НАУЧНЫЙ ЖУРНАЛ
НАУКА И МИРОВОЗЗРЕНИЕ

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PRODUCTION OF BIODEGRADABLE COMPOSITE FILMS

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Introduction

One of the notable improvements in the active packaging set is edible and degradable films. These packaging in the food industry can control or prevent the reactions that occur inside the packaging. Biodegradable film based packaging is especially important because of its natural ingredients, renewability, and the ability to avoid environmental contamination. Biodegradable films as antimicrobial and antioxidant carriers as well as other active agents to improve quality, extend shelf life, control pathogens and improve the organoleptic properties of food have many applications in the food packaging industry. Biodegradable films are a thin layer of biopolymer material (250 μm) that is located at the surface of food components and acts as a barrier against moisture, fat and gases. These films protect the food product against the growth of micro-organisms and mechanical traumas and improve the appearance, quality and longevity of the food product. The biodegradable polymers can be divided into four groups of proteins, polysaccharides, lipids and polyesters based on chemical structure. Mechanical properties, gas and moisture retention have a decisive role in the usability of biodegradable films. Water vapor deterrence, oxygen retention to prevent oxidative reactions and mechanical properties are important factors to protect food against mechanical stresses and chemical reactions.

Poly lactic acid is linear aliphatic thermoplastic polyester that can be extracted from renewable sources such as corn. Today, this polymer is widely used in packaging, textile and plastic containers. This polymer is thermoplastic and biodegradable and can be provided from renewable vegetable sources such as corn and sugar beet. PLA is known as a safe material for food ingredient and decomposes without the need for catalysts by hydrolysis of ester bonds. The rate of decomposition depends on the shape and size of the polymeric object, the isomer ratio, and the hydrolysis temperature.

Lactic acid can be produced either by chemical synthesis or by fermentation. Because of its asymmetric carbon, it has two optical isomers (L+) and (D+) that only its (L+) isomer is metabolized in humans. In the chemical production method, the optical isomers of lactic acid are produced in equal proportion. However, in the fermentation process, most of the product produced (more than 37% of it) is an isomer (L+). Different lactic acid bacteria and a variety of fungi have been used in fermentation processes to produce lactic acid. Lactic acid bacteria, however, have received more attention because of their more productivity.

The tomato is one of the richest sources of lycopene. Lycopene is a tetra-terpene that has 40 carbon atoms and 56 hydrogen atoms and is completely soluble in chloroform and ether due to its lipophilic properties. Lycopene as a carotenoid has no ring structure and has 11 conjugate and two non-conjugated bonds. Due to its non-circularity, lycopene is not a vitamin precursor [7]. Lycopene has a longer structure than other carotenoids. The presence of a long chromophore group in its polymer chain is due to the red color and strong antioxidant power of lycopene. Lycopene has a good antioxidant property among the carotenoids; its solubility at room temperature in the vegetable oil phase is 0.2 g/L. This pigment tends to shrink and precipitate in aqueous systems and becomes crystalline. This property makes it less accessible to the body. In ripe tomatoes, lycopene is present as long needle-shaped crystals. Lycopene is almost stable in plant tissue or its solid form, but becomes completely unstable after extraction from the tissue and dissolved in non-polar organic solvents [8]. So it can be stabilized by Encapsulation. Water-soluble capsules or liposomes are used for this purpose; lycopene stability is important during the preparation of samples containing this pigment or during in vitro and in vivo studies .

The use of hybrid polymers as polymeric nanocomposites is a good way to prevent the permeability of gases in food packaging. To make these polymers, nanoscale filler is dispersed throughout the polymer matrix. These fillers can include clay nano-plates, nano-silica, starch nanocrystals, cellulose nano-fibers and inorganic compounds. Polymer composites are stronger than other polymers, more resistant to flammability, have better thermal properties, lower melting point and are more resistant to moisture conditions [9].

