., 2013).

However, biopolymers suffer from some poor characteristics. Their mechanical and barrier properties are not good enough to preserve food from deterioration or keep their quality for a long time. This limitation causes natural packages to be unusable in industry. To make them applicable for food packaging researches tend to incorporate them in synthetic polymers or modify them by adding additives like fibers or nano particles. In this way produced polymer enjoys both bio and synthetic polymers advantages. Nanocomposites are mixed polymers made from biopolymers and nanoparticle components. Nanoparticles like silica, clay, talc etc. can give biopolymers desired properties including mechanical and thermal features and gas and moisture permeability to produce optimum film for packaging (Abdollahi et al., 2013; Kaur et al., 2021).

Nanocomposite edible films have at least (1-100 nm) dimension in the nanoscale. (Abdollahi et al., 2013; Khan et al., 2012). Our focus in this research was to incorporate chitin nanoparticles with various contents into CMC biopolymer to improve edible film properties (Abdollahi et al., 2013). The nanofiller includes nanoscale metals, nonochitin, and nanomaterial (Sahraee et al., 2017).

Chitin is the most abundant natural polymer (polysaccharide) after cellulose. It is also found in shrimp and crab shells. Chitin is known with scientific name  $\beta$ -(1, 4)-2acetamide-2-deoxy-D-glucopyranose. Chitin nanoparticles have a good nanofiller network for approximately 10 nm width. These particles are easily shaped. These features increase the chance of chitin application. Chitin acid hydrolysis converts it into crystalline nanowhiskers and nanofibrils forms. Chitin and chitosan as natural amino polysaccharides have multifunctional properties and are widely used in medicine and industry (Ifuku et al., 2014).

Regarding the fact that chitin has a hydrophilic nature compatible to the most of polysaccharide and protein polymers and can be dispersed homogeneously in their matrices. It has been observed that the multifunctional properties of the bionanocomposite films, such as high mechanical, thermal, barrier, antimicrobial, and antioxidant, have the potential to improve the quality and safety of the food together with no or fewer negative impacts on the environment. These classes of bionanocomposites are promising candidates to be used as novel biodegradable active packaging materials in the food sector. Nanochitins have great economic value as an efficient functional and reinforcement material for a wide range of industrial applications. Nanochitin-reinforced composite materials have shown considerable advantages in various sectors, such as food packaging and biomedical industries. Furthermore, because these materials have environmental benefits and economic feasibility, research on producing composite materials from natural polymers and understanding their structural–functional relationships should be encouraged (Sahraee et al., 2017).

In the current study, the investigated biopolymer can be used as a double-layer film, with the middle layer containing antimicrobial compounds such as essential oil, extract, or as a hybrid form of nanoparticles that contain antimicrobial compounds. The above edible film can be used for food packaging such as bread, fruit and vegetables.

The purpose of this study was to develop a CMC edible film incorporating with differentiation concentrations of nanochitin for food packaging. The effect of nanochitin on the thermal, barrier and mechanical properties of CMC matrix was assessed.

## 2. Material and Methods

### 2.1. Materials

CMC was obtained from Pharayandsazan Arian Persian Company (Pharayandsazan Arian Company, Iran). Nanochitin gel was supplied from Nano Novin Polymer Company (Nano Novin Polymer Company, Iran) and glycerol was received from Merck Company (Merck, Germany).

### 2.2. Bacterial strains

*Staphylococcus aureus* ATCC 25923, *Escherichia coli* PTCC 1399, *Bacillus cereus* ATCC 1274, *Pseudomonas aeruginosa* ATCC 1430, and *Salmonella enteritidis* ATCC 1231 were provided by the Iranian Research Organization for Science and Technology (Tehran, -Iran). They were grown into brain heart infusion broth (BHI Broth) at 37 °C for 24 h before the experiment.

