Reduction of some Food-Borne Pathogens in Chicken Fillets Using Aluminum and Silicon Nanocomposite

An assessment of Aluminium and Silicon Nanocomposite on some Food-Borne Pathogens associated with Chicken Fillets

Running head: Nanoparticles and improving quality of chicken fillets

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 3 foodborne pathogens.

Study design: Data collection study.

Place and Duration: All experiments were done in Food Technology Department, Benha University, Egypt; Nanomaterial Laboratory, Beni-Suef University, Egypt and Agricultural Research Center, Egypt and were done within ~~take~~ 3 months.

Methodology: All methods were collected by different references such as preparation edible film, antimicrobial activity, mode of action, challenge study and the scanning electron microscope (SEM) and mechanical properties of HPMC films were evaluated.

Results: The results obtained from this study showed that ~~In initial experiments,~~ 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 Al_2O_3-NPs and SiO_2-NPs at 80 ppm decreased the viability of the three ~~were highly decreasing the~~ 3 foodborne pathogens ~~growth~~ associated with chicken fillets stored at 4±1°C for ~~up to~~ 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 fillet during storage period.

Conclusion: This work indicated that ~~The results conducted that~~ HPMC films incorporated with nanoparticles (~ 80 nm) at 80 ppm could be enhanced the safety of refrigerated chicken fillets.

Keywords: antimicrobial activity, HPMC edible film, nanoparticles, chicken fillets, cold storage.

INTRODUCTION

Foodborne pathogen are one of the important biological hazards which caused a lot of disease, harmful in food products, and lose much money (17). According to Center for Disease Control and

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Prevention report, food-borne diseases account for approximately 48 million illnesses, 128000 hospitalizations and 3000 deaths cases, as well costed 15.6 billion $ each year in the United States (10). Five foodborne pathogens record about (88%) of food poisons: *Norovirus* (26%), *Salmonella nontyphoidal* (35%), *Campylobacter* (15%), *E. coli* (STEC) O157 (4%), and *Toxoplasma gondii* (8%). Moreover, twenty food products recalled in which exposure occurred in one state such as apple cider, bread, chicken, drink mix, ground beef, muffins, pork, raw tuna, and roast beef. (10).

Recently, nanotechnology have many applications in food sector particularly food industry, quality and safety (3). These applications used to improve food safety and extend shelf-life of food products (6). Nanoparticles one of the most shape utilized in food safety as antimicrobial and supplementation. As well, inorganic nanoparticles as antimicrobial have taken more attention against food-borne pathogens i.e. aluminum oxide nanoparticles (Al_2O_3-NPs) and silica oxide nanoparticles (SiO_2-NPs) (16).

Al_2O_3-NPs food grad are non-toxic, active against food-borne pathogens and permitted by FDA. Al_2O_3 NPs at 1000 mg ml^{-1} significantly affected ~~against the~~ *Escherichia coli* growth in ready to eat foods (22). One study demonstrated Al_2O_3-NPs incorporated with polyvinylidene fluoride films reduced the *E. coli* growth (33). A study conducted by the author (29) reported that iOther study found aluminum oxide nanoparticles were active against *Salmonella* Typhimurium, *Listeria monocytogenes, Fusarium oxysporum, Chromobacterium violaceum,* and *Aspergillus flavus* the result obtained by (29).

Food grade SiO_2-NPs are non- toxic, anticaking, has been used as food additive and permitted by FDA (7). Oregano silane containing SiO_2-NPs has been reported to prevented biofilm formation of food-borne pathogens (14). SiO_2-NPs reduce food-borne pathogens growth and make significate changes in cell morphology such as *Salmonella enterica* (32).

Hydroxy propyl methyl cellulose (HPMC) edible film is approved by the by FDA for food packaging (21 CFR 172.8741). It has a good characters such as tasteless and odorless, transparent, and barrier (31). As well, HPMC films including poly lactic acid and incorporated with green tea extract nanoparticles improved shelf-life of fatty foods (34). Additionally, HPMC films contained TiO_2 nanoparticles was reported to inhibited *E. coli* and *S. aureus* growth (26)

In Egypt, the chicken products consumption is growing up nowadays. That is revert to high nutritional value, available un expensive, and easy cooked, however, spoiled rapidly. The aim of this work was to evaluate nanoparticles i.e. Al_2O_3-NPs and SiO_2-NPs antimicrobials against food-borne pathogens in chicken fillets.

