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EFFECT OF EDIBLE FILMS REINFORCED WITH NANOPARTICLES ON SHELF-LIFE AND QUALITY OF CHICKEN FILLETS MEAT DURING STORAGE

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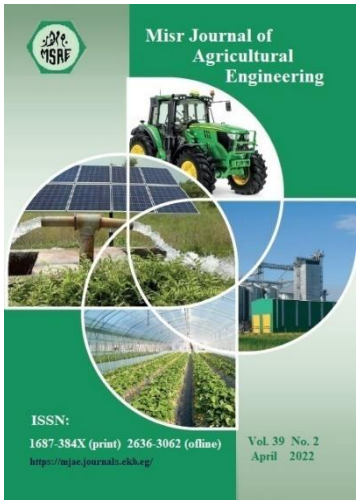


Keywords:
Antimicrobial activity; Edible film; Nanoparticles; Chicken fillets meat; Cold storage

ABSTRACT

The shelf life and quality of chicken fillets meat were examined when the meat was packed in edible films reinforced with nano-particle materials. The films are Hydroxypropyl methylcellulose (HPMC) reinforced with silver nanoparticles (Ag-NPs) and Titanium oxide nanoparticles (TiO2-NPs). Antimicrobial activity, weight loss and total protein and lipids were determined during storage. The results obtained the HPMC reinforced with nanoparticles produced a reduction of food-borne pathogens populations nearly 1.6:2.2 log_{10} CFU cm^{-2} during the challenge study. HMPC reinforced with TiO_2NPs reduced microbial growth of S. Typhimurium, E. coli, S. aureus and B. cereus nearly 1.7, 1.9, 1.9, and 1.7 log_{10} CFU cm^{-2}, respectively HMPC reinforced with Ag-NPs reduced microbial growth of S. Typhimurium, E. coli, S. aureus and B. cereus nearly 1.7, 1.6, 1.9, and 2.2 CFU cm^{-2}, respectively. The application of the coating with edible films considerably delayed the growth of microorganisms, increasing the product shelf life (7 days) compared to the control samples (2 days). Accumulated weight loss ranged from 12±0.4 to 30±0.5% for all treatment under study. The total of protein and lipids of all treatments decreased during storage.

1. INTRODUCTION

Nanotechnology has emerged as one of the most innovative technologies, with the potential to improve food quality and safety. The application of nanotechnology in the agriculture and food sectors is relatively recent, as compared to its use in drug delivery and pharmaceuticals **(Duncan, 2011)**. Nanotechnology involves the characterization, fabrication, and/or manipulation of structures, devices, or materials that are approximately 1 to 100 nm in length **(Neethirajan and Jayas, 2011).** Nanotechnology has been used to generate new products with numerous benefits for the food industry **(Duncan 2011 and Ozcalik and Tihminlioglu 2013)**, such as extending shelf life, improving food safety, and monitoring food spoilage.

Food safety is an important concept and health concern in developed and developing countries **(Hussain *et al.*, 2015).** The Center for Disease Control and Prevention Center **CDC (2017)** reported that about 179 million people get sick, 428000 hospitalized, 6000 deaths, and costed US $ 15.6 billion every year in USA from five food-borne pathogens. In addition, the World Bank report **(World Bank, 2018)** the food-borne illness in developing countries costed ~ US $ 110 billion, 600 million illness cases, and 420,000 premature deaths in Asia and Africa. Five-food-borne pathogens record about (88%) of the listed food concerned deaths: *Salmonella nontyphoidal* (35%), *Norovirus* (26%), *Campylobacter* (15%), *Toxoplasma gondii* (8%), and *E. coli* (4%) **(CDC, 2017)**.

While, poultry products have been responsible in several food-borne outbreaks over the years, these products also are prone to spoilage **(Moghimi *et al.*, 2017)**. In Egypt, the poultry products act an important food material due to high nutritive value, acceptable and relatively low cost, however, associated with food-borne outbreaks **(Noori *et al.*, 2018).** The poultry products caused more than 2 million Americans getting sick each year and costed US $ 5.7 billion. One way to control food-borne pathogens and food spoilage is application of antimicrobials in spray and/or dips **(Basumatary *et al.*, 2018).** However, in these methods, the efficiency of the antimicrobial agents restricted due to uncontrolled migration into the food components **(Quintavalla and Vincini, 2002)**. One novel approach to overcome these problems is utilizing of active packaging (antimicrobial packaging) as a promising type.

Hydroxypropyl methylcellose (HPMC), the cellulose ether, is one of the most common used cellulose derivatives (**Aydogdu *et al.*, 2019).** It is widely used in food, pharmaceutical and many other industries due to its good film forming properties, highly stability, excellent biocompatibility and biodegradability (**Wrona *et al.*, 2017)**.