### 2.3. Preparation of carboxymethyl cellulose (CMC) films

For 1% (w/v) CMC films, 1.0 g of CMC powder was added to 100 mL of distilled water at 70 °C under stirring for 45 min. After complete mixing of the solution, glycerol was added (based on CMC weight, 50% v/w) as the plasticizer. The mixed solution was cast in the center of the plate with 177 cm<sup>2</sup> circular area and 15 cm diameter and was dried for 25 h at 30 °C. The edible films were ingathered from the plate, stored in the package at 50% relative humidity (RH), and kept at 25°C for 48 h. To produce composite films, desired nanochitin concentrations (0.1%, 0.5%, and 1% w/w based on solid polymer) were blended with CMC solution, then the solution was homogenized at 2300 rpm (IKA T25-Digital Ultra Turrax, Stavfen Germany). Next, 50 mL of the nanochitin/CMC suspension was poured into plates and then dried at 30°C for 20h. The dried nanochitin film were cut and peeled from the plate and held in polyethylene bags until analyses (Savadekaret al., 2012; Shojaee-Aliabadi et al., 2013).

### 2.4. Film characterization

#### 2.4.1. Film thickness

Film thickness was measured by using a digital micrometer (Mitutoyo No 293-766, Tokyo, Japan) which was used later in mechanical properties and water vapor permeability calculations. At least eight measurements were randomly carried out from different segments of the film.

#### 2.4.2. Mechanical properties

The film samples were cut and partied in a desiccator with 53% RH for 48 h at 25 °C entailing saturated solutions Mg (NO<sub>3</sub>)<sub>2</sub> before testing. Elongation at break (EB) and tensile strength (TS) were determined with a Testometric Machine M350-10TC (Testometric Co., ltd., Rochdale, Lance., England) according to ASTM D882. The initial grip and cross-head speed of separation were regulated to 50 mm and 50 mm/min, respectively. The TS (MPa) was determined by dividing the maximum load (N) by the initial cross-sectional area (m<sup>2</sup>) and EB (%) was measured by dividing the extension as a

fraction of the treatment by the initial length of the sample (Shojaee-Aliabadi et al., 2013).

#### 2.4.3. Water vapor permeability (WVP)

WVP of the sample was determined gravimetrically according to ASTM (2007). The circular cup was sealed stiff and filled using anhydrous calcium chloride desiccant (0 % RH), and the surface of the cup was sealed film with molten paraffin. Then the cells were held in a desiccator maintained at 25 °C and 52.8% RH containing magnesium nitrate. The various humidity levels of the films on both sides expressed a vapor pressure response of 1753.55 pa. Weight changes of cups were measured every 1.0 h during the test, using a digital scale (Sartorius Co., Model 120S, Germany ± 0.0001 g). Finally, WVP and water vapor transition (WVTR) were calculated as following equation (1) and (2):

$$\text{WVTR} = \text{Curve slope} / \text{Film area} \quad (1)$$

$$\text{WVP} = \text{Thickness} \times \text{WVTR} / \text{Pressure different} \quad (2)$$

#### 2.4.4. X-ray diffraction (XRD)

XRD patterns were obtained by a Philips x'pert MPD Diffract meter using Cu k- $\alpha$  radiation with wavelength of 1.7889 Å, operated at 40 kV and 40 mA. Samples were tested between  $2\theta = 10-100^\circ$  with speed of 1°/min at room temperature. Sample angular crystallinity range was obtained as a rapport between crystalline peak areas and the total peak area (Panaitescu et al., 2016).

#### 2.4.5. Differential scanning calorimeter (DSC)

Thermal properties of CMC/nanofiller were studied by differential scanning calorimeter (DSC, 500-B). Heating rate was 10 °C / min at a temperature range from 25 to 300 °C (Azeredo et al., 2010).

#### 2.4.6. Water solubility (WS)

Dried films were plunged into 50 mL of distilled water at 25 °C for 12 h. Subsequently, films were filtered by Whatman NO.1 paper and come through filter paper, and dried in oven at 110 °C to reach constant weight. WS was calculated by using the following equation (3) (Dashipour et al., 2015):

$$\text{WS} (\%) = \text{Initial dry weight} - \text{final dry weight} \times 100 / \text{Initial dry weight} \quad (3)$$

#### 2.4.7. Moisture content (MC)

To this film (3cm×1cm), empty cachets pans were placed for 1h in the oven at 110 °C to reach a constant weight. The samples were located in the cachets pan and weighed finally.

The cachets pans were measured in the oven at 110 °C to reach the constant weight. Moisture contents of sample were measured by equation 4 (Dashipour et al., 2015; Deng et al., 2020)

$$\text{MC}_{\text{wb}} = \text{wet sample weight} - \text{Dry sample weight} \times 100 / \text{wet sample weight} \quad (4)$$

#### 2.4.8. Moisture absorption (MA)

The nanocomposite films (2cm×2cm) were put into a desiccator containing CaCO<sub>2</sub> (RH=0%) for 7 days. The film was placed in a desiccator containing saturated NaNO<sub>3</sub> mixed solution (RH 74%).

