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Preparation and Application of LDPE/ZnO Nanocomposites for Extending Shelf Life of Fresh Strawberries

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Summary

Strawberries have a very short post-harvest life mostly due to their relatively high water content, intense metabolic activity and susceptibility to microbial rot. Antimicrobial low-density polyethylene nanocomposite films containing ZnO nanoparticles at different mass fractions were prepared by melt mixing and followed by compression moulding using a hot press machine. Fresh strawberries were packed in nanocomposite films and stored at 4 °C. Their microbial stability, ascorbic acid content and titratable acidity were evaluated after 0, 4, 8, 12 and 16 days of storage. Microbial growth rate was significantly reduced up to 16 days as a result of the use of nanocomposite packaging material containing ZnO nanoparticles. By increasing the ZnO nanoparticle mass fraction to 5 %, the antimicrobial activity of the film increased. All packages containing the ZnO nanoparticles kept the microbial load of fresh strawberries below the level that affects shelf life (5 log CFU/g) up to 16 days. The lowest degradation of ascorbic acid content (6.55 mg per 100 g), and loss of acidity (0.68 %) were observed in packages containing 3 % of ZnO nanoparticles with 10 % polyethylene-grafted maleic anhydride.

Key words: strawberry, ZnO nanoparticles, nanocomposite, antimicrobial packaging

Introduction

Strawberries are highly perishable mainly due to fungal decay. Their post-harvest shelf-life at low temperatures (0–4 °C) is approx. 5 days (*1,2*). Among fresh fruits and vegetables, strawberries are especially interesting because they are fragile and sensitive to mould, which causes significant loss on the market (*3*). The loss of strawberries during storage can reach 40 % (*4*). Therefore, many strategies have been developed to reduce the strawberry loss (*5*). Due to the shortcomings of the existing technologies, as well as to consumer demands, the development of alternative, preferably non-thermal approaches to processing of fresh produce is needed (*6–8*). Although some nonthermal approaches, such as pulsed electric field, high hydrostatic pressure, infrared, ultraviolet and ultrasonic treatments, for decontamination of fresh whole fruits are currently used (*9–14*), they have relatively limited commercial applications because they are energy-intensive, difficult to adapt, require costly equipment and lack suitable industrial scale processing units (*8,15*). Nanotechnology has recently been introduced in the food packaging industry to provide solutions to challenges such as short shelf life and to improve antimicrobial packaging (*8,16*). Antimicrobially active packaging is a new generation of food packaging based on nanocomposites made by incorporating metal nanoparticles into polymer films (*17*). It ensures microbial safety of food, and can extend the shelf life of products (*18*). There is a better interaction between polymer matrix and filler in

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the nanocomposite polymers than in conventional composites. The uniform distribution of nanoparticles in the polymer matrix increases the contact surface of the matrix with the particles, which leads to mechanical and thermal improvement (*19*). Low-density polyethylene (LDPE) is widely used because of its properties such as acceptable flexibility, transparency, low cost, easy processability and thermal stability (*20,21*). ZnO nanoparticles have several industrial uses (*22*). ZnO has found many applications in daily life, *e.g.* in drug delivery, cosmetics and medical devices (*23*) due to its strong antimicrobial effect against a broad spectrum of microorganisms (*24*). Moreover, it is currently listed as generally recognized as safe (GRAS) material by Food and Drug Administration (FDA) (*25*). The antimicrobial activity of ZnO nanoparticles may be related to several mechanisms including the induction of oxidative stress in microorganisms and release of Zn ions from the surface of nanoparticles that bind to the cell membrane, which may cause its degradation. However, the mechanism of toxicity is still only partially understood (*26*). Since ZnO nanoparticles are thermally stable and thermal processing is used to produce the LDPE film, melt mixing can improve the properties of the nanocomposite (*27,28*). The purpose of this study is to investigate the application of nanocomposite packaging consisting of LDPE film and ZnO nanoparticles as a new approach to preservation and prolonging shelf life of fresh strawberries.

