



## ORIGINAL ARTICLE

# The Effects of Nano Zinc Oxide Shape on Optical Characteristics of Tapioca Starch Films and *In vitro* *Escherichia coli* Microbial Growth Kinetics

Naser Tamimi<sup>1</sup>, Abdorreza Mohammadi Nafchi<sup>\*2,3,4</sup>, Hamid Hashemi Moghaddam<sup>1</sup>, Homa Baghaie<sup>4</sup>

<sup>1</sup>Department of Chemical Engineering, Damghan Branch, Islamic Azad University, Damghan, Iran

<sup>2</sup>Food Technology Division, School of Industrial Technology, Universiti Sains Malaysia, 11800 USM, Penang, Malaysia

<sup>3</sup>Cluster of Green Biopolymer, Coatings & Packaging, School of Industrial Technology, Universiti Sains Malaysia, 11800 USM, Penang, Malaysia

<sup>4</sup>Department of Food Science and Technology, Damghan Branch, Islamic Azad University, Damghan, Iran

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## KEYWORDS

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Transparency

**ABSTRACT:** This study aimed to investigate the effects of zinc oxide nanoparticle shape on the optical characteristics of tapioca starch films and the microbial growth kinetics of *Escherichia coli*. For this purpose, nanorods, nano-spheres, and nanoparticles of ZnO at 0.5, 1.0, and 2.0% levels were incorporated into the tapioca starch film solution by solvent casting method. The results showed that tapioca starch-based films were colorless, and by adding different morphology of ZnO nanoparticles and increasing nanoparticles concentrations, the lightness and transparency of the films decreased, and  $a^*$ ,  $b^*$  and  $\Delta E$  increased significantly ( $p < 0.05$ ). The bionanocomposite films containing nano-ZnO represented antibacterial activity against *E. coli*. Their action was directly related to their concentration. With increasing the nano-ZnO concentration, the antibacterial activity increased, and the microbial growth kinetics tended downward. The morphology of nano-ZnO had a remarkable effect on their antibacterial activity, so the highest activity was related to ZnO nano-spheres.

## INTRODUCTION

One of the critical issues in the food industry is contamination and microbial spoilage of the foods [1]. Therefore, solutions and new technologies have been studied to improve the quality and safety of food products during handling, transportation, and storage. One of the best and most important ways to maintain the quality and enhance the safety and stability of foods is to use active packaging films [2, 3].

Despite the widespread use of plastic films in the packaging industry, due to the non-biodegradability and

toxicity of these synthetic packaging, today's efforts to replace biopolymer-based films instead of synthetic types are growing [4]. Biodegradable films can be produced using natural biopolymers such as carbohydrates, proteins, and fats. These natural packaging films are both inexpensive and environmentally friendly [5]. Starch is one of the major natural biopolymer materials for packaging applications because this biopolymer is abundant, cheap, available, biodegradable, and non-toxic [6, 7]. However, starch-based films alone have poor

\*Corresponding author: amohammadi@usm.my (A. Mohammadi Nafchi)  
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mechanical and thermal properties, low moisture resistance, and lack antimicrobial activity. One of the ways to improve the properties of these biopolymer films is to use nano-fillers in the film matrix [8].

Bio-nanocomposites are active packaging films that consist of two important components, including a biopolymer matrix (continuous phase) and filler agents (dispersed phase) with nano-metric dimensions (<100 nm) [9]. Previous research has confirmed that incorporating metal and metal oxide nanoparticles into biopolymer-based packaging films enhances the barrier, strength, mechanical, thermal resistance properties, and stability of films against moisture and gases [10]. Various metal dioxide nano-fillers have been used to improve biopolymer films, one of the most important of which is zinc oxide nanoparticles (nano-ZnO) [11-13].

Nano-ZnO can be manufactured using micro-emulsion, sonochemical, precipitation, and thermal decomposition [14, 15]. These nanoparticles have unique properties and are safe and non-toxic [16]. Previous research has shown significant antimicrobial activity of the films containing nano-ZnO on Gram-positive bacteria, Gram-negative bacterial, and molds [17-20].

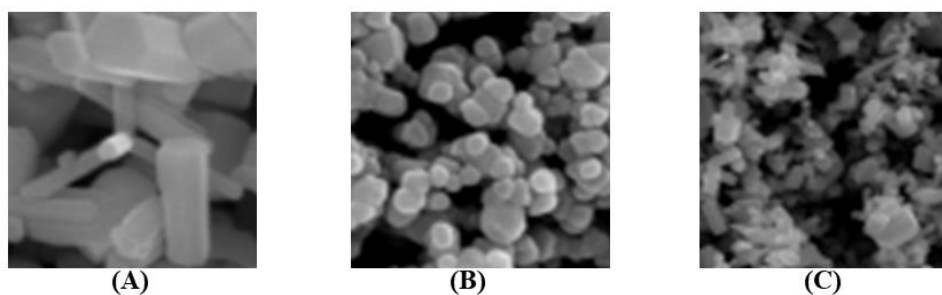
Nano-fillers used in food packaging systems have different shapes, such as nanoparticles, nanorods,

nanotubes, and nanofibrils [2], and the shape and size of nanoparticles are key factors in their permeability and diffusion into the bacterial cell [21]. Based on our knowledge, the effects of nano ZnO shape on the growth rate *E.Coli* was not investigated. Therefore, the aim of this study was to investigate the effect of ZnO morphology on the optical characteristics of tapioca starch film and the *E. coli* growth kinetics.