The MA of films was calculated using the formula (Chaichi et al., 2017):

$$\text{MA} (\%) = \text{final weight} - \text{initial weight} \times 100 / \text{initial weight} \quad (5)$$

#### 2.4.9. Antibacterial activity

The antimicrobial activity was obtained by agar-diffusion method. Films (6mm diameter) were loaded on sterile plates containing MHA (Mueller Hinton Agar). The MHA culture was made from 100 µl of previously prepared MHB (Mueller Hinton Broth) culture (10<sup>8</sup> CFU/mL) of the bacteria. Petri dishes of samples were incubated for 24 h at 30 °C (Shojaee-Aliabadi et al., 2013).

$$\text{Antimicrobial Index} = \text{Area zone of inhibition} - \text{Area of film} / \text{Area of film} \quad (6)$$

#### 2.5. Statistical analysis

Each test was replicated three times and the statistical analysis was carried out using SPSS software version 20. Mean and standard deviations obtained from the analysis and significance were determined by Duncan's ( $p < 0.05$ ) test.

### 3. Results and Discussion

#### 3.1. Water vapor permeability

To prevent migration of water to the food, is the most important property of edible films packaging. The combination of nanofillers and polymers can be used as a method to inhibit water transfer (de Moura et al., 2009). Table 1 shows the WVP of CMC-nanochitin at different concentrations. The WVP of neat CMC film (control film) was  $0.78 \times 10^{-10} \text{ g s}^{-1} \text{ m}^{-1} \text{ Pa}^{-1}$ , WVP of CMC-nanochitin effective decrease when 0.1, 0.5 and 1% of nanochitin were added nanoparticles. Salaberria et al. (2015) reported the addition of chitin nanofiber into (5-20%) into thermoplastic starch shares for water molecules a tortuosity path to pass across. They express two reasons: (i) the existence of intensive NH<sub>2</sub> groups and more affinity with OH groups for water at the surface nanochitin (ii) also the existence of chitin nanofiber cause to agglomerate, and consequence decrease the operative value of nanochitin in polymer matrix (Salaberria et al., 2015). Oun et al. (2017) reported that the WVP value of CMC film was  $1.98 \times 10^{-9} \text{ g m} / \text{m}^2 \text{ Pa s}$ . The WVP values of CMC film reinforced with chitin nanocrystals (ChNCs) up to 1 wt. % decreased. This phenomenon was attributed to created tortuous path and hinder the movement of water molecules for less hydrophilic ChNCs in the network CMC. With a higher content of ChNCs (5 and 10 wt. %) WVP values increased. This increase in values of CMC/ChNCs was due to the agglomeration of ChNCs. Qin et al. (2016) studied that the WVP of maize starch (MS) film was  $5.32 \times 10^{-12} \text{ g} / \text{m}^1 \text{ Pa s}^1$ . The addition of chitin nano-whiskers (0.5, 1, 2, and 5%) in MS film decreased from  $2.54 \times 10^{-12} \text{ g} / \text{m}^1 \text{ Pa s}^1$ . At low CNWs content, the CNWs dispersed well and had fewer path for vapor permeation in the network film.

#### 3.2. Mechanical properties

Table 1 shows the data of nanochitin/CMC on mechanical properties of edible films. The tensile strength (TS) of CMC (control films) was found to be 4.33 MPa but the mixed nanochitosan films with enhanced content of 0.1 to 0.5 % w/w, increased the TS amount

up to 4.98 and 24.59 MPa respectively. This may be interaction between nanochitin and CMC. When high content of nano chitin (1% w/w) was added, the TS decreased to 15.54 MPa, the percentage elongation demonstrating for the pure CMC film to be 201.73, and 92.45, 15.02 for the 0.1 and 0.5% w/w of nanofiller addition respectively. When more nanochitin (1% w/w) is mixed with the film matrix, EB value to be inclined and trend to plateau.

Table 1. Mechanical properties of nanochitin (NC) content of the edible films.