Nano-sized titanium dioxide is an ideal photocatalyst. Ultraviolet photons are very energetic and in most cases can easily damage objects. Titanium dioxide absorbs ultraviolet radiation through its photocatalytic properties, which can create an antibacterial coating on the surfaces and also prevent the passage of radiation. These special properties have made titanium dioxide nanoparticles a good choice for use in sunscreen materials. Another major use of this substance is the removal of unpleasant odor and decomposition of organic and inorganic toxins and harmful and pathogenic microorganisms in water and wastewater. Titanium dioxide nanoparticles have high hydrophilicity [10].

TiO2 has good antimicrobial activity and lycopene pigment has suitable antioxidant activity, so these materials were used to modification of biodegradable PLA film. The PLA/TiO_2/Lyc film is an antimicrobial and antioxidant film that can be used in food packaging. Also, as the lycopene pigment color is changed under oxidation conditions, this f ilm can also be used for smart packaging of oxidation-sensitive food products such as oils.

Materials

Polylactic acid was purchased from Khatam polymer Co. (Tehran, Iran). Titanium dioxide nanoparticles with 99.9% purity were purchased from Pishgaman Nano Materials Co. (Mashhad, Iran). Escherichia coli strain and Staphylococcus aureus and Mueller Hinton's culture medium were provided by the Iranian Industrial Microorganisms Collection. Acetic acid and glycerol were obtained from Charlotte Company.

Extraction of Lycopene Pigment from Tomato

Tomato samples were purchased from Urum-ada Co. (Urmia, Iran) and thoroughly washed. The skin and tomato seeds were manually separated, and the remainders were homogenized by a 50 Hz, 400 W power home mixer. Homogenous samples were dried in oven at 40 °C until moisture content was constant. To extract lycopene, 100 g of dried tomato was added to 200 mL of hexane/acetone/ ethanol mixed solvent at a ratio of (2:1:1). The mixture was stirred for 15 min with a shaker and then smoothed with a Buchner funnel. Then 200 mL of the hexane/acetone/ethanol mixture was again added to the residual pulp and stirred for 30 min. This mixture was also filtered by Buchner funnel and the obtained solution was added to the previous solution. The solvent (400 mL) containing lycopene was transferred to the separator funnel and 100 mL of water was added to it and stirred slowly. This solution was left for 15 min to separate the lipophilic and hydrophile phases. The solvent containing lycopene was separated and transferred to a rotary evaporator under vacuum and the solvent was removed at 35 °C under vacuum. During all these stages the containers were covered with aluminum foil to protect the lycopene from the negative effects of light.

Polylactic acid/TiO_2/Lycopene Film Preparation

To prepare poly lactic acid film, 1 g of poly lactic acid granule was added to 50 mL of chloroform. The resulting mixture was stirred on the magnetic stirrer for 8 h. Then the desired concentrations of lycopene pigment (0, 1.5 and 3% W/V) titanium dioxide nanoparticles (0, 0.0075 and 0.015% W/V) were added to the mixture according to the statistical design (Table 1). The resulting mixture was placed on a magnetic stirrer for 20 min. Finally, it was homogenized with a homogenizer for 2 min at 12,000 RPM. Then, 40 ml of the solution was poured into 10 cm diameter glass plates and kept for 4 h at room temperature under a chemical hood. After the solvent evaporated, the films were removed from the plates and placed at room temperature for 24 h to completely remove the solvent residue.

Film Thickness

Micrometer (Mitutoyo-Co, Japan) with 0.001 mm accuracy was used to determine the film thickness. Measurements were made at five different points of the film and then their

Table 1 List of Experiments in the CCD

Film	A: Lycopene (%)	B: TiO_2 (%)
F1	0.0	0.0075
F2	1.5	0.0075
F3	1.5	0.015
F4	1.5	0.0075
F5	1.5	0.0075
F6*	3.0	0.00
F7	1.5	0.0075
F8*	0.0	0.015
F9*	0.0	0.00
F10*	3.0	0.015
F11	1.5	0.0075
F12	1.5	0.00
F13	3.0	0.0075

averages were calculated. The calculated mean thickness was used to determine permeability to water vapor

