Immobilization of Synthesized Silver Nanoparticles Using Mango Peel Extract on Low Density Polyethylene Surface and its Application as Biologically Active Packages

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ABSTRACT

This work focuses on surface modification of polyethylene films by ammonium persulphate/ferric nitrate mixtures and coating of silver nanoparticles on the surface of treated film. For this purpose, the surface of polyethylene film was treated with ammonium persulphate/ferric nitrate mixtures and then the modified polymeric film was immersed in a solution of silver nanoparticles that was synthesized by plant extract reduction of silver salt using mango peel extract. The antimicrobial activity of silver/polyethylene nanocomposite was evaluated and then it was used as an active package to extend shelf life of strawberry during storage at 4°C. The obtained results showed that, silver/polyethylene nanocomposite film has high antimicrobial properties against *Listeria monocytogenes*, *Pseudomonase aeroginosa and Fusarium oxysporum*. Finally, the strawberry storage indicated that, strawberry rapped in silver/polyethylene nanocomposite had values of weight loss, pH and total aerobic bacteria count as well as yeast and mold count lower than other samples(unwrapped and wrapped in native polyethylene).

Keywords: silver/polyethylene nanocomposite, nanosilver, LDP and strawberry.

INTRODUCTION

Strawberries are a good source of natural antioxidants (Wang & Lin, 2000). In addition to the usual nutrients, such as vitamins and minerals, strawberries are also rich in anthocyanins, flavonoids, and phenolic acids (Heinonen *et al.*, 1998). Nowadays, consumption of fresh fruits and vegetables has attracted increasing attention due to their high nutritional values. Nevertheless, the major problem with fruits and vegetable is their perishable nature causing a great deal of troubles (Han *et al.*, 2004). Thus, there is an urgent need to have alternative technologies to minimize the undesirable physicochemical and physiological changes of strawberries during storage. Many techniques have been studied in order to extend the shelf life of fresh produces for example, low temperature and high relative humidity, controlled and modified atmosphere packaging, etc. However, each has advantages and disadvantages.

Active packaging is an innovative area, which causes food products to have better sensorial features and extended shelf-life, thus ensuring enhanced food quality and safety (Soares *et al.*, 2009). One of the most modern active packaging systems is polymer/metal nanocomposite materials (Mbhele *et al.*, 2003). There are several polymers have been used for the preparation of polymer/metal nanocomposite such as polyethylene (PE) (Dehnavi *et al.*, 2013), ethylene vinyl alcohol (EVOH), and polypropylene (PP) (Muriel-Galet *et al.*, 2012). Low-density polyethylene (LDPE) is a thermoplastic polymer that is widely utilized in food packaging because of acceptable flexibility, transparency, easy process ability, thermal stability, environmental recyclability, and inexpensive properties (Park & Jin, 2001).

Two methods have been used traditionally to prepare the polymer/metal nanocomposite materials, including fillers dispersed in the polymer matrix and metal fillers formed in the polymer matrix from a metal complex (Clemenson *et al.*, 2007). Several compounds have been employed as antibacterial agents in fabrication of antibacterial films. Among them, silver nanoparticles have widely been used as an antimicrobial agent (Chen *et al.*, 2013), due to their high thermal stability and long-term antimicrobial activity against a broad spectrum of bacteria, viruses, and fungi.

Silver is a nontoxic, safe inorganic antibacte-

rial agent that is capable of killing about 650 types of diseases causing microorganisms (Jeong *et al.,* 2005). The bactericidal effect of silver has been attributed to their small size and high surface to volume ratio, which allows them to interact closely with microbial cell membranes and is not merely due to the release of metal ions in solution (Ruparelia *et al.,* 2008). Synthesis of silver nanoparticles was extensively studied employing chemical and physical methods, but the development of reliable technology to produce nanoparticles is an important aspect of nanotechnology (Natarajan *et al.,* 2010). Although existing chemical and physical methods have successfully produced well defined silver nanoparticles, these processes are usually costly and involve the use of toxic chemicals. In addition, synthesis of silver nanoparticles using chemical methods could still lead to the presence of some toxic chemical species being adsorbed onto the surface of nanoparticles which may cause adverse effects in their applications. The plants or plant extracts, which act as reducing and capping agents for nanoparticles synthesis, are more advantageous over other biological processes (Valli & Vaseeharan, 2012), because they eliminate the elaborated process of culturing and maintaining of the cell, and can also be scaled up for large-scale nanoparticle synthesis (Saxena *et al.,* 2012). Moreover, plant-mediated nanoparticles synthesis is preferred because it is cost-effective, environmentally friendly, a single-step method for biosynthesis process and safe for human therapeutic use (Kumar &Yadav, 2009). Different parts of plant materials such as mango peel extract has been studied so far for the synthesis of silver nanoparticles in different sizes and shapes (Yang &Li, 2013).