New trends and perspective in nanotechnology has facilitated the path to use nanoparticles in the packaging industry **(Li *et al.*, 2016)**. Nanoparticles with sizes below 100 nm, in one or more dimensions, can increases the surface area the activity. Therefore, these particles are of importance because of their high activity and efficiency. Nowadays, nanoparticles are applied to improve the functional properties of nanocomposite films **(Guimarães *et al.*, 2015 and Rhim *et al.*, 2006).** Silver nanoparticles (AgNPs) have been used widely as a promising reinforcement agent in polymer matrices because of its unique optical, magnetic, catalytic and thermal properties in addition to the broad spectrum antimicrobial activity **(Duncan, 2011).** Moreover, AgNPs synthesized by greener methods are considered to be safe for food packaging applications **(Shankar and Rhim, 2017).**Titanium dioxide (TiO_2) is inexpensive, inert and non-toxic and is widely used as anti-radiation and anti-microbial due to its photocatalytic activity in biodegradable films. When TiO_2 is combined with polymer matrix, it exhibits proper chemical and mechanical durability and provides preservation against pathogenic and spoilage bacteria, aroma, deterioration and allergens in the presence of ultraviolet radiation. It has been used in different products as a mean to block light and to create a bright appearance **(El-Wakil *et al.*, 2015, Li *et al.*, 2011 and Zhou *et al.*, 2009).**

Fresh poultry products such as chicken breast are very convenient for consumption due to their availability and nutritional and sensory characteristics but they have a short shelf life (2–4 days) due to their high water activity and propensity to microbial contamination and spoilage. The main aim of this study is to prolong the shelf life of chicken meat and keep it

quality by using edible films reinforced with nano-particle as coating during storage. To achieve that: physical and chemical of these films were studied. Microbial activity, weight loss and chemical composition during storage were also determined.

2. MATERIALS AND METHODS

2.1. Materials:

Silver nanoparticles (Ag-NPs), Titanium oxide nanoparticles (TiO_2-NPs) and glycerol were purchased from Nano Gate Company, Cairo, Egypt. Hydroxypropyl methyl cellulose (HPMC) was supplied from G.I.D.C industrial Estate, India. *Bacillus cereus* (ATCC7464), *Salmonella Typhimurium* (ATCC14028), *E. coli* (ATCC87939), and *Staphylococcus aureus* (ATCC 6538) was activated at Microbiological Resources Centre, Cairo, Egypt. All strains were cultivated twice on Tryptic Soy Agar (TSA) at 37 °C for 24 -48 h, and kept at 10±1°C till using **(Salari *et al.*, 2018)**. As shown in Table (1), the compounds of bio-composite films were made from Distilled water, HMPC, glycerol, Ag-NPs and TiO_2 -NPs.

Table 1: Constitutes of HMPC films reinforced with nanoparticles.

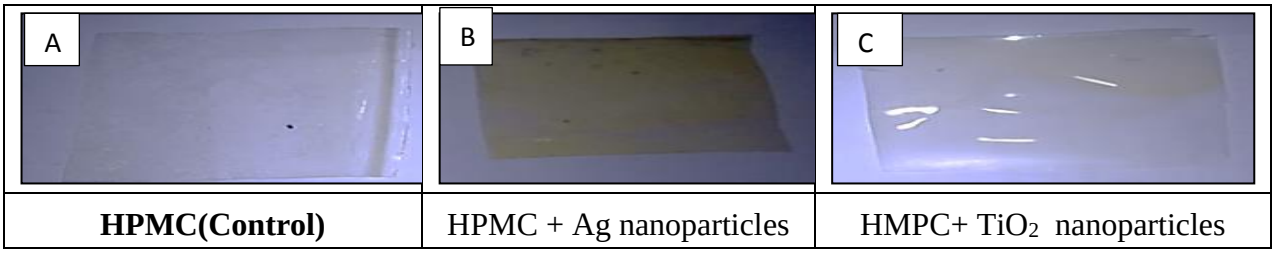
Films	Distilled water (ml)	HMPC (g)	Glycerol 30% (ml)	Ag-NP (ppm)	TiO_2-NPs (ppm)
HMPC-Control	1000	40	10	-	-
HMPC-AgNPs	1000	40	10	80	-
HMPCTiO_2NPs	1000	40	10	-	80

HPMC: hydroxyl propyl methyl cellulose film, HMPC-AgNPs: hydroxyl propyl methyl cellulose films reinforced with silver Nanoparticles, and HMPC-TiO_2 NPs: hydroxyl propyl methyl cellulose films reinforced with titanium oxide nanoparticles.

2.2. Methods:

2.2.1. Preparation of bio-composite films based on hydroxypropyl methyl cellulose (HPMC) reinforced with nanoparticles:

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 **(de Moura *et al.*, 2009).** AS show as in Fig. (1), Images of HMPC films **(A)** without the addition of antimicrobials; **(B)** with the addition of 50 nm Ag nanoparticles; **(C)** with 10 nm TiO_2 nanoparticles.



HPMC(Control)	HPMC + Ag nanoparticles	HMPC+ TiO_2 nanoparticles

Fig. (1): Images of HMPC films **(A)** without the addition of antimicrobials; **(B)** with the addition of 50 nm Ag nanoparticles; **(C)** with 10 nm TiO_2 nanoparticles.

2.2.2. Mechanical properties of edible composite films reinforced with nanoparticles:

2.2.2.1. Film thickness:

Thicknesses of films were measured with a digital micrometer (Mitutoyo Manufacturing Co. Ltd., Japan, sensitivity ±0.001 mm at 5 random positions on the film, following WVP and preceding tensile tests. WVP and mechanical properties were calculated based on average thickness.