All over Goals

Improve the quality and safety of chicken fillets.

Development the packaging systems.

Extending the shelf-life of products.

Discovering a new antimicrobial.

2. MATERIALS AND METHODS

2.1. Bacterial strains

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Three bacterial strains utilized in this work were *Bacillus cereus* (ATCC 10876), *Staphylococcus aureus* (ATCC 11988), and *S.* 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 (18).

2.2. Antimicrobials agents

Food-grade aluminum 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 follow. Briefly, 4 % of HPMC was dissolved in 100 mL distilled water at 70 ºC with stirring at 1000 rpm/min for 2 h. A 1`mL of glycerol 30% was added with stirring at 1000 rpm 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 utilized (26).

2.4. Antimicrobial activity of nanoparticles against food-borne pathogens

Antimicrobial activity of nanoparticles was evaluated by disk diffusion method. In briefly, different concentration of nanoparticles i.e. 20, 40 and 80 ppm against food-borne pathogens. Add 10µl from bacterial strains. Then, 100µl from nanoparticles agent were added. Afterward, the dishes put in incubator at 37°C for 48 h. At the end of incubation time clear zones were appeared and measured by ruler (24).

2.5. Mode of nanoparticles action against bacterial strains

The mode of action was done according to (15) with slightly modification. Briefly, 2 ml of sterilized Tryptic Soy Broth (TSB) were added. 1 ml of bacterial strain and 1 mL of antimicrobial were added. After that, the tube was incubated ~~the tubes overnight~~ at 37°C for 24 h. Then, the pellets were collected by centrifuge at 2500 rpm for 10 min. Then, examined by scanning electron microscope after spread the cells onto a glass slices pre-washed with ethanol and acetone, and drying at 37 °C for 15 min.

2.6. Challenge study

Raw chicken fillets were purchased from local Cairo, Egypt. The fillets were transferred in ice box to laboratory, and freshly used. The fillets were cut down (5 × 5 cm) sections under sterilized conditions. Then, the samples treated with ultraviolet light (UV) for 15 min to decrease bacterial population. Chicken fillets were inoculated overnight 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 room temperature 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 remain microbial colonies. 1mL was spread plated in duplicate onto brilliant green agar for *S.* Typhimurium, paird 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 (21).

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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 aluminum 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 (11).

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2.8. Film solubility and thickness characterization

The solubility of films in water were studied. Thickness was determined by using digital micrometer model 7326 (Mitutoyo Manufacturing, Tokyo, Japan) at 6 different positions on the film according to (30).

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 (11).

2.10. Water vapor permeability

Water vapor permeability was evaluated by ASTM E96-92 gravimetric method with some modifications to measure the relative humidity (RH) of HPMC films according to (12). Water vapor permeability was calculated according to follow 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 vapor partial pressure on the film underside. p^3 was water vapor partial pressure at the film underside, y the average film thickness. Water vapor permeability of each film was measured as the mean and standard deviations of 5 replications.

2.11. Gases vapor permeability (O2 and CO2)

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

The gas permeability (P) was calculated according to (12).

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 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 ≤ 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 (28). All challenge experiments were performed in triplicate, using 3 samples per treatment. Multiple comparisons were carried out applying least significant difference and Tukey's test.