Sample	WVP ( $g\ s^{-1}\ m^{-1}\ Pa^{-1}\ \times\ 10^{-10}$ )	TS (MPa)	EB (%)
CMC	0.78±0.005 <sup>a</sup>	4.33±0.18 <sup>a</sup>	201.73±0.1 <sup>a</sup>
CMC/0.1% NC	0.36±0.01 <sup>b</sup>	4.98±0.01 <sup>b</sup>	92.45±0.61 <sup>b</sup>
CMC/0.5% NC	0.34±0.03 <sup>bc</sup>	24.59±0.05 <sup>c</sup>	15.02±0.39 <sup>c</sup>
CMC/1% NC	0.30±0.04 <sup>c</sup>	15.54±0.41 <sup>d</sup>	53.94±0.78 <sup>d</sup>

NC: nanochitin

CMC: carboxymethyl cellulose

WVP: water vapor permeability

TS: tensile strength

EB: Elongation at break

a-b, means average values ± standard deviation, with different low letters in all columns are significantly different ( $p < 0.05$ )

Chang et al. (2010) obtained the effect of chitin nanoparticles (0-5 wt %) in starch film on the mechanical properties had an increased tensile strength and decreased percentage elongation, though at higher concentration (many than 5wt %) aggregation of chitin nanocomposite had a negative efficacy on mechanical properties (Chang et al., 2010). Similarly, Sahraee et al. (2017b) reported that used gelatin polymer on nanochitin. Due to improving of nanochitin on mechanical properties may be attributed higher stiffness and density of chitin in collation to protein matrix, also filling interval of amorphous regions, author a strong network and increase hydrogen bond and crystallinity in polymer matrix. Too with enhanced concentration of nanofiller polymer, cause to aggregate due to interaction of polymer and nanofiller.

### 3.3. X-ray diffraction (XRD)

The XRD plot of CMC and CMC/NC nanocomposite films is shown in Fig. 1. Native CMC (without nanoparticles) film displayed peak at  $2\theta = 23.94^\circ$ . Recent studies have reported that the characteristic crystalline peak of NC at around  $2\theta = 19.2^\circ$  (Chen et al., 2018; Shankar, et al., 2015). The peaks of CMC film with 0.1, 0.5, 1% of NC exhibited characteristic around at  $22^\circ$ . With an increase in the content of NC, the intensity of peak increased compared to the CMC film (Shankaret al., 2015). The relative crystallinity of the NC films showed that with increase NC concentration (0.1% to 1%) increased crystallinity of the nanocomposite film (Qin et al., 2016). Salaberria et al. (2015) reported that the increase of thermoplastic starch-based chitin nanocrystals (5%, 10%, and 20%) concentration, seemed to be an increase in crystallinity (retrogradation process), a consequence of the nucleating trace of chitin nanocrystals.

Ji et al. (2017) studied that the relative crystallinity at higher content chitin nano whiskers (0%, 2%, 4%, 6%, and 8%) increased. The crystallinity of nanofilm with increased concentration and compared to control film decreased, which is similar to the mechanical results.

Chang et al. (2010) reported that as compared crystallinity of chitin and chitin nanoparticles (CNP), degree crystallinity CNP decreased. During the production of CNP, some ingredients of crystallite commutation to region amorphous, and the percent crystallinity of chitin is higher than that of pure chitin.

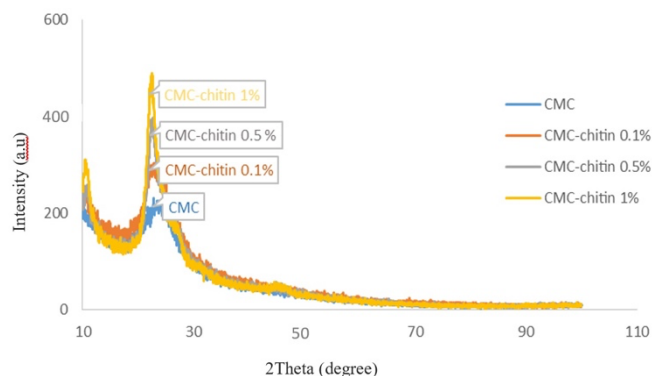


Fig. 1 XRD pattern for the carboxymethyl cellulose (CMC) with different concentrations (0.1, 0.5, and 1 %) of nanochitin (NC).