Mango is consumed all over the world. The production of this fruit is very high. After consumption of the pulp, the peel is generally discarded. In the literature there are a few applications of this peel (Zainuri *et al.,* 2012). This study hypothesized that the polymers composing mango peel such as polysaccharide, lignin, flavonoid, hemicellulose and pectin (Wilkinson *et al.,* 2011) could be applied in the synthesis of silver nanoparticles.

The main goal of the present study is to produce phyto-nanosilver using mango peel extract in order to prepare silver/polyethylene nanocomposite film. In addition, the antibacterial properties of the nanocomposite films against *Pseudomonase aeroginosa* as a model Gram-negative bacterium and *Listeria monocytogenes* as a model Gram-positive bacterium and *Fusarium oxysporum* were determined. Finally, evaluation the effect of wrapping strawberry by silver/polyethylene nanocomposite films on its physicochemical parameters during storage.

MATERIALS AND METHODS

Materials:

Blown films LDPE having a thickness of 100 μm and a density of 0.92 g/cm^3, strawberry and mango peels were purchased from local market in Kafrelshiekh governorate, Egypt. All microorganisms' strains *Listeria monocytogenes*, *Pseudomonase aeroginosa and Fusarium oxysporum* were kindly provided by the Plant Pathology Department, Faculty of Agriculture, Kafr El-sheikh University. Nutrient agar medium (NAM) and potatoes dextrose agar (PDA) used in the microbial tests were purchased from Merck Co. Ltd. (Darmstadt, Germany). The applied reagents were of the highest purity available and purchased from the Sigma-Aldrich Chemical Company (St. Louis, Mo., USA).

Preparation of peel extract

Mango peels were washed thoroughly with distilled water. Such peels (100 g) were added to 250 mL distilled water and crushed by a juicer. The extract was filtered through a cheese cloth and stored at −4°C for further experiments.

Biosynthesis of silver nanoparticles

To optimize the synthesis route for producing the silver nanoparticles (AgNPs), three ml of aqueous peel extract were added to the 27 ml aqueous solution of 1 mM $AgNO_3$. The reduction of silver ions takes place within 30 min at 80°C. The colour change of the solution viz., brownish–orange colour was observed, indicating the formation of silver nanoparticles **(Yang & Li, 2013).**

Modification of LDP plastics

All raw LDP plastics which were cut at the dimensions of 4×4×0.1 cm (L×W×H) were cleaned overnight by acetone to remove the oil and impurities on the surface. The LDP was then immersed in a solution composed of 0.5 M ammonium persulphate solution and 1 M ferric nitrate solution at the volume ratio 1:1. It was then stirred continuously to modify the substrate under various time periods at 6 hrs. Modified LDP was immersed in biosynthesis silver nanoparticles solution for 3 hrs. After that,

LDP was removed and placed in a circular oven (controlled at 100°C) for 20 min (Wu *et al.*, 2012).

Antimicrobial test

Antimicrobial efficiency of the silver/polyethylene nanocomposite films against *Listeria monocytogenes and Pseudomonas aeroginosa* was carried out by agar diffusion method as described by Sadeghnejad *et al* (2014). The agar diffusion method was performed using nutrient agar medium in case of bacteria or potatoes dextrose agar in case of fungi. The test was initiated by pouring the media onto sterilized Petri dishes and was allowed to solidify. 100 µL of incubated testing microbial solution (10^8 CFU/mL) was spread uniformly over the plate. Each film sample of 1.5 cm × 1.5 cm in size was placed on the medium surface. The Petri dishes were incubated for 1 day at 37°C. The clear zone formed around the samples was recorded as an indication of inhibition of the microbial species. Control experiments were performed with uncoated PE.

Storage experiment

Strawberry was selected for uniformity, shape, colour, and size, and any blemished or diseased fruits were discarded. The fruits (≈ 35 g) were randomly distributed into three groups. The first one was left without rapping, while the second one was rapped with native polyethylene and the last one was rapped with modified PE at 4°C for 12 days. Each three days, some parameters were determined.