Moisture

To measure the moisture content, 0.2 g of the film samples were first conditioned in a desiccator containing magnesium nitrate (25 °C) for 48 h and (m1). The film was then dried at 105 °C in an oven for 6 h and its weight was measured again (m_2). The moisture content was calculated as follows

$$MC_{wb} = \left(\frac{m_1 - m_2}{m_1}\right) \times 100$$

where MC is moisture contents (%), m_1 and m_2 are the film weight before and after drying respectively.

Water Vapor Permeability (WVP)

Water vapor permeability (WVP) of films was measured according to the Farshchi et al. method [13]. Falcons with a diameter of 2 cm were used for this purpose. 5 g of calcium chloride was poured into the falcons. A piece of film was placed on the Falcon cap with paraffin melted. The falcons were then weighed and placed in a desiccator containing saturated NaCl (RH = 75%). The weight of the falcons was measured every 6 h. The amount of water vapor transferred from the films was determined by Falcon weight loss. The falcon's weight loss curve was plotted over time and after calculating linear regression, the slope of the resulting line was calculated.

By dividing the weight loss slope of each vial to the film surface exposed to water vapor transmission, water vapor transmission rate (WVTR) and water vapor permeability (WVP) were calculated according to the following equations.

$$WVTR = \frac{Slope}{A} \quad (2)$$

$$WVP = \frac{(WVTR \times L)}{\Delta P} \quad (3)$$

WVTR: Water vapor transmission rate (kg/m^2 s), L: film thickness (m), ΔP: relative water vapor pressure difference between film sides (Pa), A: film surface (m^2).

Antioxidant Activity

Determination of DPPH free radical scavenging activity was performed in polylactic acid films according to Karimi Sani et al. method [14]. Briefly, 25 mg of each film sample was dissolved in 4 mL of methanol with continuous stirring, then vortexed for 3 min and centrifuged at 2300 RPM for 10 min. 2 mL of the film extract was mixed with 0.2 mL of 1.0 mM DPPH methanol solution. The resulting mixture was stirred well for 5 min and then kept in the dark at room temperature for 1 h. The absorbance of the samples was read at 517 nm with UV–Vis spectrophotometer (Model T60 UV, USA).

Color Determination

CIE colorimeter (Minolta CR300 Series, Minolta Camera Co. Ltd., Osaka, Japan) was used to determine the surface color of the film samples. Results were expressed as light dark (L*), green-red (a*), and blue-yellow (b*). The whiteness index (WI) and the total color difference (ΔE) were calculated as follows:

$$WI = 100 - \left[(100 - L)^2 + a^2 + b^2\right]^{0.5}$$

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

Antibacterial Activity

The agar diffusion method was used to determine the antibacterial property of the films. The films were cut as discs (15 mm diameter) and then placed on Mueller Hinton Agar plates containing Escherichia coli H7: 0157 and Staphylococcus aureus(105 CFU/mL). Then the plates were incubated at 37 °C for 24 h. After this period, the radius of growth inhibition zone was measured by a precise caliper and its area was reported in millimeters [15].

FT-IR Test

Perkin Elmer instrument (Spectrum Two, USA) was used for the FT-IR test. The film samples were powdered and mixed with dry potassium bromide. Thin tablets less than 1.0 mm thick were prepared from mixed powder to identify functional groups. The passing spectra of the samples were recorded and analyzed in the range of 400–4000 cm^{-1}

Scanning Electron Microscopy (SEM) Test

Scanning electron microscope (Philip, Netherlands) was used to study the microscopic structure and morphology of PLA/Lyc/TiO_2 films. For preparation of the sample, the samples were first glued onto a metal base with a doublesided carbon adhesive and then coated with gold particles. Imaging of the samples was done by electron microscopy at various magnifications

X-Ray Diffraction (XRD) Test

X-ray diffraction test was used to study the structure of the f ilms. X-ray measurements were performed on the films using a diffractometer (Karlsruhe, Germany) operating at a wavelength of 0.154 nm. The samples were exposed to X-rays at 40 kV and 40 mA. Radiation scattering was recorded in the range of (2θ) from 10 to 70° with a scan rate of 1°/min [14].