2.2.2.2. Tensile strength (TS), elongation at break (EAB), and Young`s elastic modulus (EM):

The TS, EAB, and EM of composite edible film were determined according to **Nur Hazirah *et al.*, (2016)**. An Instron Universal Testing Instrument (Model 1011) was used to determine film TS and %E. Testing film specimens were rectangular strips 38 mm long and 5.79 mm wide as suggested in ASTM D683M **(ASTM, 1993)**. A strain rate of 50 mm/min was used. All film strips were equilibrated for one week to 52±2% RH in a cabinet using saturated magnesium nitrate solution at room temperature (25±1 °C). At least four replicates of each MC film were tested. All three tests were performed in edible composite films. Values for TS, EAB, and EM were calculated using:

$$TS\left(\text{MPa}\right)=\frac{F_{\max}\left(N\right)}{A\left(m^2\right)} \quad (1)$$

Where: F_{max} is the max load (N) needed to pull the sample apart and A is the cross sectional area m^2 of the film sample.

$$EAB\left(\%\right)=\frac{l_{\max}}{l_0}\times 100 \quad (2)$$

Where: l_{max} is the film elongation (mm) at that moment of rupture and l_o is the initial grip length (mm) of the sample.

$$EM\left(\text{MPa}\right)=\frac{stress}{strain} \quad (3)$$

Where: stress is load (N) divided by area (mm^2) and strain is change in length (mm) divided by original length (mm).

2.2.2.3. Water vapor or permeability (WVP):

WVP of films was determined gravimetrically at 25±1 °C using a modified ASTM E96-80 **(ASTM, 1983)** procedure. The test film was sealed to a glass dish containing anhydrous calcium chloride (Merck, Darmstadt, Germany), 0% RH, and the dish was placed in a desiccator maintained at 52±2% RH with saturated magnesium nitrate (Merck, Darmstadt, Germany). The water vapor transferred through the film and absorbed by the desiccant was determined by measuring the weight gain. WVP was calculated from the following equation:

$$WVP=C\frac{x}{A\Delta P} \quad (4)$$

Where: WVP is in g/msPa, x is the film thickness (m), A is area of the exposed film (m^2), DP is the water vapor pressure differential across the film (Pa), and C is the slope of the weight gain of the dish, to the nearest 0.0001 g, versus time. Generally, ten weighing were taken over a 7–10 h period. Slopes were calculated by linear regression and correlation coefficient (r^2) for all reported data were 0.99 or greater. At least three replicates of each film type were tested for WVP.

2.2.3. Antibacterial activities of nanoparticles against foodborne pathogens:

Antimicrobial activity of nanoparticles was evaluated by disk diffusion method on tryptic soy agar media (TSA). In briefly, different concentrations 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 **(Salari *et al.*, 2018).**

2.2.4. Packaging of chicken meat samples:

First, fresh chicken samples were cut into 10 g then, it was packaged by edible films such as HMPC film, and HMPC film reinforced with Ag-NPs and TiO_2-NPs. The control samples (untreated) were immersed in distilled water. All samples were stored under cold storage Conditions of 10 °C and RH% was 24 up to 9 days. Overall quality of samples was evaluated by microbiological and physicochemical analyses at 0, 3, 6, and 9 days of storage **(Takma and Korel, 2019).** As shown in Fig. (2), Photographs of chicken fillets samples stored in different films (HMPC, HMPC AgNPs and HMPC-TiO_2NPs) compared to untreated samples (Control) at 0, 3, 6 and 9 days.

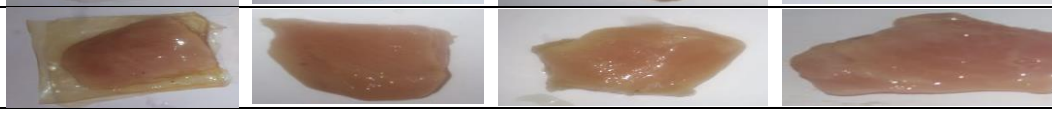
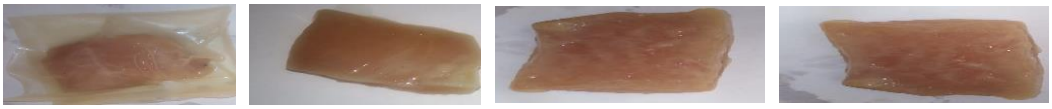
Treatments	Storage time (days) 0	3	6	9
Untreated (Control)				
HMPC-Control				
HMPC-AgNPs				
HMPC-TiO_2NPs				

Fig. 2: Photographs of chicken fillets samples packaged with HMPC films and HMPC films reinforced with nanoparticles at 10^{o} c for 9 days.

2.2.5. Effect of HPMC films reinforced with nanoparticles on shelf life and quality of chicken fillets under cold storage at 10 °C for 9 days:

2.2.5.1. Determination of weight loss (WL):

WL was calculated according to **Licodiedoff *et al.* (2016)** through the difference between the initial weight and the weight obtained at each storage time (Eq. (5)):

$$WL\ \% = \frac{w_o - w_t}{w_t} \times 100 \quad (5)$$

Where:

W_0 is the initial weight, g

W_t is the weight after 9 days, g

2.2.5.2. Determination of total protein and lipids:

Total lipids, crude protein were determined according to the methods of the Association of Official Analytical Chemists described in **(AOAC, 2005).**

2.2.5.3. Microbiological analysis of meat samples:

The HPMC films were utilized to cover raw chicken fillets at 10±1°C up to 9 days to extent shelf-life of fresh chicken fillets. The chicken fillets were cut down (2×2cm) sections under

sterilized conditions. Then, the samples treated with ultraviolet light (UV) for 15 min to decrease the bacterial population. Chicken fillets were inoculated for 24 h by aseptically diluted cultures of *S. Typhimurium, S. aureus, E. coli* 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 packaged with HPMC films (2×2cm) reinforced with nanoparticles. While, the control samples covered by control HPMC films. After 0, 3, 6, and 9 days of cold storage, the samples were tested to determine microbial colonies. One mL 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* oxford listeria ager for *E. coli* to demonstrate microbial growth. Resulting colonies were counted after 1 to 2 day of incubation at 37°C, populations recorded by $\log_{10}$, and expressed as $\log_{10}$ CFU/cm^2 according **(Nguyem *et al.*, 2008 and Trinetta *et al.*, 2010)**.

