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Impact of Aluminum Oxide and Silica Oxide Nanocomposite on Foodborne Pathogens in Chicken Fillets

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Authors' contributions

This work was carried out in collaboration among all authors. Authors AGO and MKM designed the study, did the experiments and analyses and wrote the first draft of the manuscript. Authors AIE and AAA managed the analyses of the study. Author MHM performed the statistical analysis and managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Nanotechnology is an innovative technology for improving food quality and safety.
Aims: The aim of this study was to evaluate the efficacy of hydroxy propyl methyl cellulose (HPMC) films containing nanoparticles against three foodborne pathogens.
Design of the Study: *This study was designed using two nanoparticles i.e. (Al_2O_3-NPs and SiO_2-NPs), edible film (HPMC), and three foodborne pathogens i.e. *Bacillus cereus*, *Staphylococcus aureus,* and *Salmonella* Typhimurium. Both nanoparticles were evaluated against foodborne pathogens as well applied in chicken fillets.
Place and Duration: All experiments were done in the Food Technology Department, Benha University, Egypt; Nanomaterial Laboratory, Beni-Suef University, Egypt; and Agricultural Research Center, Egypt and were done within three months.
Methodology: The preparation of edible films, the antimicrobial activity, mode of antimicrobial action, challenge study, and scanning electron microscopy had been carried out in different laboratories. As well the mechanical properties of the HPMC films were evaluated.
Results: The results obtained from this study showed that the nanoparticles (~80 nm) at 80 ppm were active against *Bacillus cereus*, *Staphylococcus aureus,* and *Salmonella* Typhimurium compared with 20 and 40 ppm. The HPMC films including Al_2O_3-NPs were active against *B. cereus* than *S. aureus* and *S. typhimurium*, while the SiO_2-NPs were more effective against *S. typhimurium* and *B. cereus* compared with *S. aureus*. In challenge studies, HPMC films including

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Al_2O_3-NPs and SiO_2-NPs at 80 ppm decreased the viability of the three-foodborne pathogens associated with chicken fillets stored at 4±1°C for 15 days, as compared with the control sample. HPMC films incorporated with nanoparticles inhibited the microbial population ~ 2-3 log_{10} CFU/cm^2 over the chicken fillets during storage period.

Conclusion: This work indicated that, HPMC films incorporated with Al_2O_3-NPs and SiO_2-NPs (~80 nm) at 80 ppm could be reduce the microbiological loads of the refrigerated chicken fillets.

Keywords: Antimicrobial activity; HPMC edible film; nanoparticles; chicken fillets; cold storage.

1. INTRODUCTION

Foodborne pathogens are one of the important biological hazards which causes a lot of diseases, harmful in food products and leading to lose much money [1]. According to Center for Disease Control and Prevention report, foodborne diseases account for approximately 48 million illnesses, 128000 hospitalizations and 3000 deaths cases each year. as well, cost 15.6 billion $ each year in the United States [2]. Five foodborne pathogens record about (88%) of food poisons: *Norovirus* (26%), *Salmonella nontyphoidal* (35%), *Campylobacter* (15%), *E. coli* (STEC) O157: H7: (4%), and *Toxoplasma gondii* (8%). Moreover, twenty food products recalled from markets in which exposure occurred in one state such as apple cider, bread, chicken, drink mix, ground beef, muffins, pork, raw tuna, and roast beef [2].

Recently, nanotechnology have many applications in the food sector particularly the food industry, quality and safety [3]. These applications used to improve food safety and extend the shelf-life of food products [4]. Nanoparticles one of the most types utilized in food safety as antimicrobial and supplementation. As well, inorganic nanoparticles as antimicrobial have taken more attention against foodborne pathogens i.e. aluminium oxide nanoparticles (Al_2O_3-NPs) and silica oxide nanoparticles (SiO_2-NPs) [5].

Al_2O_3-NPs food grade are non-toxic, active against foodborne pathogens and approved by FDA as indirect additive [6]. Al_2O_3 NPs at 1000 mg ml^{-1} significantly inhibits *Escherichia coli* growth in ready to eat food products [7]. One study demonstrated Al_2O_3-NPs incorporated with poly vinylidene fluoride films reduced the *E. coli* growth [8]. A study conducted by the author [9] reported that, aluminium oxide nanoparticles were active against *Salmonella* Typhimurium, *Listeria monocytogenes, Fusarium oxysporum, Chromo bacterium violaceum,* and *Aspergillus flavus*.