## MATERIALS AND METHODS

### Materials

Native tapioca starch was prepared from SIM Supply Company Sdn. Bhd (Penang, Malaysia). ZnO nanorods (Figure 1a) and ZnO nano-sphere (Figure 1b) were gifts from Universiti Sains Malaysia, School of Physics (Penang, Malaysia). ZnO nanoparticles (a mixture of nanoparticles with dimensions of 40-100 nm) (Figure 1c) and glycerol (as plasticizer) were supplied by Sigma Chemical Company. (USA). *Escherichia coli* O157:H7 culture was obtained from the Organization of Scientific and Industrial Research (Tehran, Iran). All chemicals and mediums used in this research were based on analytical grade and prepared by Merck Company (Germany).



**Figure 1.** The microscopic structures of (A) ZnO nano-rods, (B) ZnO nano-sphere, (C) ZnO nanoparticles. (Magnification 50X)

### Preparation of bionanocomposite films

To prepare the nano-ZnO solutions, ZnO nano-sphere, nanoparticles, and nanorod at levels of 0.5, 1.0, and 2.0% w/w (based on the dry matter) were first poured into distilled water and heated at 60°C for 60 min by a hot plate. After that, the mixtures were placed on a stirrer for 24 h without heat to create homogeneous solutions and then homogenized at 40°C for 45 min in an ultrasonic bath (BANDELIN SONOREX digitec, Germany).

To prepare the film solutions, tapioca starch (4% w/v) and glycerol (40% w/w based on the dry matter) were added to nano-ZnO solutions. The starch dispersions containing the nano-ZnO were heated to 85°C and kept for 45 minutes to complete gelatinization (stirring at 500 rpm), then cooled to 40°C. The control sample also was prepared without nano ZnO by the above method. A proper amount of solutions (90 g) were casted on Plexiglas plates (16 × 16 cm<sup>2</sup>) to form the films. The

bionanocomposite films were dried for one day under controlled conditions (50% RH and 30°C) [22]. Finally, the starch films were peeled off from the plates at room temperature and placed in a desiccator (RH = 55±5%) until the experiments were performed [23].

#### ***The thickness of the films***

The thickness of the bionanocomposite films was measured using a digital micrometer (QLR IP54, America) at five films points with an accuracy of 0.001 mm [7].

#### ***Color measurement***

The color parameters of the bionanocomposite films, including lightness ( $L^*$  index), redness-greenness ( $a^*$  index), and yellowness-blueness ( $b^*$  index), were measured using a colorimeter (Minolta CM-3500D, Osaka, Japan) [24]. Before testing, the device was calibrated by a standard white screen. Color indexes were determined with five replications for each film sample. Total color changes ( $\Delta E$ ) of the film samples were also calculated using the following equation:

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

#### ***Transparency of the films***

The film was cut into 4 × 1 cm pieces, and its thickness was recorded. The film piece was then placed in a transparent wall inside the quartz cell of the spectrophotometer (Shimadzu, Japan), and its absorbance was measured at 600 nm. The transparency of the bionanocomposites was calculated using the following equation:

$$\text{Transparency} = \frac{A_{600}}{X}$$

where  $A_{600}$  and  $X$  are the absorbances at 600 nm and the average thickness of the film (mm). The transparency of the films was expressed in  $\text{mm}^{-1}$  [25].

#### ***Antibacterial activity of films against E. coli by dynamic method***

The antibacterial activity of the bionanocomposite films against *E. coli* O157:H7 was investigated according to the shake flask method [26]. In this test, *E. coli* O157:H7 grown in Mueller Hinton Broth was used.  $2 \times 10^5$  CFU/mL of this bacterium was added to the Mueller Hinton Broth medium (100 mL) and incubated for 11 h at 37 °C. Every 1 h, the absorbance was read by a UV/Visible spectrophotometer (Shimadzu, Japan) at 600 nm, and bacterial growth was obtained. The experiment was repeated 3 times, and the mean values were used.