Differential Scanning Calorimetry (DSC) Test

DSC instrument (Netzsch 200 F3, Germany) was used to measure the thermal properties of the films. The device was calibrated by indium and silver. Aluminum containers were used as the reference and nitrogen as atmosphere. Samples with an approximate weight of 0.03 g at 0 °C/min rate were heated in the temperature range of 0–200 °C. From the thermal pattern obtained, the melting temperature (Tm) of the glass transition temperature (Tg) was determined.

Statistical Analysis

In this study, the central composite design according to Table 1 was used to investigate the effect of two variables, including TiO2 and lycopene pigment concentration on the film properties. Response surface methodology was used to investigate the following characteristics: moisture, thickness, water vapor permeability, antioxidant properties and color characteristics as dependent variables that were affected by the independent variables. These features were fitted with linear and quadratic models at 95% probability level. Data analysis and charting were performed by Design Expert 11.0.0 software. Then, factorial design was used to investigate the effect of titanium dioxide alone and lycopene pigment alone as well as the simultaneous effect of titanium dioxide and lycopene pigment on the following properties: Antimicrobial, FT-IR, SEM, XRD and DSC. For this purpose, F6, F8, F9 and F10 treatments were selected from Table 1

Result and Discussion

For studying the effect of lycopene concentration (A) and TiO2 concentration (B) on the properties of PLA based film, the response surface method (RSM) was used. The following characteristics were studied based on RSM; thickness, moisture, WVP, DPPH scavenging activity and color properties. Mathematical models were used to estimate the effects of lycopene concentration (%) and TiO2 concentration (%) on the PLA based film characteristics. Mathematical models and relations between independent variables and responses were investigated and reported in Table 2.

Table 2 Obtained models and estimated regression coefficients for the responses

Response	Equations	R^2	R^2_{Adj}
Thickness	$=0.21 + 0.01 * A + 0.0043* B + 0.0032 * AB - 0.01* A^2 + 0.0011 * B^2$	0.93	0.88
Moisture	$= 8.74 + 0.06 * A +0.33 * B +0.02 * AB +1.15 * A^2 - 0.11 * B^2$	0.96	0.93
WVP	$= 1.32 - 2.65 * A - 1.85 * B$	0.5	0.41
DPPH	$= 22.06 + 18.19 * A +1.57 * B +0.24 * AB +7.14 * A^2 +1.40 * B^2$	0.98	0.97
L*	$= 56.61 - 8.56\ A - 1.24\ B + 2.46\ AB + 2.75\ A^2 + 2.82\ B^2$	0.97	0.64
a*	$= 17.64 + 8.56\ A + 1.94\ B - 2.68\ AB - 2.05\ A^2 - 3.97\ B^2$	0.75	0.58
b*	$= 65.50 + 24.44\ A - 2.47\ B - 4.78\ AB - 35.75\ A^2$	0.99	0.99
ΔE	$= 88.63 + 11.55\ A - 0.77\ B - 19.87\ A^2$	0.58	0.44
WI	$= 19.18 - 22.70\ A + 0.51\ B + 5.29\ AB + 25.67\ A^2 + 2.85\ B^2$	0.96	0.93