Food grade SiO_2-NPs are non- toxic, anticaking, had been used as a food additive and permitted by FDA [10]. Oregano silane containing SiO_2-NPs had been reported to prevent biofilm formation of foodborne pathogens [11]. SiO_2-NPs reduce foodborne pathogens growth and make significate changes in cell morphology such as *Salmonella enterica* [12].

Hydroxy propyl methyl cellulose (HPMC) edible film approved by FDA for food packaging (21 CFR 172.8741). It have a good characters such as tasteless and odourless, transparent, and barrier [13]. As well, HPMC films including polylactic acid and incorporated with green tea extract nanoparticles improved shelf-life of fatty foods [14]. Additionally, HPMC films contained TiO_2 nanoparticles were inhibited *E. coli* and *S. aureus* growth [15].

In Egypt, chicken products consumption is increasing nowadays for many reasons: such as, highly nutrition value, easily nutrients absorption in human body, high productivity, low price and availability, and easy to cook according to FAO [16].

The aims of this study were (a) to improve the quality and safety of chicken fillets; (b) the development of the food packaging systems; and (c) extending the shelf-life of chicken fillets, by using (d) novel food grade products such as antimicrobials.

2. MATERIALS AND METHODS

2.1 Bacterial Strains

Three bacterial strains utilized in this work were purchased from the American Type Culture Collection (ATCC) i.e. *Bacillus cereus* (ATCC 10876), *Staphylococcus aureus* (ATCC 11988), and *Salmonella.* Typhimurium (ATCC 14028). The strains activated at Food Technology Department, Benha University, Egypt. All strains were cultivated twice on Tryptic Soy Agar (TSB; Bio-life company, Italy) at 37°C for 24 h, and kept at 4°C till using [17].

2.2 Antimicrobials Agents

Food-grade aluminium oxide nanoparticles (Al_2O_3-NPs), and silica oxide nanoparticles (SiO_2-NPs) at (~80 nm) were obtained from Nanomaterial Laboratory, Beni-Suef University, Egypt.

2.3 Preparation of Hydroxy Propyl Methyl Cellulose (HPMC) Films

Hydroxy propyl methyl cellulose films (HPMC) were prepared according to the following. Briefly, 4 g of HPMC was dissolved in 100 mL distilled water at 70ºC with stirring at 1000 rpm/min for 2 h. A 1`mL of glycerol 30% was added with stirring at 1000 rpm/min for 30 min. The nanoparticles were added and stirred at 1000 rpm/min for 15 min. The solution was sterilized at (121ºC/15 min). Then, casted and dried, as well, kept under cold storage till utilizing [15].

2.4 Antimicrobial Activity of Nanoparticles against Foodborne Pathogens

Antimicrobial activity of nanoparticles was evaluated by disk diffusion method on tryptic soy agar media (TSA). In briefly, different concentration of nanoparticles i.e. 20, 40 and 80 ppm against foodborne pathogens. Add 10µl from bacterial strains. Then, 100µl from nanoparticles agent were added. Afterwards, the dishes put into the incubator at 37°C for 48 h. At the end of incubation period, clear zones were appeared and measured by ruler [18].

2.5 Mode of Nanoparticles Action against Bacterial Strains

The mode of action was done according to He et al. [11] with slight modifications. Briefly, 2 mL of sterilized Tryptic Soy Broth (TSB) were added. 1 mL of bacterial strain and 1 mL of antimicrobial agent were added. After that, the tubes were incubated at 37°C for 24 h. Then, the pellets were collected by centrifuge at 2500 rpm/min for 10 min. Finally, all glass slices were prepared by washing by acetone and methanol, then spread the cells onto glass slices with drying at 37ºC for 15 min and examining by scanning electron microscopy.