### 3.4. Differential scanning calorimeter (DSC)

The DSC thermograms obtained for the CMC (control) film and chitin 1% (CMC/NC 1%) are presented in Fig. 2. Thermal property revealed that did difference was significant between CMC (206.31 °C) and CMC/nanochitin 1% (or chitin1%) (204.81 °C). Shankar et al. (2015) reported that the TGA results as compared between chitin and nanochitin show that chitin was high thermostable than nanochitin. It may be caused by the presence of sulfate groups through chitin nanofibrils pending acid hydrolysis. This sulfate group's presence on the external of chitin leads to dehydration and decreases thermal stability.

The thickness of CMC films increased with the addition of nanochitin, which varied from 0.013 to 0.015 mm, which caused the increase in solid volume.

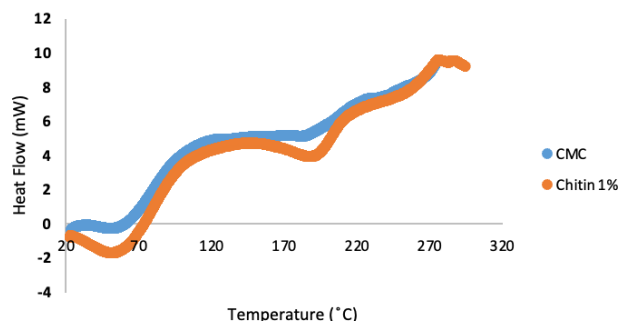


Fig. 2 The DSC thermograms of CMC and chitin 1% (CMC/ NC 1%) nanocomposite.

### 3.5. Physical properties

Table 2 shows the results of varied nano chitin-based CMC film on the physical properties. The thickness of CMC films increased with the addition of nanochitin ranging from 0.013 to 0.015 mm, which caused the increase in solid volume (Shojaee-Aliabadi et al., 2013). This report was similar Shojaee-Aliabadi et al. (2013) Kappa-

carrageenan edible film with nanoclay 3-15% ranging from 0.032-0.037  $\mu\text{m}$ .

The WS of CMC pure films was 91%. When nanoparticle was added, this value decreased in different concentrations of nanofiller. This can decrease amino groups which facilitate the reaction with hydrogen bond molecules (Rubentheren et al., 2015). Similarly in this study was reported by Abdollahi et al. (2013) on alginate biopolymer and cellulose nanofiller and Sanuja et al. (2015) on chitosan-neem oil and nano zinc oxide, because the reaction between polymer matrix and nanofiller and also decrease hydrophilic group (Abdollahi et al., 2013; Sanuja, Agalya et al., 2015). Sahraee et al. (2017a) also reported that the reduced WS with increase content nano chitin (3%, 5%, and 10%) based gelatin film.

MC of the nanofiller films was observed lower than that of neat films. Shojaee et al. (2013) reported that in reduce effect MC with increased concentration nanoclay on polymer kappa-carrageenan, can be regarded dispersion process of the nanofiller, that decreased the usability of hydroxyl groups to interplay with water, hence, decreased the films moisture content.

Dehnad et al. (2014) similar obtained results on chitosan and nanocellulose. The MA of control film was 36.12%, the mix of chitin/CMC decreased from 31.31% to 16.28%, with an addition of the nanofiller content from 0.1 to 1% nanofiller. Chaichi et al. (2017) expressed that this may be a strong interaction between filler-matrix. Furthermore, good homogenization of nanofiller at this surface.

Table 2. Effect of nanochitin (NC) content on properties of CMC films.

Sample	Thickness			
	(mm)	WS (%)	MC (%)	MA (%)
CMC	0.013 $\pm$ 0.001 <sup>a</sup>	91 $\pm$ 1.20 <sup>a</sup>	22 $\pm$ 0.11 <sup>a</sup>	36.12 $\pm$ 1.02 <sup>a</sup>
CMC/0.1% NC	0.014 $\pm$ 0.0005 <sup>a</sup>	64 $\pm$ 0.36 <sup>b</sup>	21 $\pm$ 0.45 <sup>a</sup>	31.31 $\pm$ 1.13 <sup>b</sup>
CMC/0.5% NC	0.014 $\pm$ 0.003 <sup>a</sup>	49 $\pm$ 0.24 <sup>c</sup>	19 $\pm$ 0.62 <sup>b</sup>	31.17 $\pm$ 0.04 <sup>b</sup>
CMC/1% NC	0.0158 $\pm$ 0.004 <sup>a</sup>	17 $\pm$ 0.34 <sup>d</sup>	14 $\pm$ 0.25 <sup>c</sup>	16.28 $\pm$ 0.11 <sup>c</sup>