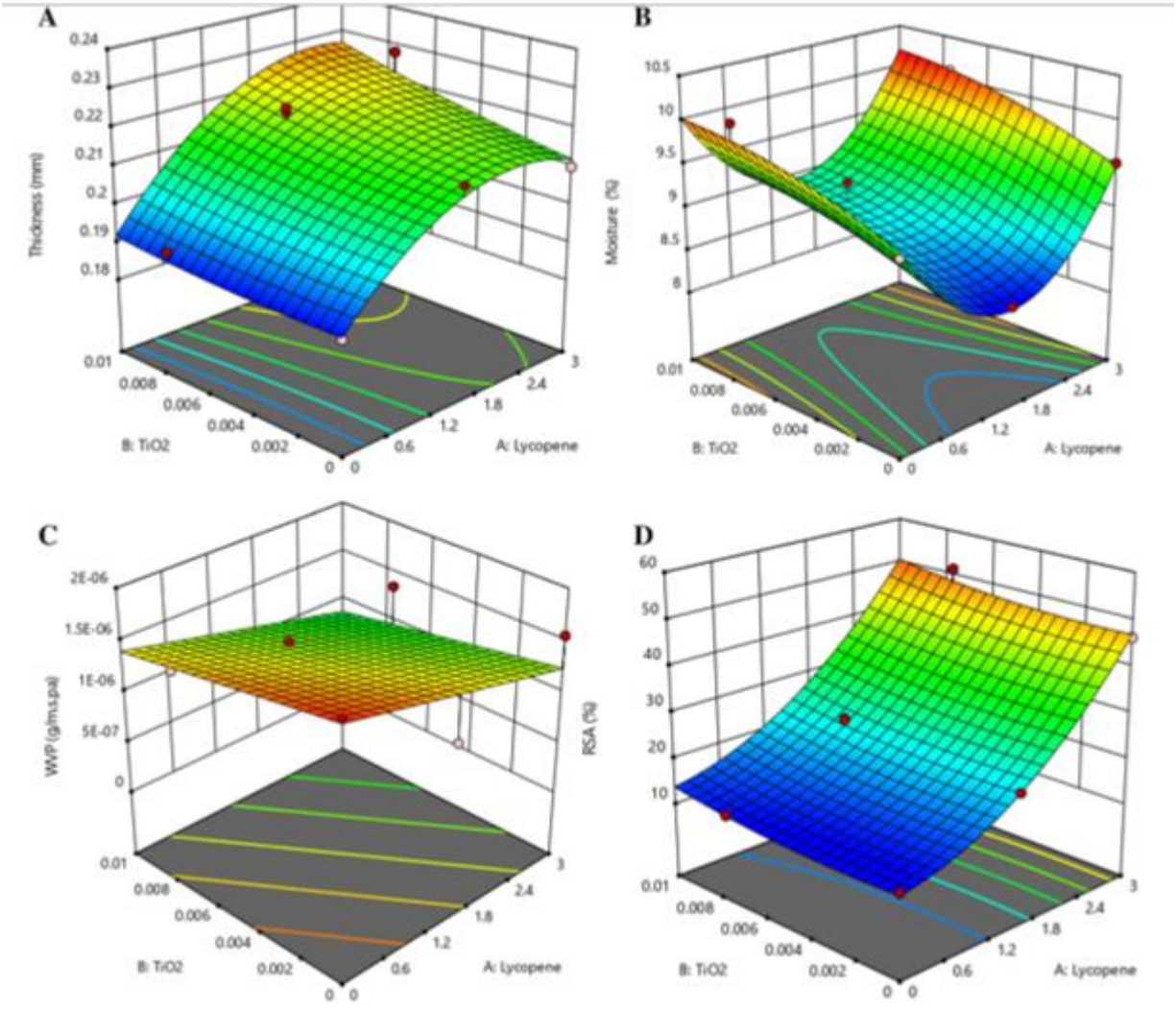
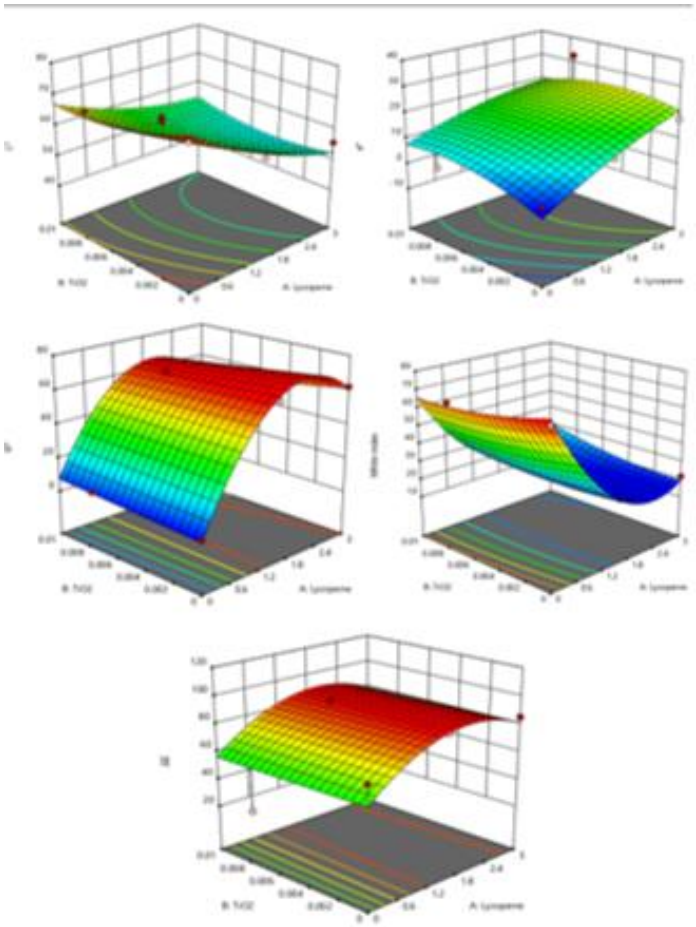