Materials and Methods

Preparation of antimicrobial nanocomposite films

Film-grade LDPE resin pellets (LF0200, melt flow index (MFI) 2 g per 10 min, density 0.92 g/mL, softening point 94 °C), polyethylene-grafted maleic anhydride (PE-g-MA) containing 1 % malic anhydride and antimicrobial agents including ZnO nanoparticle powder with an average particle diameter of about 10–30 nm were obtained from Pishgaman Nano Material, Tehran, Iran. The film-grade LDPE resin pellets (0.9 kg) were mixed directly with the ZnO nanoparticles (0.1 kg) and the mixture was fed into a twin-screw extruder machine with a screw diameter of 55 mm and a screw length/diameter ratio of 30 mm (Brabender GmbH & Co. KG, Duisburg, Germany) to be cut into masterbatch granules. Prior to compression moulding, the masterbatch and film-grade LDPE blends were again dried overnight in the vacuum oven at 50 °C. It was particularly important to complete this step before compression moulding because the remaining water in the blend may cause the formation of air bubbles in the samples. Appropriate amounts of masterbatch resins were then added to pure LDPE resin pellets and put into a single-screw blowing machine with a screw diameter of 45 mm and a length/diameter ratio of 28 mm (Venus Plastic Machinery, Chia-Yi Region, Ming Shung Shian, Taiwan) to make the final nanocomposite. Hot press (Polystat 200T, Brabender GmbH & Co. KG) with an attached water-cooling system was used to make the final nanocomposite films (0.09 mm thick) with the desired nanomaterial mass fractions (1, 3 and 5 % of ZnO nanoparticles, 3 % ZnO nanoparticles with 10 % PE-g-MA as a compatibilizer, and pure LDPE film as a control). The compression moulder was heated to 180 °C for 30 min prior to use. The material was first pressed at low pressure for 4 min, followed by a high-pressure cycle at 1034 kPa for 6 min. The samples were then cooled under pressure (483 Pa) for 5–7 min. Film thickness was measured using a micrometer (Mitutoyo Corp, Kawasaki, Kanagawa, Japan) and reported as the average of five readings taken at five different points on the film sample.

Transmission electron microscopy analysis

Cross-section samples for transmission electron microscopy (TEM) were prepared by ultramicrotomy and the slices were placed on a standard amorphous formvar/carbon film on a copper grid (300 mesh). Dispersion quality of nanomaterials into the polymer matrix film was monitored using transmission electron microscope (Zeiss EM 10 C, 80 kV; Merck, Darmstadt, Germany).

Determination of mechanical properties

Young's modulus and elongation at break of the LDPE (control) and LDPE nanocomposite films were tested according to ASTM (American Society for Testing and Materials) D882-12 method (*29*) using universal material-testing machine (Zwick Testing Machines Ltd, Leominster, Herefordshire, UK) at 25 °C and 40 % relative humidity (RH). Five identical specimens, in the shape of a strip of approx. 50 mm in length, 10 mm in width and 0.05 mm in thickness, were tested for each sample. The speed of the moving clamp was 500 mm/min.

Preparation of packages for fresh strawberries

Strawberries (*Fragaria ananassa* cv. Paros) were harvested at the last stage of commercial ripeness. Early in the morning they were transported to the post-harvest laboratory and were sorted to obtain homogeneous batches based on colour, size, absence of injuries and ripeness. Packages were prepared with a manual heat sealer using antimicrobial nanocomposite and pure LDPE films, 15 cm×10 cm in size. They were immediately wrapped in aluminium foil and sanitised at 95 °C for 2 min. After cooling under a sterile laboratory hood, fresh strawberries were selected randomly and immediately packed in 15 cm×10 cm nanocomposite and pure LDPE films.