3. RESULTS AND DISCUSSION

3.1. Physical and Mechanical properties of edible composite films reinforced with nanoparticles:

As shown in Table (2), the thickness, Tensile strength (TS), elongation at break (EAB), Young`s elastic modulus (EM), and water vapor permeability were evaluated, HPMC films containing Ag-NPs and TiO_2-NPs. The thickness results of control film (HMPC), HMPC-AgNPs, and HMPC-TiO_2NPs were 0.30, 0.19, and 0.12 µm, respectively. The tensile results showed that, HPMC film reinforced with Ag NPs and TiO_2-NPs were higher value than HPMC films without nanoparticle (control), the results values were 39.24, 143.87and 157.92 MPa, respectively. On the other hands, elongation was tested, the results obtained that, the HPMC film reinforced with Ag NPs and TiO_2-NPs were higher value than HPMC films without nanoparticle (control), the results values were 2%, 35% and 42%, respectively. In addition to, Young`s elastic modulus was evaluated, the results show that, HPMC film reinforced with Ag NPs and TiO_2-NPs were less value than HPMC films without nanoparticle (control), the results values were 19.62, 4.11 and 3.76 MPa, respectively. The WVP results showed that, HPMC film reinforced with Ag NPs and TiO_2-NPs were less value than HPMC films without nanoparticle (control), the results values were 0.5076×10^{-3} and 0.4596×10^{-3}, and 0.4504×10^{-3} (g/msPa), respectively. That is due to (a) the nanoparticles ability to filling pore between HPMC film structures. (b) The water evaporates permeability during film formation (c) Hence, the increased surface area reinforces the (d) film thickness and biodegradable. These results are in agreement with those obtained **by Ahmadi *et al.* (2012), Osorio *et al.* (2011) and Sievens-Figueroa *et al.* (2012).**

Table (2): Physical and mechanical properties of HPMC films reinforced with Ag-NPs and TiO_2-NPs

Samples	Thickness (µm)	Tensile (MPa)	Elongation (%)	Young ′s elastic modulus (MPa)	WVP ($\times 10^{-3}$ g/msPa)
Control-Control	0.30	39.24	2	19.62	0.5076
HMPC-AgNPs	0.19	143.87	35	4.11	0.4596
HMPCTiO_2NPs	0.12	157.92	42	3.76	0.4504

HPMC: Hydroxy propyl methyl Cellulose, Ag-NPs: silver Nanoparticles and (TiO_2 -NPs): titanium oxide nanoparticles.

3.2. Antibacterial activities of nanoparticles against foodborne pathogens:

Tables (3 and 4) show the antibacterial activity of inorganic nanoparticles i.e. silver nanoparticles (Ag-NPs) and titanium oxide nanoparticles (TiO_2-NPs) against foodborne pathogens such as *Bacillus cereus, E. coli Salmonella Typhimurium* and *Staphylococcus aureus* were evaluated. The results showed that Ag-NPs and TiO_2-NPs at 80 ppm were effective against foodborne pathogens i.e. *B. cereus, E. coli,* S. *typhimurium* and *S. aureus,* than 20 and 40 ppm respectively, the results were partially agreement with **by Khezerlou *et al.* (2018) and Ejaz *et al.* (2018)**. Moreover, AgNPs at 80 ppm were more effective against *B.Cereus* and *E.Coli* these results agreement with data those reported by **Nanda *et al.* (2009).** As well, TiO_2-NPs at 80 ppm were more active against *B.cereus* and *S. Typhimurium* these results were similar to the results those obtained by **Martinez-Gutierrez *et al.* (2010)**. AgNPs and TiO_2- NPs incorporated composite films demonstrated strong antibacterial activity against both the Gram-positive and Gram-negative food borne pathogenic bacteria.

Table (3): Antibacterial activity of Ag-NPs (~10 nm) and TiO_2-NPs nanoparticles (~50 nm) at different concentration against foodborne pathogens.

Bacterial strains	Ag-NPs			TiO_2- NPs		
	20ppm	**40ppm**	**80ppm**	**20ppm**	**40ppm**	**80ppm**
S. Typhimurium	6±0.1	7±0.1	8±0.04	8±0.02	9±0.01	10±0.01
E. coli	5±0.01	8±0.1	10±0.2	7±0.1	8±0.1	9±0.1
S. aureus	5±0.1	7±0.3	8±0.1	6±0.2	7±0.2	8±0.2
B. cereus	7±0.1	8±0.2	9±0.1	8±0.1	9±0.2	11±0.2

Values were presented as mean ± standard deviation (SD) included n=3.Ag-NPs: silver Nanoparticles and (TiO_2 -NPs): titanium oxide nanoparticles.