3. RESULTS AND DISCUSSION

3.1 Antimicrobial activity of nanoparticles against food-borne pathogens

As shown in **Table 1 and 2**. The antibacterial activity of inorganic nanoparticles i.e. aluminum oxide nanoparticles (Al_2O_3-NPs) and silica oxide nanoparticles (SiO_2-NPs) against food-borne pathogens such as *Bacillus cereus, Salmonella* Typhimurium and *Staphylococcus aureus* were evaluated. The result showed that ~~Results conducted that~~ Al_2O_3-NPs and SiO_2-NPs (~80 nm) at 80 ppm were effective against food-borne pathogens i.e. *B. cereus*, *S.* Typhimurium and *S. aureus*, than 20 and 40 ppm respectively, as reported by (9). Moreover, Al_2O_3-NPs were more active against *B. cereus* and *S. aureus* than *S.* Typhimurium that is agreement with (13). In addition, SiO_2-NPs were more active against *B. cereus,* and *S.* Typhimurium compared *S. aureus* that is partially agreement with (14). The results found 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 (4).

Furthermore, according to **Table 3,** the effect of hydroxy propyl methyl cellulose (HPMC) edible films incorporated with nanoparticles were reduced *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 agreement with data reported by (5)

3.2 Mode of action nanoparticles against foodborne pathogens

Based on the results of nanoparticles activity against food-borne pathogens, the mode of action it seems necessary. **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 cell wall. (b) As well, it produces reactive oxygen species (ROS) which allow to penetrate the cell membrane and led the cell to death. (c) Moreover, causes cell oxidative stress and formed free-radical scavenging that is led the bacteria to die that is reported by (19).

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 led to damage. (c) Additionally, cause the oxidative stress regulation in gens according to (16)

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,** demonstrated that the bacterial population was gradually increase during the storage period over 15 days, when used control films compared with the nanoparticles films. HPMC films including nanoparticles reduced the food-borne pathogens growth approximately 2:3 log_{10} during the challenge study. 20

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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 (19), (29) and (25).

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, colorless 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 films show no pores with 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 were found to be homogeneous in all films according to (1)**.**

3.5 Mechanical properties of films

As shown in **Table. 4,** the tensile, water vapor permeability oxygen vapor permeability and carbon dioxide vapor permeability were evaluated, HPMC films containing SiO_2-NPs 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 vapor permeability, carbon dioxide vapor permeability, tensile, oxygen vapor permeability and formed 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 ration of glycerol and it is 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 can not interference with HPMC films and there is heterogenous distribution. In the control HPMC films, the transparence and thickness, was the lowest values than Al_2O_3-NPs and SiO_2-NPs films. That is refer to the color of nanoparticles and nanoparticles doses in films solution. Regarding solubility, there are non-significant results between HPMC films control and HPMC films including nanoparticles, these data agreement (2), (23) and (27).

4. CONCLUSION

The results of this investigation ~~were~~ demonstrated that HPMC films including Al_2O_3-NPs and SiO_2-NPs were active against food-borne 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 ~~are~~ stronger antimicrobial activity against food-borne pathogens than Al_2O_3-NPs. However. HPMC films incorporated with SiO_2-NPs had a better mechanical property than HPMC films included Al_2O_3-NPs. HPMC films containing nanoparticles have ~~ia~~ longer ~~ncreasing the~~ shelf-life proprerty and improve ~~the~~ chicken fillets safety and quality.