Storage of packed strawberries

Strawberries were packed in 75 packages, each containing ten pieces of fruit, of the capacity of 20 g and stored in dark and cool conditions at (4±1) °C and (75±5) % RH. Microbiological and physicochemical characteristics (ascorbic acid degradation and titrable acidity) of the samples were evaluated in triplicate immediately after packaging and after 4, 8, 12 and 16 days of storage.

Microbiological evaluations

Tenfold dilutions of the suspension of strawberries in sterile peptone water (0.1 %) were prepared. Total aerobic plate counts were determined by pouring 0.1 mL of diluted suspensions on the plate count agar (PCA; Scharlau Chemie, S.A., Barcelona, Spain) incubated at 30 °C for 3 days. Total yeast and mould counts were determined by

spreading the suspensions on the plates with potato dextrose agar (PDA; Scharlau Chemie, S.A.) containing 10 % tartaric acid, which were incubated at 25 °C for 5 days. Each measurement was performed in duplicate and the results were expressed in colony-forming units (CFU) per gram.

Degradation of ascorbic acid in strawberry samples

Ascorbic acid is easily oxidised and its degradation is mainly determined by the method that relies on the reduction of 2,6-dichlorophenolindophenol reagent. In this work ascorbic acid degradation was determined using the titrimetric method (*30*).

Determination of titrable acidity

Titrable acidity of the samples was measured with a pH meter (laboratory pH/mV/ORP meter, model BT-600, 220 V; Boeco, Hamburg, Germany) at pH=8.1 with 0.1 M NaOH. It was expressed in percentage calculated from g of citric acid per 100 g of strawberry sample (*31*).

Statistical analysis

Analysis of variance was carried out using the SAS statistical software v. 6.12 (*32*), based on completely randomised designs. Differences among the data were significant at $p<0.05$.

Results and Discussion

Transmission electron microscopy images of films

Fig. 1 shows the TEM images of LDPE films containing different mass fractions of ZnO nanoparticles. As shown, ZnO nanoparticles are well distributed in the polymer matrix. However, a slight agglomeration is observed when the mass fraction of ZnO nanoparticles is increased to 1 % (Fig. 1a) possibly because of the strong attraction of nanoparticles. The TEM images of nanocomposite LDPE film with 3 % ZnO nanoparticles and PE-g-MA show that the nanoparticles are well dispersed in the polymer matrix, with aggregates ranging from 10 to 20 nm with an average size of 16 nm (Figs. 1c and d). The agglomeration can be reduced with the addition of compatibilizer because PE-g-MA not only acts as a compatibilizer inside the composite but also as a separator of the agglomerations inside the composites (*33*). As the mass fraction of ZnO nanoparticles increases to 5 %, the quantity of the agglomerates increases and their size becomes more uneven (Fig. 1d). It is well-known that this depends on several morphological and molecular parameters of nanofillers and polymer matrix. Moreover, the dispersion of nanoparticles is another important factor that affects the performance of nanocomposites. As shown in Table 1, the LDPE film with 3 % ZnO nanoparticles and the LDPE with 3 % ZnO nanoparticles and 10 % PE-g-MA show better mechanical performances in comparison with the LDPE with 1 % ZnO nanoparticles and the LDPE with 5 % ZnO nanoparticles. This correlates well with TEM micrograph records that are shown in Fig. 1.