NC: nanochitin

CMC: carboxymethyl cellulose

WS: water solubility

MC: Moisture content

MA: moisture absorption

a-d, means average values  $\pm$  standard deviation, with different low letters in all columns are significantly different ( $p < 0.05$ )

### 3.6. Antibacterial activity

The antibacterial activity of pure CMC and CMC/NC is shown in Table 3. Neat CMC film did not inhibition zone around the disk, nanochitin acts as antibacterial activity against *E. coli* and *S. aureus*, and no inhibition zone was observed for *B. cereus*, *S. typhimurium* and *P.aeruginosa*. Shanker et al. (2015) reported that polymer/nanochitin presumably creates flocculate the bacteria and prohibits growth bacteria through loss oxygen and nutrients. The other antibacterial mechanism includes interaction between amino group's chemical chitin structure and the anionic groups, bacteria cell wall, which leads to disrupt and membrane permeability of cell membrane activity (Sahraee et al., 2017a). Oun et al. (2017) reported that the antimicrobial activity of chitin nanowhiskers combination CMC sowed that low antimicrobial activity and any antimicrobial activity effect against *E. coli* and *L. monocytogenes*, respectively.

Also, Li et al. (2016) reported that the antimicrobial activity of chitin nanofibers reinforced CMC film against *S. aureus* and *E. coli*. The zone inhibition for 5 and 10 wt% were 0.2/0 and 0.6/0.2 against gram negative (*E. coli*) and gram positive (*S. aureus*). Sahraee et al. (2017b) studied the antifungal activity of gelatin film reinforced nanochitin (0, 3%, 5%, and 10%) against *Aspergillus niger*. Increasing content of nanochitin 3 and 5% enhanced the inhibition zone, 6.56 mm and 15.72 mm, respectively. At concentration 10% the inhibition smaller zone was 4.78 mm.

Table 3. Antimicrobial activities of CMC/nanochitin (NC) film in testing.

Film	Inhibition zone (mm)				
	<i>P. aeruginosa</i>	<i>S. enteritidis</i>	<i>E. coli</i>	<i>B. cereus</i>	<i>S. aureus</i>
CMC	0	0	0 <sup>a</sup>	0	0 <sup>a</sup>
CMC/0.1% NC	0	0	1.33 $\pm$ 0.01 <sup>b</sup>	0	0 <sup>a</sup>
CMC/0.5% NC	0	0	1.66 $\pm$ 0.00 <sup>c</sup>	0	0 <sup>a</sup>
CMC/1% NC	0	0	3.66 $\pm$ 0.03 <sup>d</sup>	0	3.00 $\pm$ 0.70 <sup>b</sup>

NC: nanochitin

CMC: carboxymethyl cellulose

a-b, means average values  $\pm$  standard deviation, with different low letters in all columns are significantly different ( $p < 0.05$ )

## 4. Conclusion

In this study, different concentrations of NC (0.1, 0.5, and 1% w/w) were incorporated to the CMC matrix for the reinforcement of film. It was found that an increase of NC up to 1% significantly improved the mechanical properties of composites compared to the control film and also WVP decreased for film containing 1%. In addition, by increasing the amount of NC up to 1%, physical properties of composite films reduced significantly. The value of WS, MC, and MA were 17%, 14%, and 16% respectively. Analysis of thermal properties indicated no significant effect of NC on glass transition temperature. The Crystallinity percent CMC/NC was lowest toward CMC with addition nanofiller into polymer. The X-ray Scherrer formula crystalline size in highest content for CMC/NC 1% and CMC were 0.77 nm and 0.31 nm. Antimicrobial activity CMC/NC exhibit only was against *E. coli* (3.66 mm) and *S. aureus* (3.00 mm). Therefore, based on the overall results, it was concluded that 1% NC /CMC film had the best properties and can be considered as an effective strengthening strategy to use CMC based materials for food packaging applications.

## Conflict of interest

The authors declare that there is no conflict of interest.

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