2.6 Challenge Study

Raw chicken fillets were purchased from local market in Cairo, Egypt. The fillets were transferred in the ice box to the laboratory, and freshly used. The fillets were cut down (5 × 5 cm) sections under sterilized conditions. Then, the samples treated with ultraviolet light (UV) at 260 nm for 15 min to decrease the bacterial population. Chicken fillets were inoculated for 24 h by aseptically diluted cultures of *S. typhimurium*, *S. aureus* and *B. cereus* approximately 5 $\log_{10}$ CFU/cm^2 on the surface. After impregnation, the samples were kept at 25 ± 1ºC for 20 min to allow cell attachment. Then, raw chicken fillets were coated with HPMC films (5 × 5 cm) incorporated with nanoparticles. Control samples covered by control HPMC films. After 0, 3, 6, 9,12 and 15 days, the samples were tested to determine the remain microbial colonies. 1mL was spread plated in duplicate onto brilliant green agar for *S. typhimurium*, paired parker (M043) for *S. aureus*, *Bacillus cereus* agar base (M833) for *B. cereus* to demonstrate microbial growth. Resulting colonies were counted after 24:48 h incubation at 37°C, populations measured by $\log_{10,}$ and expressed as $\log_{10}$ CFU/cm^2 [19].

2.7 Scanning Electron Microscope (SEM) of HPMC Films

Hitachi S-4700 scanning electron microscope (Hitachi, Toronto, Ontario, Canada) was used to study the morphology of nanoparticles and films. The samples were deposited onto aluminium specimen stubs using double-stick carbon tabs (Ted Pella Inc,. Redding, CA, USA) and coated with gold/palladium on an ion sputter coated (Denton Vacuum Inc., Moorestown, NJ, USA) for 45 s at 20 mA. All samples were examined using an accelerating beam at a voltage of 1.5 kV. Magnifications of 40,000x ;and 60,000x were used [20].

2.8 Film Solubility and Thickness Characterization

The solubility of films in water were studied. Thickness was determined by using digital micro meter model 7326 (Mitutoyo Manufacturing, Tokyo, Japan) at 6 different positions on the film according to [21].

2.9 Tensile of HPMC Films Determination

The tensile of films were determined by Texture Analyzer TA.XT2 (Stable Micro System, Surrey, UK), according to the ASTM Standard Method D 88283 (initial grip separation = 50 mm and cross-head speed = 100 mm/min) according to de Moura et al. [20].

2.10 Water Vapour Permeability

Water vapour permeability was evaluated by ASTM E96-92 gravimetric method with some modifications to measure the relative humidity (RH) of HPMC films according to Du et al. [22]. Water vapour permeability was calculated according to following relation: WVP=$\frac{WVTR}{(P2-P3)}y$

Where WVTR was obtained from the slope of the weight loss rate through the film surface and p^2 was the water vapour partial pressure on the film underside. p^3 was water vapour partial pressure at the film underside, y the average film thickness. Water vapour permeability of each film was measured as the mean and standard deviations of 5 replications.

2.11 Gases Vapour Permeability (O2 and CO2)

The gas vapour permeability was determined at 30ºC in a designed stainless cell by gas testing instrument, model Witt Ox baby headspace gas analyzer (O_2/CO_2) following the method described by the following equation: P = $\frac{Q.X}{A.t\Delta p}$

The gas permeability (P) was calculated according to Du et al. [22].

Where, P is the permeability of gas, (m^3/m. day. mmHg), Q is the quantity of gas diffused m^3, X is the thickness of the film, A an area of the film, m^2, t is the time, day and Δp is the pressure difference across the film.

2.12 Statistical Analysis

The challenge study, statistical analyses for bacterial growth were carried out utilizing one-way ANOVA with a significate value of $P \leq 0.05$ by using SPSS software, var. 18 (IBM; Armonk, N.Y., U.S.A.). Results were analyzed as a completely randomized design according to Steel et al. [23]. All challenge experiments were performed in triplicate, using 3 samples per treatment. Multiple comparisons were carried out applying the least significant difference and Tukey's test.

3. RESULTS AND DISCUSSION

3.1 Antimicrobial Activity of Nanoparticles against Foodborne Pathogens

As shown in Table 1 and 2. The antibacterial activity of inorganic nanoparticles i.e. aluminium oxide nanoparticles (Al_2O_3-NPs) and silica oxide nanoparticles (SiO_2-NPs) against foodborne pathogens such as *Bacillus cereus, Salmonella* Typhimurium and *Staphylococcus aureus* were evaluated. The results showed that Al_2O_3-NPs and SiO_2-NPs (~80 nm) at 80 ppm were effective against foodborne pathogens i.e. *B. cereus*, *S. typhimurium* and *S. aureus*, than 20 and 40 ppm respectively, the results were partially agreement with [24]. Moreover, Al_2O_3-NPs were more active against *B. cereus* and *S. aureus* than *S. typhimurium,* the results were partially agreement with the author [25]. In addition, SiO_2-NPs were more active against *B. cereus,* and *S. typhimurium* compared *S. aureus* that is not it at all by Gkana et al. [26]. The results indicated that the Al_2O_3-NPs were more active against spores and gram-positive than gram-negative bacteria, while SiO_2-NPs more effective against gram-negative and spores compared with gram-positive bacteria. The results are agreement with data reported by Allen [27].