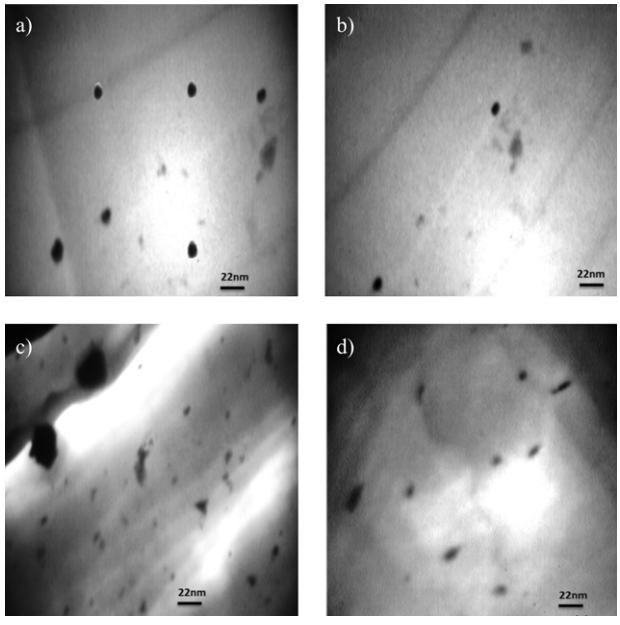

Fig. 1. Transmission electron microscopy images of antimicrobial nanocomposites: a) LDPE films with 1 % ZnO nanoparticles, b) LDPE films with 3 % ZnO nanoparticles, c) LDPE films with 3 % ZnO nanoparticles and 10 % PE-g-MA, and d) LDPE films with 5 % ZnO nanoparticles

Table 1. Effect of ZnO nanoparticles on the mechanical properties of LDPE films

Film type	Young's modulus	Tensile strength	Elongation at break
	MPa	MPa	%
Pure LDPE	$(237.5\pm3.5)^{e}$	$(10.07\pm0.04)^{dc}$	$(150.5\pm3.6)^{a}$
LDPE with 1 % ZnO	$(367.5\pm2.1)^{c}$	$(9.1\pm0.1)^{d}$	$(28.1\pm2.5)^{d}$
LDPE with 3 % ZnO	$(384\pm6)^{b}$	$(13.4\pm0.2)^{b}$	$(51.4\pm1.2)^{c}$
LDPE with 5 % ZnO	$(326\pm6)^{d}$	$(10.9\pm0.8)^{c}$	$(45.3\pm4.8)^{dc}$
LDPE with 3 % ZnO and 10 % PE-g-MA	$(671.5\pm2.1)^{a}$	$(23.7\pm0.6)^{a}$	$(78.8\pm5.7)^{b}$

Values are expressed as mean±standard deviation. Values with different letters in superscript in the same column are statistically different at $p<0.05$

Mechanical properties of nanocomposite films

Stress-strain curves from tensile tests of LDPE/ZnO nanocomposites are shown in Fig. 2. Tensile strength, elongation at break and Young's modulus were determined from stress-strain curves as shown in Table 1. A significant increase can be observed in Young's modulus of all the LDPE/ZnO nanocomposites, while the elongation at break decreased significantly in all the tested films. There were no significant differences in the tensile strength between pure LDPE films and the LDPE with 1 and 5 % ZnO nanoparticles, while the LDPE with 3 % ZnO nanoparticles had a greater tensile strength, Young's modulus and elongation at break, suggesting that the fine nanoparticles reinforced the films and were orientated along the direction of stress, which contributed to the increase of tensile strength. The highest values of Young's modulus (671.5 MPa), tensile strength (23.7 MPa) and

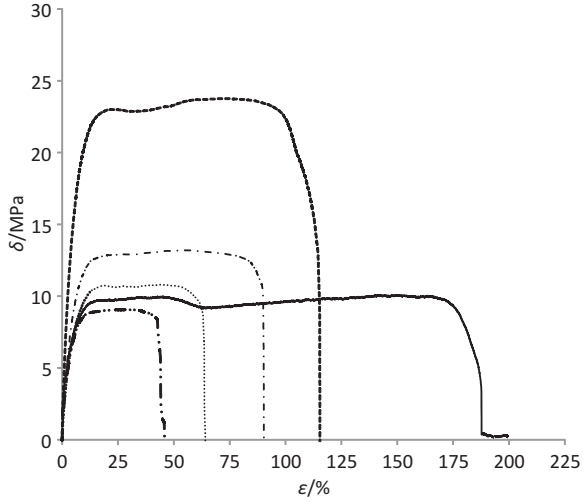

Fig. 2. Stress-strain diagrams of pure LDPE films (—), LDPE films with 1 % ZnO nanoparticles (–···–), LDPE films with 3 % ZnO nanoparticles (–·–), LDPE films with 5 % ZnO nanoparticles (···) and LDPE films with 3 % ZnO nanoparticles and 10 % PE-g-MA (– – –)