Weight loss, pH value, titratable acidity, total microbial count, yeast and mould count were determined by the methods described in AOAC (2000). Finally, to quantify the silver ion release from the coated LDPE films, a sample of 6 cm × 10 cm was immersed in 125 mL of de-ionized water at room temperature. At a defined period of time, 10 mL of the solution was taken and the silver content was analyzed by atomic absorption spectroscopy. Finally, silver ion release from the coated LDPE films was quantified according to the method described by Chapman and Pratt (1978) as follow: A sample of 6 cm × 10 cm of Ag/LDP was placed inside a beaker and 10 mL 16 M of nitric acid solution was added for digestion purpose. It was then heated at 90°C for 10 min and the residual solution after filtration was measured by atomic absorption spectroscopy (Zeiss FMD3) for quantifying the concentrations of silver which can be converted into weight percentage over LDP.

Statistical Analysis:

Each experiment was replicated and each parameter was analyzed in duplicate. The data recorded were analyzed using SPSS version 17.0 (SPSS, Chicago, III, and U.S.A). Two way analysis of variance was applied and the data were tabulated. The level of significant effects were tested by comparing mean values using the least significant difference (LSD) test at 1% level (Snedecor & Cochran, 1967).

RESULTS AND DISCUSSION

Antimicrobial activity

Modified LDPE by nanosilver possessed noticeable inhibitory against *Listeria monocytogenes, Pseudomonas aeroginosa* and activity against fungi (*Fusarium oxysporum).* From the data given in Table (1), it is clear that, modified LDPE by nanosilver gave the highest wide inhibition zones (6.14mm), followed by that of native PE.

Generally, all tested modified LDPE by nanosilver showed effective antimicrobial activity against Gram- positive bacteria higher than that of Gram-negative bacteria (*Pseudomonas aeroginosa*).

Weight loss:

Water is the most abundant nutrient in fruits. However, maximum amount of water content varies between individual fruit of the same types because

Table 1: Antimicrobial activity of native and surface modified LDPE films

Type of LDPE	Inhibition zone(mm)		
	Listeria monocytogenes (G^+)	*Pseudomonas aeroginosa* (G^-)	*Fusarium oxysporum*
Native	0.00^{Ab}	0.00^{Ab}	0.00^{Ab}
Modified by nanosilver	6.14^{Aa}	3.57^{Ba}	2.39^{Ca}

Means with different superscripts (capital letters in the same row and small letters in the same column) differ significantly ($P < 0.01$).

of structural difference. It may be also affected by cultural conditions, which influence structural differentiation (Salunkhe *et al.*, 1991). Weight is considered one of great important properties because it can cause fruit shriveling and advance senescence. It mostly depends on relative humidity surrounding the fruit, but can also be associated with a slight reduction in flesh firmness (Ishaq *et al.*, 2009).

Fig. (1) shows that, unwrapped samples had the highest weight loss during storage, followed by samples wrapped with native PE, while the lowest weight loss was detected in case of samples wrapped with modified PE .These results are in agreement with those reported by Aharoni *et al.* (2007) who stated that, plastic film materials are known to reduce water loss during prolonging storage. The reduction in water loss observed in the PE bags plays a key role by serving as a tight barrier to water evaporation. This explains why samples kept unwrapped lost high amount of water because they offered less resistance to water loss.

It should be also noted from the same Fig. that, weight loss ranged from 0.00 to 24 % in unwrapped samples, from 0.00 to 18.6% in samples wrapped by native PE and from 0.00 to 13.2% in samples wrapped by modified PE

pH value

As shown in Fig. (2), pH value increased in strawberry fruits from 3.40 at zero time to 4.4,4.1 and 3.8 at the end of experiment in in case of unwrapped strawberry, strawberry wrapped with native PE sheets and the third ones wrapped with modified PE sheets, respectively. Ayala-Zavala *et al.* (2007) reported that pH values increased during storage period in strawberry fruits. The increase in pH values seems to be normal during the postharvest life of strawberry fruits.

Titratable acidity

Titrable acidity is an important factor in maintaining the quality of some fruits and vegetables, which is directly related to the concentration of organic acids % in these products. Organic acids exist as free acids, anions (malate) or combined as salt (potassium bitartarte) and esters such as isopentyl acetate (Kays, 1991).

Fig. (3) showed that titratable acidity content decreased significantly during storage for all investigated samples. The results of Tano *et al.* (2008) are in the same line with the results obtained in the present study.