Furthermore, according to Table 3, the effect of hydroxy propyl methyl cellulose (HPMC) edible films incorporated with nanoparticles were decreased *B. cereus, S. aureus* and *S. typhimurium* population growth. The results showed that, Al_2O_3-NPs were inhibited *B. cereus* and *S. aureus* growth than *S. typhimurium*. Although, SiO_2-NPs less effective against *S. aureus* than *B. cereus,* and *S. typhimurium*. The results were similar to the results obtained by Ansari et al. [28].

Table 1. Antibacterial activity of Al_2O_3-NPs and SiO_2-NPs nanoparticles (~80 nm) at different concentration against foodborne pathogens

Bacterial strains	Al_2O_3-NPs			SiO_2-NPs		
	20 ppm	40 ppm	80 ppm	20 ppm	40 ppm	80 ppm
S. typhimurium	9±0.3	11±0.3	13±0.2	11±0.3	15±0.2	18±0.3
S. aureus	8±0.3	12±0.3	14±0.3	12±0.3	13±0.3	16±0.3
B. cereus	ND	12±0.3	15±0.3	13±0.3	15±0.3	18±0.3

ND: Not Detect; Al_2O_3-NPs: Aluminum oxide nanoparticles; SiO_2-NPs: Silica oxide nanoparticles

Table 2. Antibacterial activity of Al_2O_3-NPs and SiO_2-NPs nanoparticles (~80 nm) at 80 ppm against foodborne pathogens

Bacterial strains	Nanoparticles agents	
	Al_2O_3-NPs	SiO_2-NPs
S. typhimurium	13±0.2	18±0.3
S. aureus	14±0.3	16±0.3
B. cereus	15±0.3	18±0.3

Al_2O_3-NPs: Aluminum oxide nanoparticles; SiO_2-NPs: Silica oxide nanoparticles

Table 3. Antibacterial activity of HPMC film incorporation with nanoparticles (~80 nm) at 80 ppm against foodborne pathogens

Bacterial strains	HPMC films incorporation nanoparticles	
	Al_2O_3-NPs	SiO_2-NPs
S. typhimurium	16±0.2	22±0.4
S. aureus	17±0.3	20±0.3
B. cereus	18±0.3	22±0.4

HPMC: Hydroxy propyl methyl cellulose; Al_2O_3-NPs: Aluminum oxide nanoparticles; SiO_2-NPs: Silica oxide nanoparticles

3.2 Mode of Action Nanoparticles against Foodborne Pathogens

The mode of action it seems necessary because it presents all changesets in bacterial cells. Fig. 1, illustrated that, Al_2O_3-NPs were highly effective against gram-positive than gram-negative bacteria, this is reverting to the Al_2O_3-NPs action as follows, Al_2O_3-NPs interact with bacteria membrane and made changes in cell morphology such as (a) the formation of 'pits' in their cell wall. Moreover, made disruption and drastic in the cell wall. (b) As well, it produces reactive oxygen species (ROS) which allow to penetrate the cell membrane and lead the cell to death. (c) Moreover, causes cell oxidative stress and formed free-radical scavenging that is lead the bacteria to die that is reported by Khezerlou et al. [29].

In addition to, SiO_2-NPs more effective against gram-negative and spores than gram-positive bacteria. That is due to (a) the ability of SiO_2-NPs to make morphological changes, lose the cell to preform it in function role. (b) As well, reactive oxygen spices (ROS) generation, and lose the DNA function and lead to damage. (c) Additionally, cause the oxidative stress regulation in gens according to Hoseinnejad et al. [5].