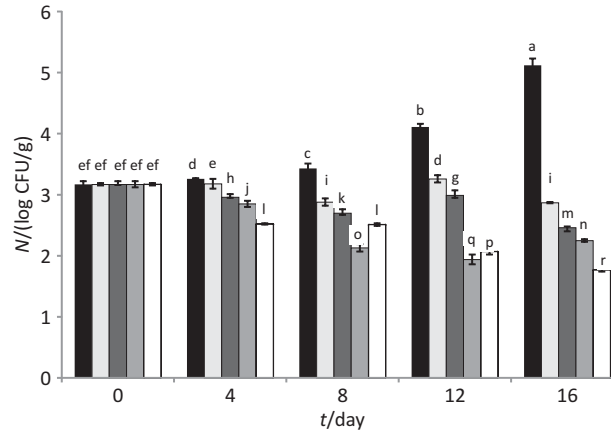

Fig. 3. Effect of packaging (■ pure LDPE films, □ LDPE films with 1 % ZnO nanoparticles, ■ LDPE films with 3 % ZnO nanoparticles, ■ LDPE films with 5 % ZnO nanoparticles and □ LDPE films with 3 % ZnO nanoparticles and 10 % PE-g-MA) on the mould and yeast population of fresh strawberries during 16 days storage at 4 °C. Different letters indicate significant differences at $p<0.05$ between the nanocomposite packagings containing different mass fractions of ZnO nanoparticles and pure LDPE packaging

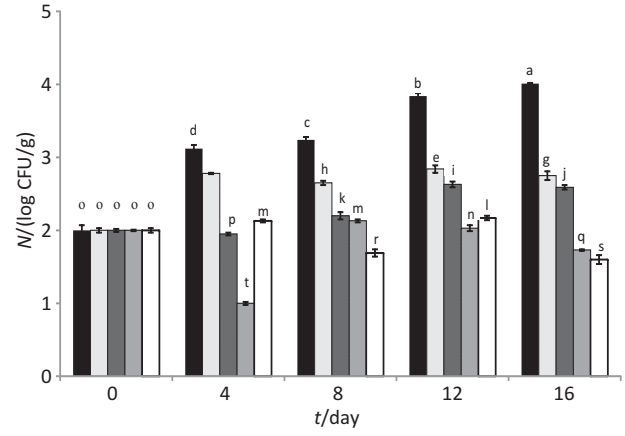

Fig. 4. Effect of packaging (■ pure LDPE films, □ LDPE films with 1 % ZnO nanoparticles, ■ LDPE films with 3 % ZnO nanoparticles, ■ LDPE films with 5 % ZnO nanoparticles and □ LDPE films with 3 % ZnO nanoparticles and 10 % PE-g-MA) on the total aerobic bacterial count of fresh strawberries during 16 days storage at 4 °C. Different letters indicate significant differences at $p<0.05$ between nanocomposite packagings containing different mass fractions of ZnO nanoparticles and pure LDPE packaging