As shown in Fig. (3), titratable acidity decreasal in treated samples from 0.92% to 0.52, 0.65 and 0.8% at the end of storage period for strawberry samples (unwrapped, wrapped with native PE sheets and modified PE sheets, respectively). These results are in accordance with those found by Ghasemnezhad *et al.* (2010).

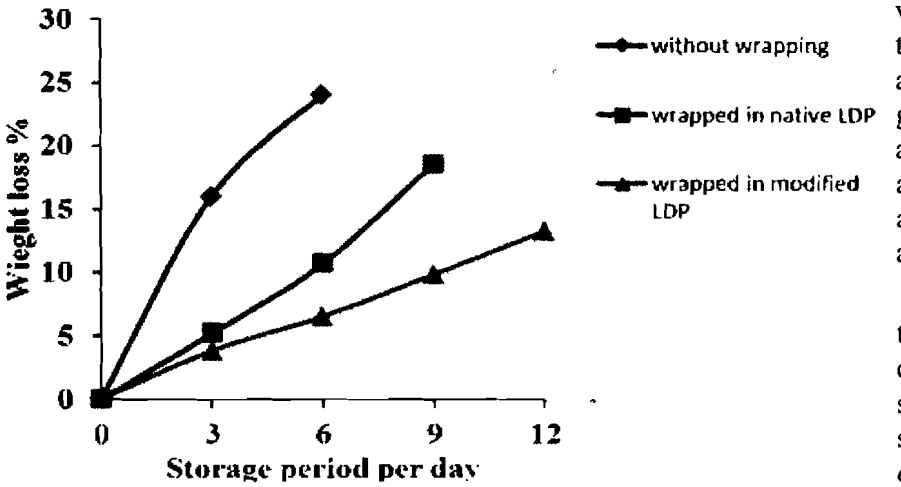

Fig.1: Effect of wrapping with native and modified PE on strawberry fruits weight loss (%) during storage period at 4°C

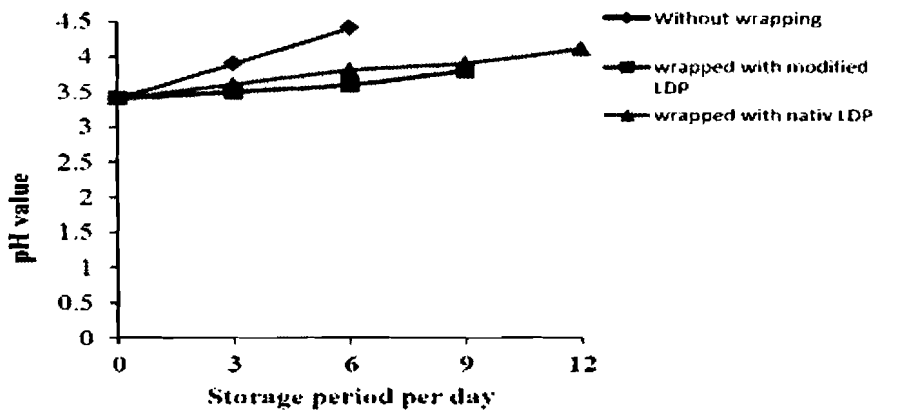

Fig. 2: Effect of wrapping with native and modified PE on pH value of strawberry fruits during storage period at 4°C

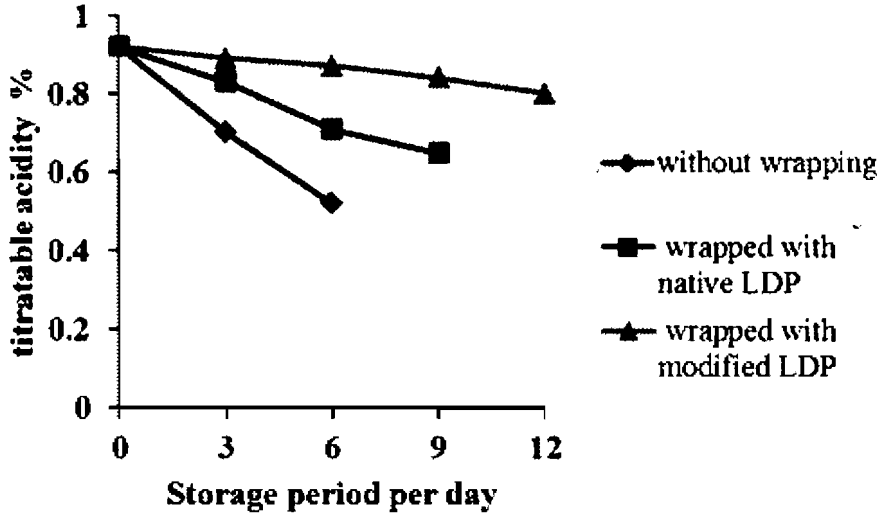

Fig. 3: Effect of wrapping with native and modified PE on titratable acidity % of strawberry fruits during storage period at 4°C.