Table (4): Antibacterial activity of Ag-NPs (~10 nm) and TiO_2-NPs nanoparticles (~50 nm) at 80 ppm against foodborne pathogens

Bacterial strains	Nanoparticles agents	
	Ag-NPs	**TiO_2-NPs**
	80ppm	**80ppm**
S.Typhimurium	8±0.04	10±0.01
E.coli	10±0.2	9±0.1
S.aureus	8±0.1	8±0.2
B.cereus	9±0.1	11±0.2

Values were presented as mean ± standard deviation (SD) included n=3.Ag-NPs: silver Nanoparticles and (TiO_2 -NPs): titanium oxide nanoparticles.

3.3. Effect of HPMC films reinforced with nanoparticles on shelf life and quality of chicken fillets under cold storage at 10^0C for 9 day:

3.3.1. Weight loss (WL):

Table (5) shows the weight loss (WL%) of chicken fillets meat that stored in edible films of HMPC, HMPC-AgNP, and HMPC-TiO_2NPs compered to untreated sample (control) during 9 days at 10^0C. it is indicated that the weight loss of all samples increased during storage time.

The weight loss ranged from 12 -36% for all treatments. The values results weight loss of untreated sample (control) was 20%, 28% and 36% at 3, 6, and 9 days, respectively. In addition to, the values result of samples packaged with HMPC were 18%, 22%, 30% at 3, 6, and 9 days, respectively. Additionally, the values result of samples packaged with HMPC-AgNPs were 16%, 20%, and 28% at 3, 6, and 9days, respectively. The weight loss of samples packaged with HMPC-TiO_2NPs recorded 12%, 18%, and 24% at 0, 3, 6, and 9 days, respectively.

Weight loss during cold time storage time showed differences between untreated samples (control) and samples packaged with edible films, as shown in Fig. (3). Control fillets (untreated) had a higher weight loss than samples packaged with edible films during the 9 days of storage. Thus, the antibacterial packaging application had different impact on weight loss of chicken fillets meats (**Garavito *et al.*, 2020**).

Regression analysis was carried out to find a relationship between weight loss and storage time (0-9) days for chicken fillets meat stored in different edible films (HMPC, HMPC-Ag-NPs, and HMPC-NPs). The follows obtained:

- $(WL_1) = 25.633\ln(ST) + 0.6342$ $R^2 = 0.9949$ (6)
- $(WL_2) = 20.861 \ln(ST) + 0.927$ $R^2 = 0.977$ (7)
- $(WL_3) = 19.397\ln(ST) + 0.5891$ $R^2 = 0.9806$ (8)
- $(WL_4) = 17.026 \ln(ST) - 0.0277$ $R^2 = 0.9972$ (9)

Where:

WL is the weight loss, g

ST is the storage time, day

WL_1 is the weight loss for untreated (Control), g

WL_2 is the weight loss for HMPC film, g

WL_3 is the weight loss for HMPC Ag-NPs film, g

WL_4 is the weight loss for HMPC TiO_2-NPs film, g

Table (5): weight loss of chicken during storage at 0, 3, 6 and 9 days.

Samples	Initial Weight, g	Weight loss at day, g				Accumulated Weight loss, %			
		0	3	6	9	0	3	6	9
Untreated (control)	10	10	8.0±0.0	7.2±0.4	6.4±0.5	0	20±0.0	28±0.4	36±0.5
HMPC-Control	10	10	8.2±0.4	7.6±0.5	7.0±0.7	0	18±0.4	22±0.5	30±0.7
HMPC-AgNPs	10	10	8.4±0.5	8.0±1.0	7.2±0.8	0	16±0.5	20±0.8	28±0.8
HMPC-TiO_2NPs	10	10	8.8±0.4	8.2±0.4	7.6±0.9	0	12±0.4	18±0.9	24±0.9

Values were presented as mean ± standard deviation (SD) included n =5. HMPC: hydroxyl propyl methyl cellulose film, HMPC-AgNPs: HMPC film reinforced with silver Nanoparticles, and HMPC-TiO_2NPs: HMPC film reinforced with titanium oxide nanoparticles.

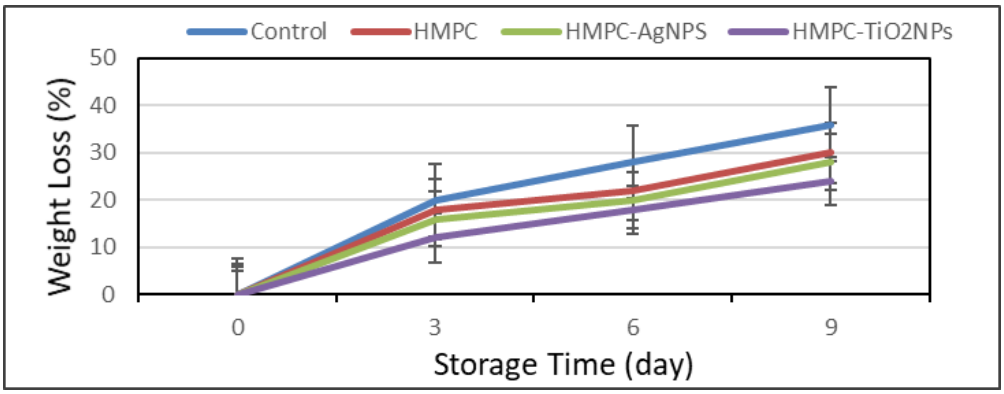


Fig. (3): Accumulated weight loss (%) of chicken fillets meat with and without the antimicrobial packaging stored at 10 °C for 9 days.