elongation at break (78.8 %) were obtained in the LDPE films with 3 % ZnO nanoparticles and 10 % PE-g-MA compared with other nanocomposite films (Table 1). The addition of PE-g-MA into nanocomposite films containing 3 % ZnO nanopartcles as a compatibilizer improved stress at break due to the significant interfacial adhesion and interaction between the LDPE matrix and ZnO nanoparticles. This improvement correlated well with the good dispersion of ZnO nanoparticles into the LDPE matrix, which enhanced the interaction between the molecules (Fig. 1c). Without the addition of compatibilizer, interfacial adhesion between the LDPE films and ZnO nanoparticles was weak, which prevented the formation of nanocomposite films (*33,34*). Based on the results shown in Fig. 1d, the high amount of nanoparticle loadings (5 %) did not contribute to the homogeneous interactive bonding with the LDPE. It reduced the reinforcing effect and mechanical properties of the nanocomposite due to poor dispersion and agglomeration of ZnO nanoparticles. The weak interaction between the particles and the matrix caused weaker mechanical properties of LDPE films with 5 % ZnO nanoparticles compared with those with 3 % ZnO nanoparticles due to the debonding of particles from the matrix prior to its plastic deformation (Table 1). It seems that LDPE films with 1 % ZnO nanoparticles have weaker elongation at break compared with other nanocomposites (Fig. 2). Poor dispersion due to agglomeration of the nanoparticles has been known to be one of the main causes of weak elongation at break (Fig. 1a).

Microbiological counts in packaged strawberries

Immediately after packaging of fresh strawberries the mean initial population of yeast and moulds was determined to be 3.17 log CFU/g and of total aerobic bacteria 2 log CFU/g. The variations in the population of yeast and moulds, and total aerobic bacteria are shown in Figs. 3 and 4, respectively. Microbial population in strawberries increased when storage time was increased to 16 days in all packages with pure LDPE films (5.12 log CFU/g of yeast and moulds and 4.01 log CFU/g of total aerobic bacteria). However, the addition of ZnO nanoparticles into packaging resulted in a significantly lower microbial load during storage than in packages with pure LDPE films. By increasing the mass fraction of ZnO nanoparticles to 5 %, the antimicrobial activity of the films increased (Figs. 3 and 4). However, it seems that the LDPE films with 3 % ZnO nanoparticles and PE-g-MA had a significantly ($p<0.05$) higher antimicrobial activity compared with other nanocomposites over 16 days of storage at 4 °C, indicating a considerable effect of PE-g-MA on the increase of antimicrobial activity of nanocomposite LDPE films.

Moreover, in the LDPE films with 3 % ZnO nanoparticles and PE-g-MA, significant decreases in the yeast and mould population were observed during 16 days of storage compared to the packages containing the same mass fraction of ZnO nanoparticles. The addition of PE-g-MA into the nanocomposite films as a compatibilizer improved the dispersion of nanoparticles into polymer matrix and caused good interaction between the LDPE matrix and ZnO nanoparticles (*33*). The mean population of yeast and moulds after 16 days of storage remained below the mean initial population (3.17 log CFU/g) in all the nanocomposite packages except for the LDPE films with 1 % ZnO nanoparticles. In the samples of LDPE films with 1 % ZnO nanoparticles, significant decreases ($p<0.05$) in yeast and mould counts were not observed during 4 days of storage compared to pure LDPE packages, indicating that the application of low mass fractions of ZnO nanoparticles is not sufficient for significant reduction of yeast and mould in a short period of time. Strawberries produce fermentation metabolites including acetaldehyde, ethanol and ethyl acetate under aerobic conditions, which can impact the flavour if they are present in higher amounts than their threshold values. Fermentation metabolism can be enhanced in fruits by several stress factors including environmental stress and microbial infections (*35,36*). There is a correlation between the amount of ethanol and ethyl acetate production and the yeast counts in strawberries during cold storage. Ethyl acetate has been reported as an off-odour compound in strawberries. Yeast and moulds are the dominant flora on strawberries; if their microbiological count greatly increases, damaged spots will appear on the strawberries. The shelf life of fresh strawberries is defined as the time needed to reach yeast population of 5 log CFU/g (*37*). The mean population of yeast and moulds remained below 5 log CFU/g after the same storage time in all the packages except for the one made of pure LDPE films. However, it seems that the incorporation of ZnO nanoparticles into the LDPE film can reduce the microbial load of fresh strawberries and extend their shelf life by up to 16 days. Antimicrobial effects of ZnO nanoparticles may be attributed to several mechanisms: (*i*) induction of oxidative stress due to the generation of reactive oxygen species (ROS), such as H_2O_2, both in the interior and exterior of the cell, which leads to an interaction with proteins, DNA and lipids, causing cell death (*38–41*); (*ii*) membrane disorganisation due to the accumulation of ZnO nanoparticles in the bacterial cell walls and also their cellular internalisation (*42*), and (*iii*) release of Zn ions that may be responsible for antimicrobial activity by binding to the membrane of microorganisms (*22*). However, the toxicity of ZnO nanoparticles is not directly related to their entering the cell, but rather to their intimate contact with the cell that causes changes in the microenvironment in the vicinity of the contact area between the organism and the particle to either increase metal solubilisation or to generate ROS, which may ultimately damage the cell membrane (*43*). Moreover, the toxicity of ZnO nanoparticles is not only affected by the light through the formation of ROS, but may also occur in the dark although its mechanism is not yet defined (*41*). Jin *et al.* (*25*) studied the application of ZnO nanoparticles in food systems in several forms (as powder, polystyrene film and polyvinylpyrolidone gel) and concluded that these particles exhibit antimicrobial effects against *Listeria monocytogenes*, *Salmonella* Enteritidis and *Escherichia coli* in liquid egg white and in culture media. Moreover, Emamifar *et al.* (*15*) showed that the application of LDPE packages containing ZnO nanoparticles prolonged the shelf life of fresh orange juice, without any negative effect on sensory attributes. In another study, the same authors (*44*) observed reduced numbers of *Lactobacillus plantaurum* in sterilised orange juice when packed in LDPE film containing ZnO nanoparticles.