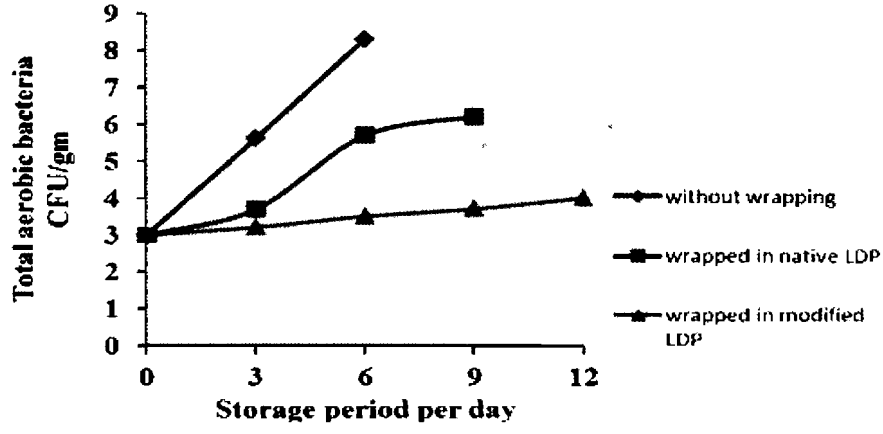

Fig. (4). Effect of wrapping with native and modified PE on total aerobic bacteria (CFU/gm) of strawberry fruits during storage period at 4°C.

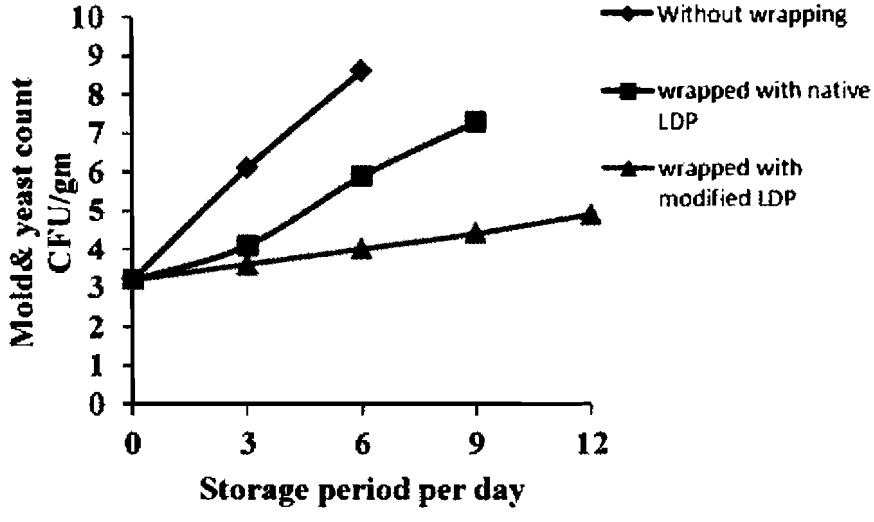

Fig. 5: Effect of wrapping with native and modified PE on mold and yeast count (CFU/gm) of strawberry fruits during storage period at 4°C

Total aerobic bacteria

Fig. (4) shows that, aerobic plate count increased significantly during storage for all investigated samples, the recorded results agree with those reported by Ruiz-Cruz *et al.* (2010).

Fig. (4) shows that aerobic plate count increased from 3.0 log CFU/g to 8.3 , 6.2 and 4 log CFU/g at the end of experiment period in case of unwrapped strawberry, strawberry wrapped with native PE sheets and strawberry wrapped with modified PE sheets, respectively. These results agree with those found by Ediriweera *et al.* (2014).

Mould and yeast count

Fig. (5) shows that, mould and yeast count increased significantly during storage for all investigated samples. The received results are in the same line with those reported by Ruiz-Cruz *et al.* (2010).