3.3.2. Changes in percent of total protein and lipid values:

Tables (6) shows the changes in percent of total protein and lipid values of chicken fillets meat during 9 day of storage at 10^0C. Changes in percent of total protein and lipid values of chicken fillets meat with respect the storage time are presented. The results values of total protein% of untreated sample (control) at zero time were 22% and 21.58% after 9 days of storage. In addition, the results values of all samples stored in active packaging (HMPC, HMPC-AgNPs, and HMPC-TiO_2NPs) were 21.94%, 21.51%, and 21, 16%, respectively after 9 days of storage. The results values of total lipid% of untreated sample (Control) at zero time were2.45% at zero time and 1.99 after 9 day of storage. Additionally, the results values of all samples stored in active packaging (HMPC, HMPC-AgNPs, and HMPC-TiO_2NPs) were 1.75%, 1.57%, and 1.93%, respectively after 9 day of storage (**Alasnier *et al.*, 2000 and Soyer *et al.*, 2010)**.

The total of protein and lipids of all samples decreased during storage, as shown in figs. (4 and 5). Difference was observed in total protein and lipid between all samples at 9 days of storage and untreated sample at zero time. In addition to, no difference was observed in total protein and lipid between samples packaged with antibacterial packaging and untreated (control) without packaging after 9 days of storage. Thus, the antibacterial packaging application had no difference impact on total protein and lipids of chicken fillets meat during storage.

Table (6): changes in percent of total protein and lipid values of chicken fillets meat during 9 d of storage at 10^0C.

Samples	Untreated (Control)		HMPC-Control	HMPC-AgNPs	HMPC-TiO_2NPs
Storage time, day	0	9	9	9	9
Protein %	22±0.05	21.58±0.01	21.94±0.02	21.51±0.02	21.16±0.02
Lipid%	2.4±0.01	1.99±0.01	1.75±0.01	1.57±0.02	1.93±0.02

Values were presented as mean ± standard deviation (SD) included n= 3. HMPC: hydroxyl propyl methyl cellulose film, HMPC-AgNPs: HMPC film reinforced with silver Nanoparticles, and HMPC-TiO_2NPs: HMPC film reinforced with titanium oxide nanoparticles.

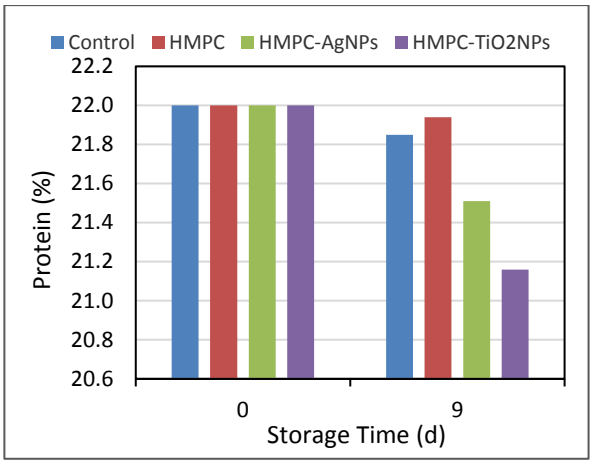


Fig 4. Changes in percent of total protein of chicken fillets meat during 9 days of storage at 10^0C.

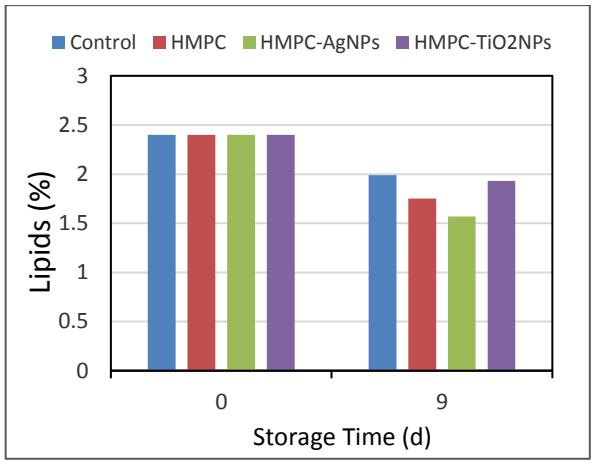


Fig 5. Changes in percent of total lipid of chicken fillets meat during 9 days of storage at 10^0C.

3.3.3. Microbiological changes:

Depending on the results of antibacterial activity of HPMC films reinforced with nanoparticles, the films were applied on raw chicken fillets at clod storage conditions (10±1°C) up to 9 days. In Figs. (6 to 9) the bacterial growth of *S. Typhimurium*, *E. coli*, *S. aureus* and *B. cereus* was increased gradually during the cold storage over 9 days, when treated with the control films compared to the packaged samples with nanoparticles. HPMC reinforced with nanoparticles produced a reduction of food-borne pathogens populations nearly 1.6:2.2 $\log_{10}$ CFU/cm^2 during the challenge study. HMPC reinforced with TiO_2NPs reduced microbial growth of *S. Typhimurium*, *E. coli*, *S. aureus* and *B. cereus* nearly 1.7, 1.9, 1.9 and 1.7 $\log_{10}$ CFU cm^{-2}, respectively. HMPC reinforced with AgNPs reduced microbial growth of *S. Typhimurium*, *E. coli*, *S. aureus* and *B. cereus* nearly 1.7, 1.6, 1.9 and 2.2 CFU cm^{-2}, respectively. These results due to some reasons: (a) the microbial growth and it is adaptation on raw chicken fillets. (b) Nanoparticles distribution on film surface and the ability of nanoparticles to penetrate the microbial cells. (c) Bacterial cells genus and species. (d) Antibodies production by bacterial cells to overcome the antimicrobial activity and protected itself from antibacterial effective. (e) The ability of antibacterial to make a significant changesets in it is mechanism, vital role and morphology. (f) Emigration system of nanoparticles to attached with raw chicken fillets. These results were similar to those results obtained by **KimiaeeSadr *et al.* (2016), Nguyen *et al.* (2017) and Khezerlou *et al.* (2018).**