3.3 Challenge Study

Based on the results of antimicrobial activity of HPMC films incorporated with nanoparticles, the films were utilized to cover raw chicken fillets at 4±1°C up to 15 days. Fig. 2, 3, and 4 reported that the bacterial population gradually grew during the storage period over 15 days, when used control films compared with the nanoparticles films. HPMC films including nanoparticles reduced the foodborne pathogens growth approximately 2:3 log_{10} during the challenge study. HPMC films include SiO_2-NPs were stronger antimicrobial against *B. cereus, S. typhimurium* and *S. aureus* than Al_2O_3-NPs on raw chicken fillets, these results are agreement with [29,9,30].

3.4 Scanning Electron Microscope of HPMC Films including Nanoparticles Agent

Fig. 5 showed that**,** the cross sections and surface appearance of the control film, which appear to be homogeneous, smooth, colourless and free of any dimples or crevices. The HPMC films incorporated with nanoparticles were completely dispersion. Al_2O_3-NPs and SiO_2-NPs loaded on films showed no pores with a smooth surface. The presence of these pores is likely due to the flocculation and coalescence of small drops during film preparation. Also, the nanoparticles distribution was found to be homogeneous in all films according to Aboud et al. [31].

3.5 Mechanical Properties of Films

As shown in Table. 4**,** the tensile, water vapour permeability oxygen vapour permeability and carbon dioxide vapour permeability were evaluated, HPMC films containing SiO_2-NPs