Ascorbic acid degradation in strawberries

Strawberries are a good source of nutritional compounds and are among fruits richest in ascorbic acid (*45*). The ascorbic acid content of fresh strawberries is from 19 to 71.5 mg per 100 g (*46*). In most strawberry cultivars, it decreases with storage time, possibly because of the activity of ascorbate oxidase, which changes ascorbic acid to dehydroascorbic acid (*47–49*). According to Fig. 5, a significant decrease in the ascorbic acid content was observed in all the experimental packages during storage at 4 °C. This overall ascorbic acid content reduction might be due to the nonbarrier properties of packaging against oxygen and the prolonged storage time (*46,50*). Loss of ascorbic acid was significantly higher in pure LDPE films than in other packages, while the rates of these changes decreased with the increase of the mass fraction of ZnO nanoparticles in LDPE films with 5 % ZnO nanoparticles. After storage for 16 days, ascorbic acid content in strawberries in pure LDPE films decreased by about 96 % (2.30 mg per 100 g), while its content in the LDPE nanocomposites with 1, 3 and 5 % ZnO, and 3 % ZnO with PE-g-MA was 4.7, 4.85, 5.25 and 6.55 mg per 100 g, respectively. Fig. 5 shows that the degradation rate of ascorbic acid content in LDPE films with 3 % ZnO nanoparticles and PE-g-MA was lower than in other samples.

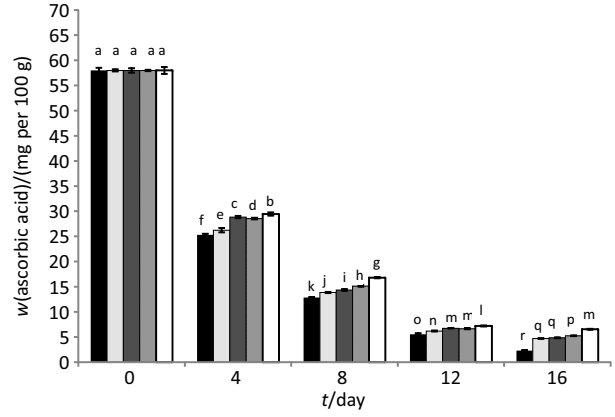

Fig. 5. Effect of packaging (■ pure LDPE films, □ LDPE films with 1 % ZnO nanoparticles, ■ LDPE films with 3 % ZnO nanoparticles, ■ LDPE films with 5 % ZnO nanoparticles and □ LDPE films with 3 % ZnO nanoparticles and 10 % PE-g-MA) on ascorbic acid content in fresh strawberries during 16 days of storage at 4 °C. Different letters indicate significant differences at $p<0.05$ between nanocomposite packagings containing different mass fractions of ZnO nanoparticles and pure LDPE packaging