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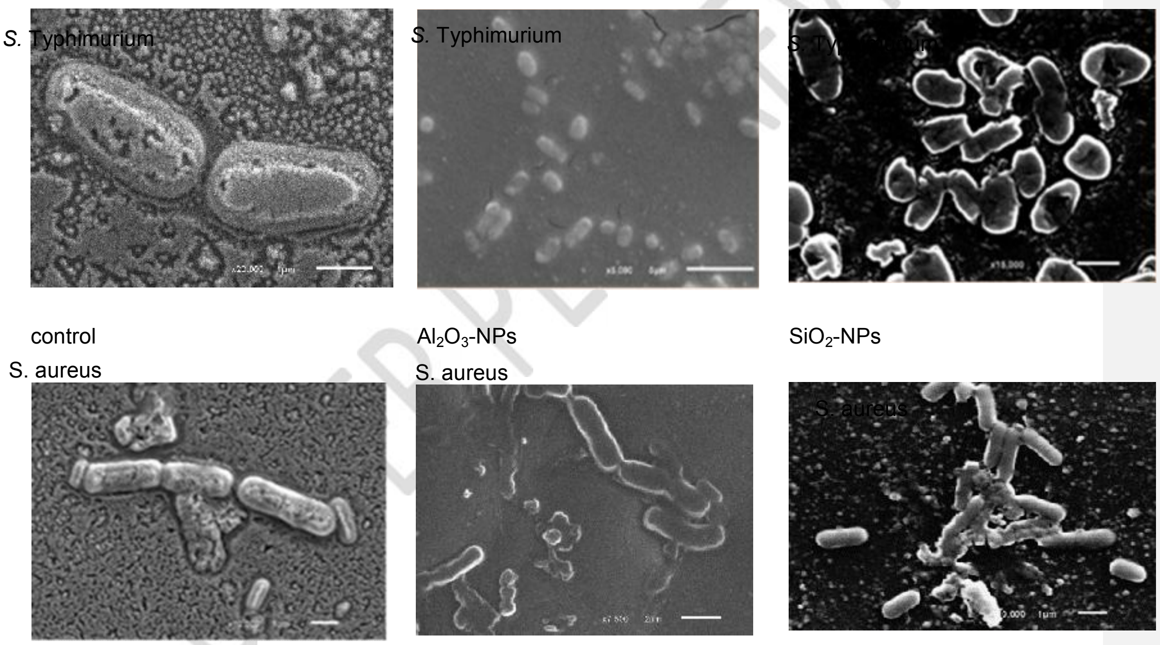
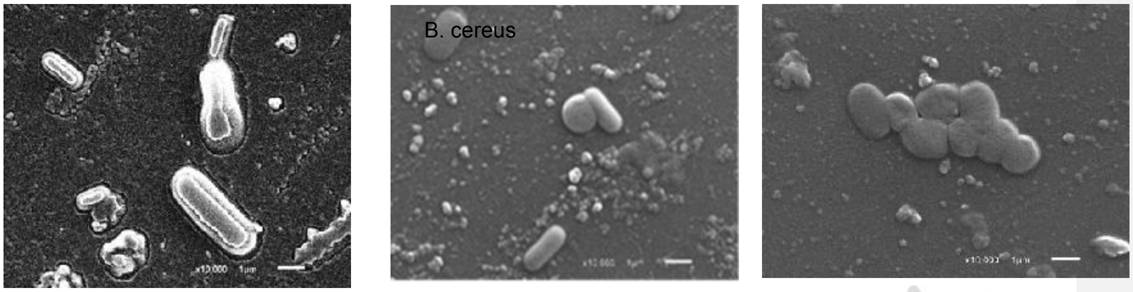


control Al_2O_3-NPs SiO_2-NPs

B. cereus B. cereus



control Al$_2$O$_3$-NPs SiO$_2$-NPs

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

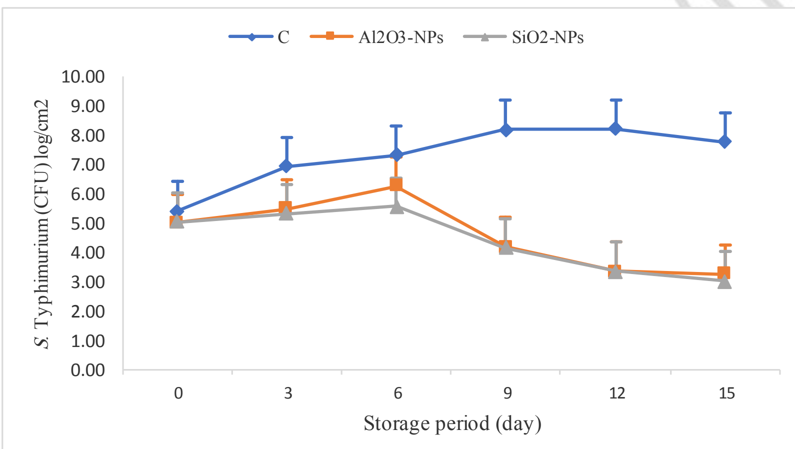


Fig. (2) Antimicrobial activity of HPMC film made with HPMC (40 g / L) and glycerol (10g/L) and incorporated with nanoparticles against *S.* Typhimurium on raw chicken fillet.