The results in the same Fig. showed that, mould and yeast count increased in strawberry from 3.2 log CFU/g to 8.6, 7.3 and 4.9 log CFU/g at the end of experiment period in case of unwrapped strawberry, strawberry wrapped with native PE sheets and wrapped with modified PE sheets, respectively. These results are in agreement with those reported by Francis *et al.* (1999).

Silver ion release from the coated LDPE films:

The amount of silver ion release from the coated LDPE films during strawberry storage was measured and presented in Fig. (6). It is

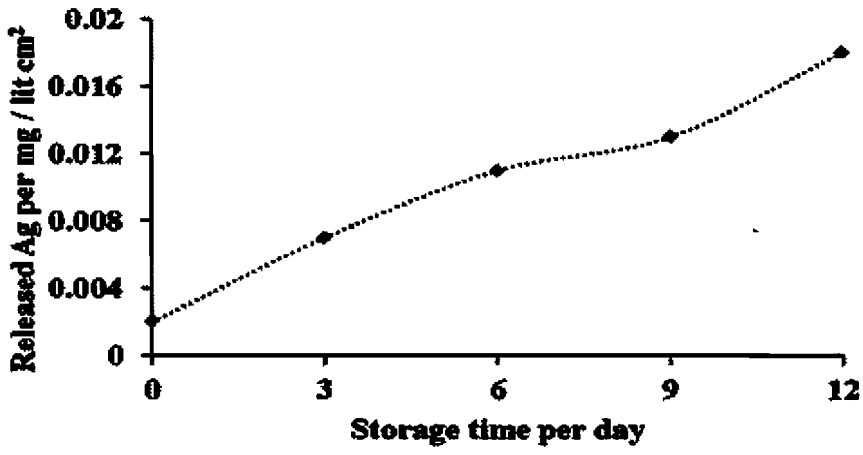

Fig. 6:The amount of silver ion release from the coated LDPE films of strawberry fruits during storage period at 4°C

observed that release of silver ion from the coated LDPE films during storage is continued with a moderate slope over 12 days. In an aqueous environment, the silver nanoparticles release silver ions. Elemental silver particles (Ag(s)) need to be oxidized by dissolved oxygen ($O_{2(aq.)}$) according to the equation below in order to release silver ions (Hoskins *et al.*, 2002):

$$4Ag_{(s)} + 4H_3O^{+} + O_{2(aq.)} \rightarrow 4Ag_{(aq.)} + 6H_2O.$$

Indeed, when the silver loaded films are in contact with water, release of silver ion occurs over a longer period of time due to the involved process of oxidation of the elementary silver nanoparticles to silver ions and a subsequent diffusion of the silver ions to the sample surface. The amounts of released silver ions from the coated films are in a range in which an antimicrobial activity has been found according to Damm *et al.* (2007).

CONCLUSIONS

Ammonium persulphate/ferric nitrate mixed solution was used to modify the surface of PE. Oxygen-containing functional groups, including –OH and C=O, have been substantially formed and they can provide better enhancement on the adhesion of nano-silver particles on the LDP substrate.

silver/polyethylene nanocomposite has a high antimicrobial properties which may be used to increase strawberry shelf life during storage at 4°C.

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تثبيت جزيئات نانو الفضة المخلقة بواسطة مستخلص قشور المانجو على أسطح البولى إيثيلين منخفض الكثافة واستخدامه كعبوات نشطة حيويا

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تناولت هذه الدراسة كيفية تعديل اسطح البولي إثيلين منخفض الكثافة بواسطة مخلوط من الأمونيوم بيرسلفيت ونترات الحديديك وايضا غمر هذه الأسطح المعدلة بواسطة محلول من نترات الفضة في حجم النانو والتى تم الوصول إليه بطريقة اختزالها بواسطة مستخلص من قشور المانجو . وقد تم تقييم خواص التضاد الميكروبي لهذه الأغلفة المعدلة والمغطاة بنانو الفضة واستخدامها كمواد تعبئة نشطة بغرض اطالة العمر التخزيني للفراولة المحفوظة على ٤ درجة مئوية ومن اهم النتائج المتحصل عليها ما يلي:

أظهرت هذة الافلام خواص عالية للتضاد الميكروبي ضد كل من

Fusarium oxysporum و *Pseudomonas aeroginosa* و *Listeria monocytogenes*

وأيضا كان معدل الفقد في الوزن والتغير فى رقم الأس الهيدروجيني والعدد الكلى للميكروبات الهوائية والفطريات أقل من المعاملات الأخرى.