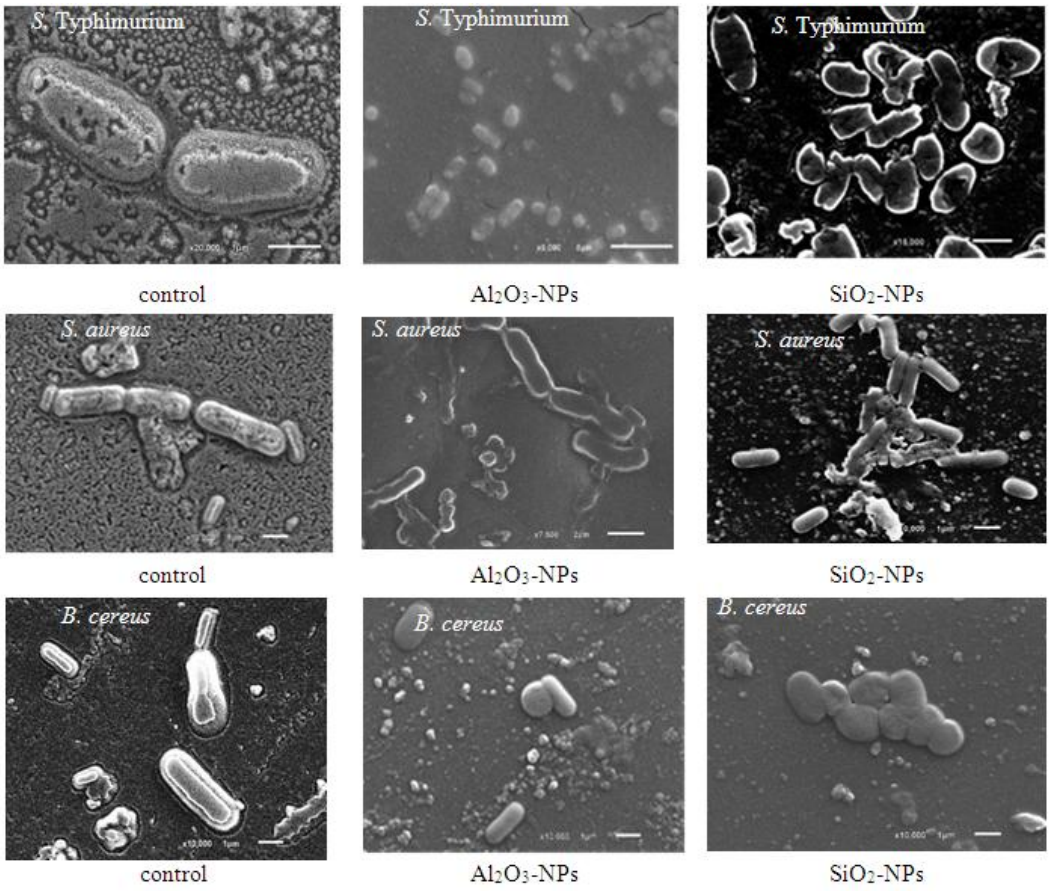

Fig.1. The mode of action of nanoparticles against foodborne pathogens using SEM

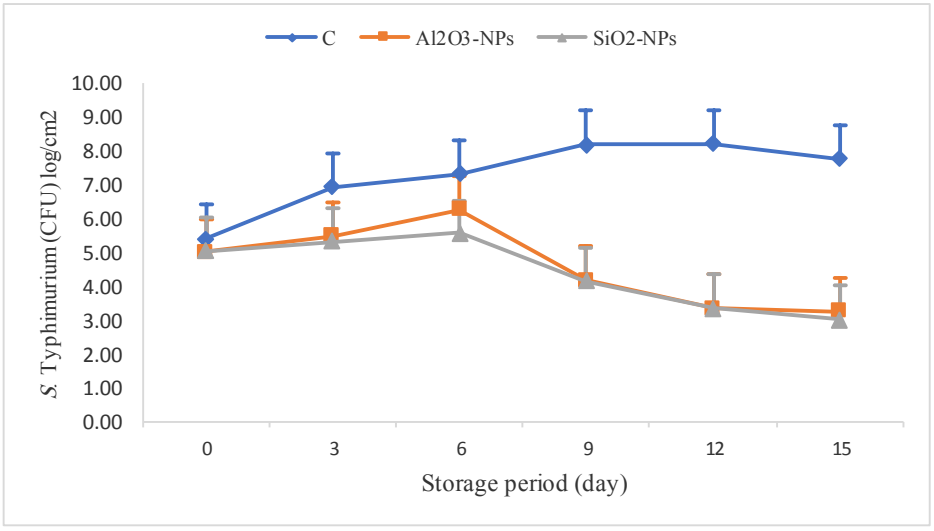

Fig. 2. Antimicrobial activity of HPMC film made with HPMC (40 g /L) and glycerol (10 g/L) and incorporated with nanoparticles against *S. typhimurium* on raw chicken fillets.

Table 4. Physical and mechanical properties of HPMC films incorporated with Al_2O_3-NPs and SiO_2-NPs

Samples	Properties (tests results)						
	Tensile (MPa)	Water vapor permeability (g mm K^{-1} Pa^{-1} h^{-1} m^{-2})	O_2 vapor permeability P (ml mm cm^{-2} s^{-1} cm Hg^{-1})	Co_2 vapor permeability P (ml mm cm^{-2} s^{-1} cm Hg^{-1})	Transparence	Thickness	Solubility
Control	38.1	0.108	0.188×10^{-8}	2.25×10^{-9}	0.065	0.5 mm	100%
HPMC- Al_2O_3-NPs	31.6	0.056	1.074×10^{-8}	1.44×10^{-9}	0.079	0.5mm	100%
HPM -SiO_2-NPs	43.17	0.541	2.17×10^{-8}	14.4×10^{-9}	0.082	0.51 mm	100%

HPMC: Hydroxy propyl methyl cellulose
Al_2O_3-NPs: Aluminum oxide nanoparticles
SiO_2-NPs: Silica oxide nanoparticles

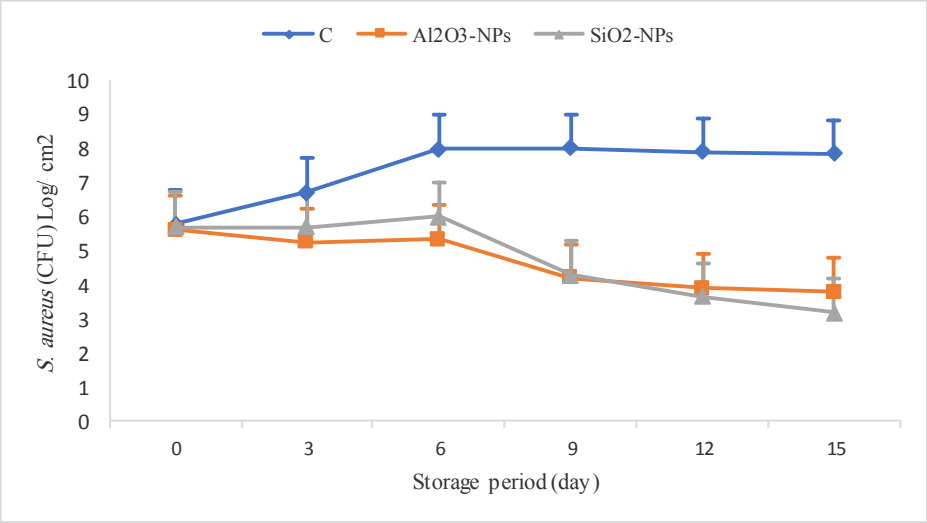

Fig. 3. Antimicrobial activity of HPMC film made with HPMC (40 g/L) and glycerol (10 g/L) and incorporated with nanoparticles against *S. aureus* on raw chicken fillets.