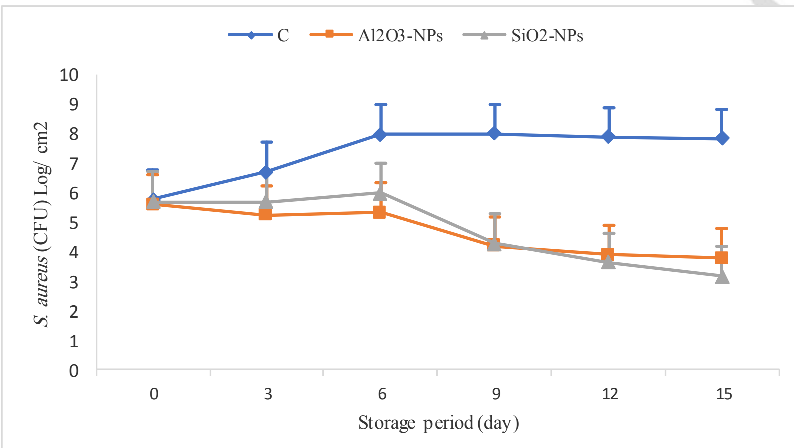


Fig. (3) Antimicrobial activity of HPMC film made with HPMC (40 g / L) and glycerol (10g/L) and incorporated with nanoparticles against *S. aureus* on raw chicken fillet.

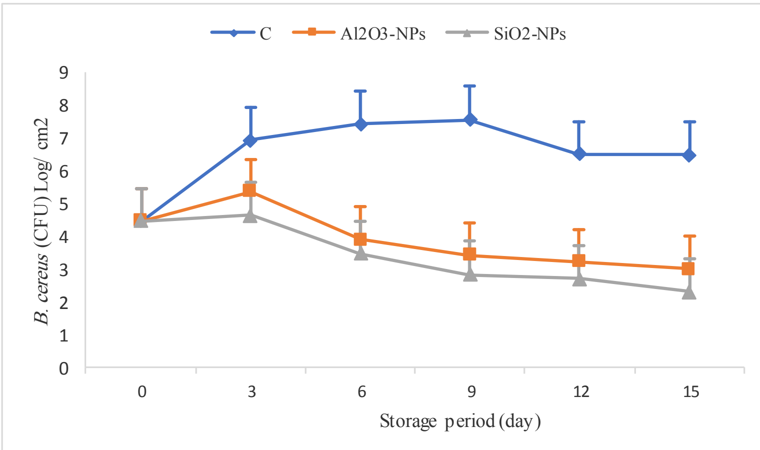
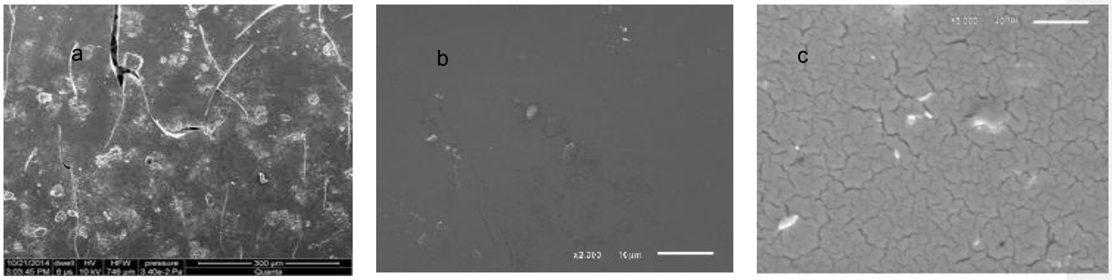


Fig. (4) Antimicrobial activity of HPMC film made with HPMC (40 g / L) and glycerol (10g/L) and incorporated nanoparticles against *B. cereus* on raw chicken fillet.



control Al_2O_3-NPs SiO_2-NPs

Fig. (5) The SEM of (a) HPMC films incorporation (b) Al_2O_3-NPs and (c) SiO_2-NPs.

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

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