4. CONCLUSION

The results of this investigation had demonstrated that HPMC films reinforced with Ag- NPs and TiO_2-NPs were active against foodborne pathogens such as *S. typhimurium*, *E. coli*, *B. cereus* and *S. aureus* in chicken fillets. Additionally, Bio-composite films reinforced with AgNPs and TiO_2NPs incorporated demonstrated strong antibacterial activity against both the Gram –positive and Gram –negative food borne pathogenic bacteria. However, HPMC films reinforced with Ag-NPs and TiO_2NPS had improved mechanical property. 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.

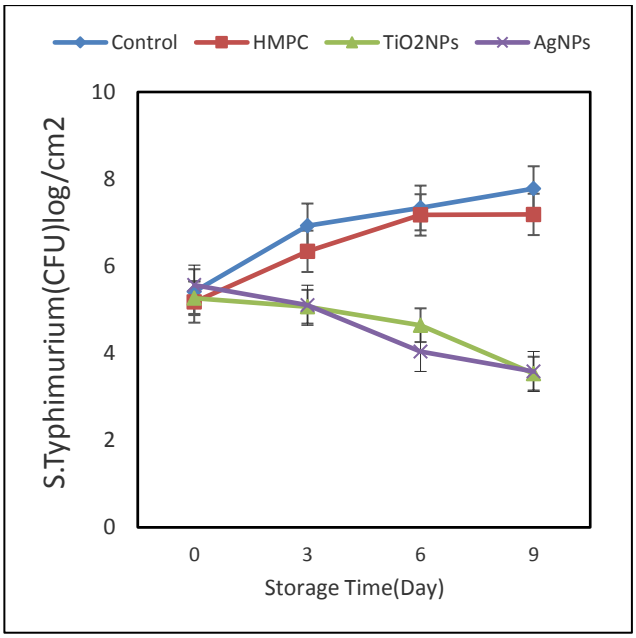


Fig 6. Antibacterial activity of HMPC edible films, made with HMPC (40 g/ L), and glycerol (10g/ L) and reinforced with different types of antimicrobials against *S.typhrimrium* on chicken fillets.

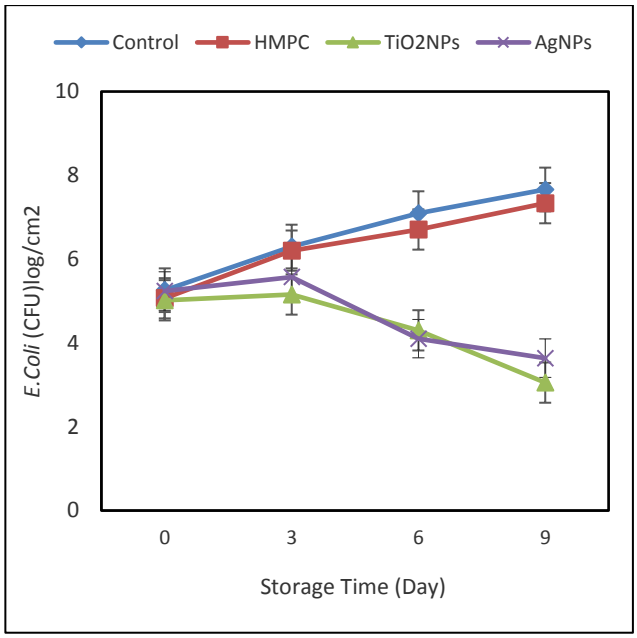


Fig 7. Antibacterial activity of HMPC edible films, made with HMPC (40 g/ L), and glycerol (10 g/ L) and reinforced with different types of antimicrobials against *E.coli* on chicken fillets.

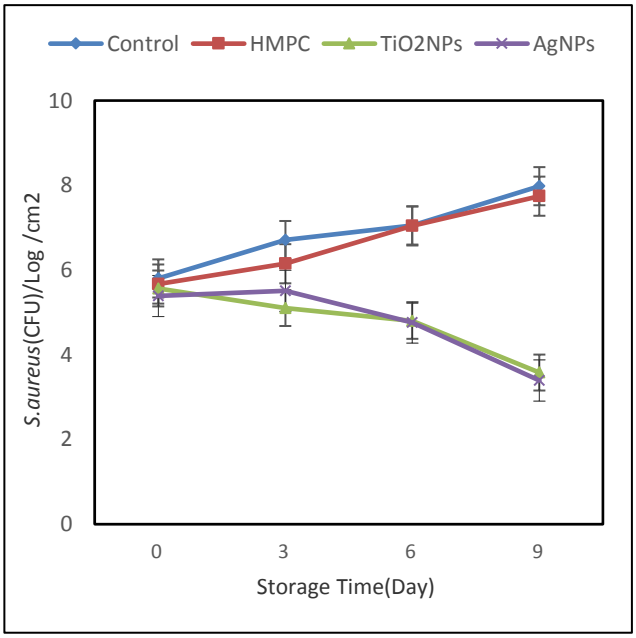


Fig 8. Antibacterial activity of HMPC edible films, made with HMPC (40 g/ L), and glycerol (10 g/ L) and reinforced with different types of antimicrobials against *S.aureus* on chicken fillets.