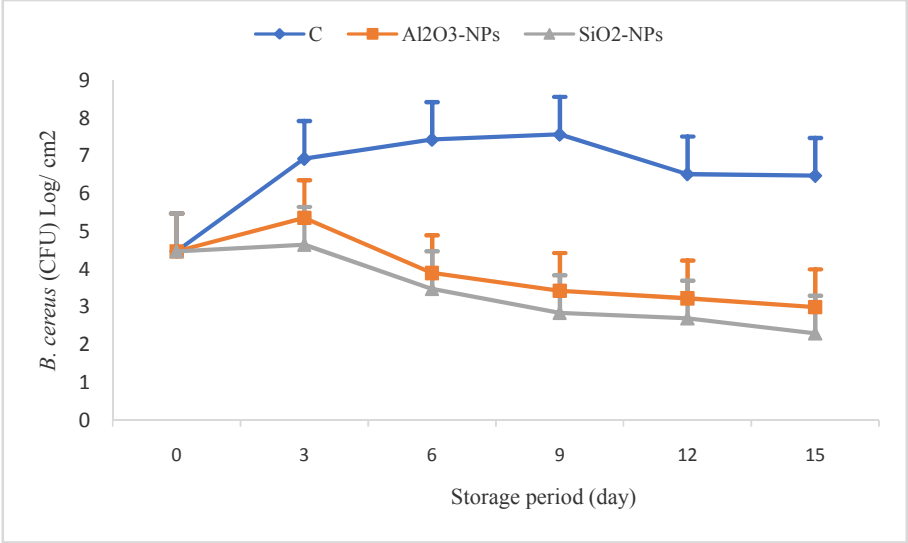

Fig. 4. Antimicrobial activity of HPMC film made with HPMC (40 g / L) and glycerol (10 g/L) and incorporated nanoparticles against *B. cereus* on raw chicken fillets.

were the highest values compared with HPMC films control and Al_2O_3-NPs films in mechanical properties. Additionally, SiO_2-NPs increased the films water vapour permeability, carbon dioxide vapour permeability, tensile, oxygen vapour permeability and formed a strong structure of films. That is due to (a) the ability of SiO_2-NPs to fill the pores between the HPMC films structure (b) HPMC diffusion with SiO_2-NPs and form homogenized structure (c) the ratio of glycerol and it is the ability to prevent water evaporation. As well, Al_2O_3-NPs were the lowest values and formed a weak structure, that is revert to the Al_2O_3-NPs cannot interference with HPMC films and there is heterogeneous distribution. In the control HPMC films, the transparency and thickness were the lowest values than Al_2O_3-NPs and SiO_2-NPs films. That is referred to the colour of nanoparticles and nanoparticles doses in films solution. Regarding solubility, there are non-significant results between HPMC films control and HPMC films including nanoparticles, there

are no previous works in this point, but these results were similar to the results were obtained by other authors [32,33,34]. Moreover, these results we had got from experimental.

4. CONCLUSION

The results of this investigation had demonstrated that HPMC films including Al_2O_3-NPs and SiO_2-NPs were active against foodborne pathogens such as *S. typhimurium, B. cereus* and *S. aureus* in chicken fillets. Additionally, nanoparticles (~80 nm) at 80 ppm showed a significant inhibition compared with 20 and 40 ppm respectively. Moreover, SiO_2-NPs has a stronger antimicrobial activity against foodborne pathogens than Al_2O_3-NPs. However, HPMC films incorporated with SiO_2-NPs had improved mechanical property than HPMC films combined with Al_2O_3-NPs. HPMC films containing nanoparticles have the potentials to increase the shelf-life of chicken fillets, by reducing the microbiological loads in the products. However, more researches are need to determine the stability of HPMC under different conditions and toxicological issues.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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