Titratable acidity of strawberries

Citric acid is the most abundant organic acid in strawberry, followed by malic acid (*51*). The titratable acidity of fresh strawberries is in the range from 0.57 to 2.26 % (*52*). The variations of acidity in strawberries are influenced markedly by time of storage, temperature, different cultivars and maturity stage of fruits (*53–56*). The titratable acidity of fruits decreased significantly during storage probably because of the increased rate of metabolism, especially respiration, which consumed the organic acid and thus decreased the acidity (*2,57–59*). In Fig. 6, a significant decrease in the titratable acidity of strawberries in all the experimental packages was observed during storage at 4 °C. The loss of titratable acidity was significantly higher in the strawberries packaged in pure LDPE films than in other packages, while the rates of these changes decreased with the increase of ZnO nanoparticle mass fraction. The higher acidity of strawberries packaged in the nanocomposite containing ZnO during up to 16 days of storage could be related to the ROS generation mechanism by ZnO nanoparticles, which leads to interaction with proteins and respiratory enzymes (*41*).

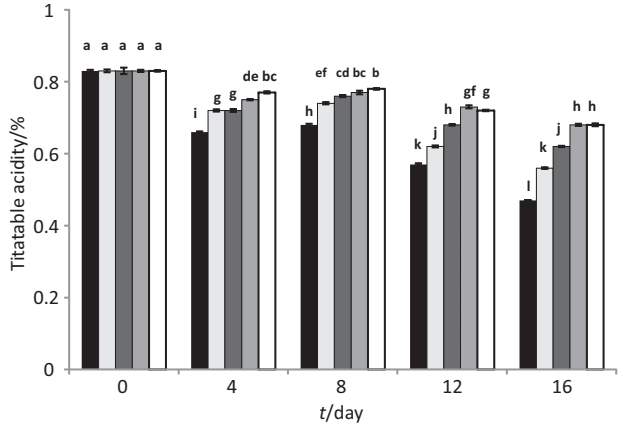

Fig. 6. Effect of packaging (■ pure LDPE films, □ LDPE films with 1 % ZnO nanoparticles, ■ LDPE films with 3 % ZnO nanoparticles, ■ LDPE films with 5 % ZnO nanoparticles and □ LDPE films with 3 % ZnO nanoparticles and 10 % PE-g-MA) on the titratable acidity of fresh strawberries during 16 days storage at 4 °C. Different letters indicate significant differences at $p<0.05$ between nanocomposite packaging containing different mass fractions of ZnO nanoparticles and pure LDPE packaging

Conclusions

This study showed a new approach to preserve and extend the shelf life of fresh strawberry at 4 °C by the application of LDPE packaging materials containing ZnO nanoparticles. These nanocomposite packaging films have a very effective antimicrobial activity because of good dispersion of nanomaterials in the polymer matrix free from agglomeration. The addition of polyethylene-grafted maleic anhydride into the polymer matrix resulted in a good dispersion of nanoparticles in the nanocomposite polymer matrix, which not only increased antimicrobial activity but also improved mechanical properties of nanocomposite polymers. The shelf life of fresh strawberries in packages containing ZnO nanoparticles was prolonged up to 16 days without any negative effects on the ascorbic acid content. However, storage of fresh strawberries in nanopackages is not sufficient for long-term storage. This study revealed that for prolonged shelf life of fresh strawberry, it is necessary to apply another treatment in combination with the nanopackages.

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