Fig. 1 3-D plots of the effect of Lycopene and TiO_2 on the thickness (**a**), moisture (**b**), WVP (**c**) and antioxidant activity (**d**) of films

chromophore group in the lycopene chain, so increasing concentration of lycopene increases a*. Also with increasing concentration of titanium dioxide a* is decreased, which may be due to photocatalytic activity and excessive absorption of UV waves. In the case of b*, the b* increased as the amount of lycopene increased and b* decreased as the amount of titanium dioxide increased. Lycopene pigments and titanium nanoparticles had a significant effect on the b* index ($p < 0.05$). Increase in lycopene increases ΔE but no increase in ΔE is observed with increase in titanium dioxide content. In the case of whiteness index, it is clear that by the increase in lycopene, the amount of white color index (WI) decreases. The presence of the chromophore in the lycopene chain reduces the lycopene whiteness and consequently reduces the film whiteness. As the concentration of titanium dioxide increases, the white color index (WI) increases. The titanium oxide nanoparticles are white and thus increase the film whiteness index.

Antibacterial Activity Study

The use of edible and biodegradable films to release antimicrobial compounds into food packaging is a form of active packaging. Figure 3 shows the results of the antimicrobial activity of PLA based films against S. aureus (A) and E. coli (B). The zone of inhibition against Escherichia coli was found to be 15 mm, 12 mm, 8 mm and 0 mm for F10, F8, F6 and F9, respectively. The zone of