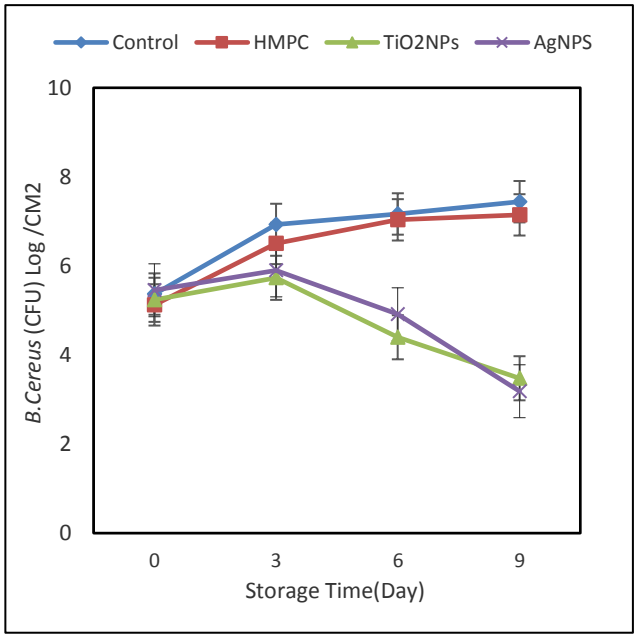


Fig 9. Antibacterial activity of HMPC edible films, made with HMPC (40 g/ L), and glycerol (10 g/ L) and reinforced with different types of antimicrobials against *B.cereus* on chicken fillets.

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تأثير الأغشية المأكولة المدعمة بجزيئات النانو على الجودة والعمر التخزيني للحوم فيليه الدواجن اثناء التخزين

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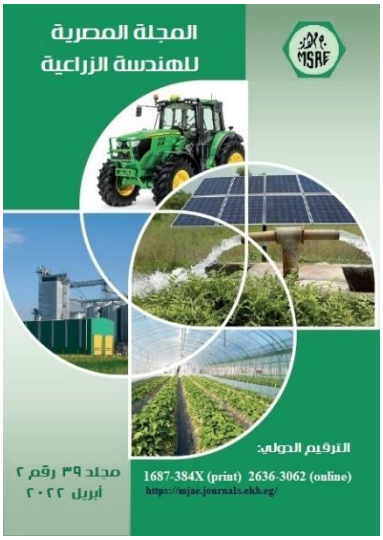

الكلمات المفتاحية:

نشاط مضاد للميكروبات ، غشاء صالح للأكل ، جزيئات نانوية ، لحم فيليه دجاج.

الملخص العربى

إن الهدف من هذه الدراسة هو تقييم فعالية أغشية هيدروكسي بروبيل ميثيل سيليوز المعززة بالجسيمات النانوية ضد أربعة مسببات الأمراض المنقولة بالغذاء، وقد صممت هذه الدراسة باستخدام جسيمتين نانويتين هما الفضه النانويه واكسيد التاتينوم النانويه، غشاء صالح للأكل (هيدروكسي بروبيل ميثيل سيليوز) وأربعة مسببات الأمراض المنقولة بالغذاء مثل الباسلس سريوس والايكولاي والاستفيلكوسس ايريوس السالمولانا تيفرميويوم. تم تقييم كلا الجسيمتين النانويتين ضد مسببات الأمراض المنقولة بالغذاء وكذلك تم تطبيقها في شرائح الدجاج. أظهرت النتائج التي تم الحصول عليها من هذه الدراسة أن الجسيمات النانوية عند ٨٠ جزء في المليون كانت نشطة ضد الباسلس سريوس والايكولاي والاستفيلكوسس ايريوس والسالمولانا تيفرميويوم مقارنة بـ ٢٠ و٤٠ جزء في المليون. ومع ذلك، فإن أغشية هيدروكسي بروبيل ميثيل سيليوز المقواة بالفضة النانويه واكسيد التاتينوم النانويه حسنت الخصائص الميكانيكية. تم تقييم خصائص الأغشية الصالحة للأكل المحضرة من هيدروكسي بروبيل ميثيل سيليوز وهيدروكسي بروبيل ميثيل سيليوز المعززة بالجسيمات النانوية من هذا التقييم، تم تحضير أغشية مضادة للبكتيريا لتحديد تأثيرها على العمر الافتراضي والتغير في الخصائص الفيزيائية والكيميائية والميكروبيولوجية لشرائح صدور الدجاج تحت ظروف التبريد. تم تعبئة العينات الطازجة بأغشية صالحة للأكل على أساس HMPC وتخزينها عند ١٠ درجة مئوية لمدة ٩ أيام. خلال هذا الوقت ، تم تحديد التغيير في فقدان الوزن والدهون والبروتين ونمو الكائنات الحية الدقيقة. تم تحقيق انخفاض في فقدان الوزن في العينات المعالجة مقارنة بالعينات غير المعبأة. أدى تطبيق الطلاء بأغشية صالحة للأكل إلى تأخير كبير في نمو الكائنات الحية الدقيقة ، مما أدى إلى زيادة العمر الافتراضي للمنتج (٧ أيام) مقارنةً بعينات التحكم (يومين).