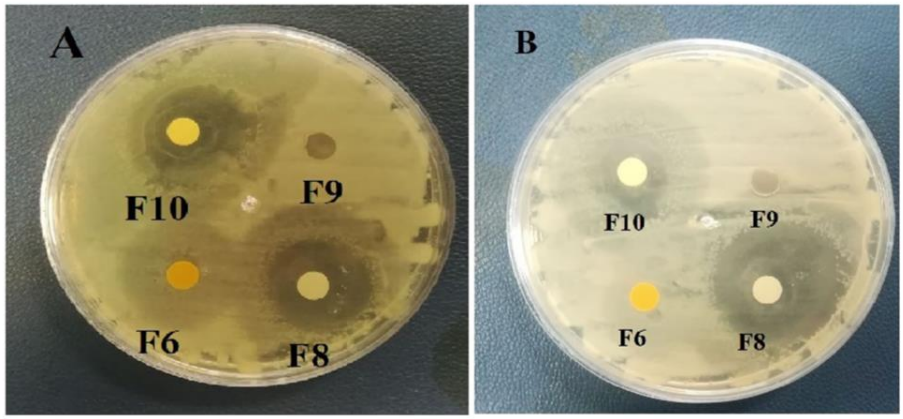


Fig. 3 Antibacterial activity of pure PLA (F9), PLA/3% Lycopene (F6), PLA/0.015 TiO_2 (F8) and PLA/0.015 TiO_2/PLA/3% (F10) against *Staphylococcus aureus* (**a**) and *Escherichia coli* (**b**)

inhibition against Staphylococcus aureus was found to be 18 mm, 15 mm, 10 mm and 0 mm for F10, F8, F6 and F9, respectively. As can be seen, the pure PLA film showed no inhibition zone to Gram-negative and Gram-positive bacteria. Various factors affect the antimicrobial activity of active films, including the nature of the bacterium, the film matrix characteristics, the method and the conditions of film production [24]. With the addition of titanium dioxide nanoparticles and lycopene pigment, the diameter of the inhibition zone against Gram-positive and Gram-negative bacteria increased. It should be mentioned that the film containing titanium dioxide nanoparticles without the presence of lycopene shows antioxidant activity due to the antimicrobial activity of titanium dioxide nanoparticles through the destruction of the bacterial cell wall. These results are in consistent with the results of Ramos et al. [25]. The film containing lycopene without the presence of titanium oxide nanoparticles also shows a good antioxidant property because of the high antimicrobial properties of the lycopene that mainly is related to carotenoid compounds in lycopene structure. The film containing titanium oxide and lycopene nanoparticles has the highest antibacterial activity due to the synergistic effect of titanium oxide and lycopene. Antimicrobial agents had a greater effect on Gram-positive bacteria than on Gramnegative bacteria, in the Gram-positive bacteria the main molecule of the cell wall is peptide and glycan and a small amount of protein. Therefore, due to the absence of extra outer membrane in the Gram-positive bacteria, antimicrobial agents penetrate more easily. Whereas the cell wall of Gram-negative bacteria has a more complex structure with different polysaccharides, proteins and lipid-based peptidoglycan [26]. As a result, Gram-negative bacteria contain an additional hydrophilic membrane that is embedded with specific lipopolysaccharide molecules that act as a barrier against hydrophilic compounds and reduce the effect of antibacterial agents on Gram-negative bacteria.

Conclusion

Nanotechnology has shown great potential for making significant changes in the food packaging industry. The use of titanium dioxide and the antioxidant pigment of lycopene improve the overall performance of polylactic acid and is a factor to extend its use as biodegradable packaging.

Considering the results of this study, the combination of small amounts of titanium dioxide and lycopene pigment to the lattice matrix had a significant improvement in the physical and chemical properties of the film. Proper interaction between the polymer matrix and lycopene had good effect on the decreasing of WVP of film. The compatibility of lycopene pigment with poly lactic acid and its antioxidant effects for the production of activated film provided satisfactory results. Lycopene has a strong antioxidant capacity due to the long chromophore group in its chain, so adding of lycopene to the film increased antioxidant activity significantly. According to the DSC results, the interactions between the pigments, nanoparticles and the polylactic acid, and the formation of dense crystalline regions in the PLA matrix increased the polymer melting point. The film containing titanium oxide and lycopene nanoparticles had antibacterial activity due to the synergistic effect of titanium oxide and lycopene. Antimicrobial film had a greater effect on Gram-positive bacteria than on Gram-negative bacteria. Finally, it should be mentioned that that the prepared film had color change ability in different oxidation condition, so it can be used as an antimicrobial and antioxidant film with suitable color properties for active and intelligent packaging in the food industry.

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