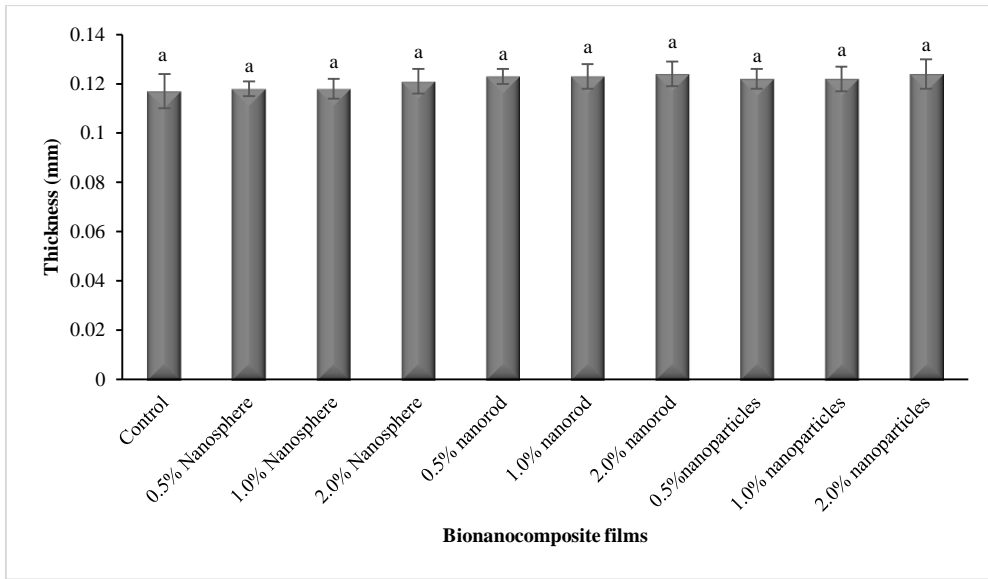
#### ***Statistical analysis***

Data were analyzed using one-way analysis of variance and followed by the Duncan's post hoc test at 5% level of probability using IBM SPSS 22.0 (IBM Corp., Armonk, NY, USA) software.

## **RESULTS**

#### ***The film thicknesses***

Figure 2 shows the mean thickness values of tapioca starch films containing different shape of nano-ZnO. The mean thickness values of different bionanocomposite films ranged from 0.117-0.124 mm. The addition of nano-ZnO caused a slight increase in the thickness of starch films; however, this change wasn't statistically significant.

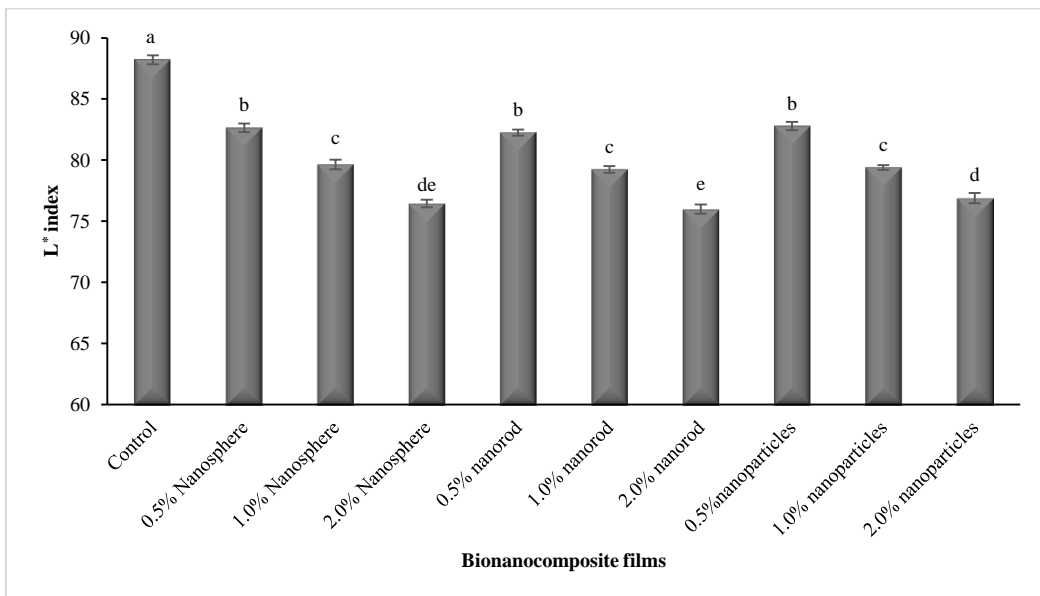


**Figure 2.** Comparison of different concentration and different nano ZnO shape on thickness (mm) of tapioca starch films. Bars show mean±SD. The same letters on the bars represent no significant differences at the 5% probability level.

**The film color**

The color indexes of tapioca starch-based bionanocomposite films containing different forms and concentrations of nano-ZnO, including L\* (lightness), a\* (redness-greenness), b\* (yellowness-blueness), and ΔE (total color changes), are compared in Figures 3, 4, 5, and 6, respectively. The form of nano-ZnO and their concentration significantly affected the color indexes of tapioca starch-based film. The control sample had the

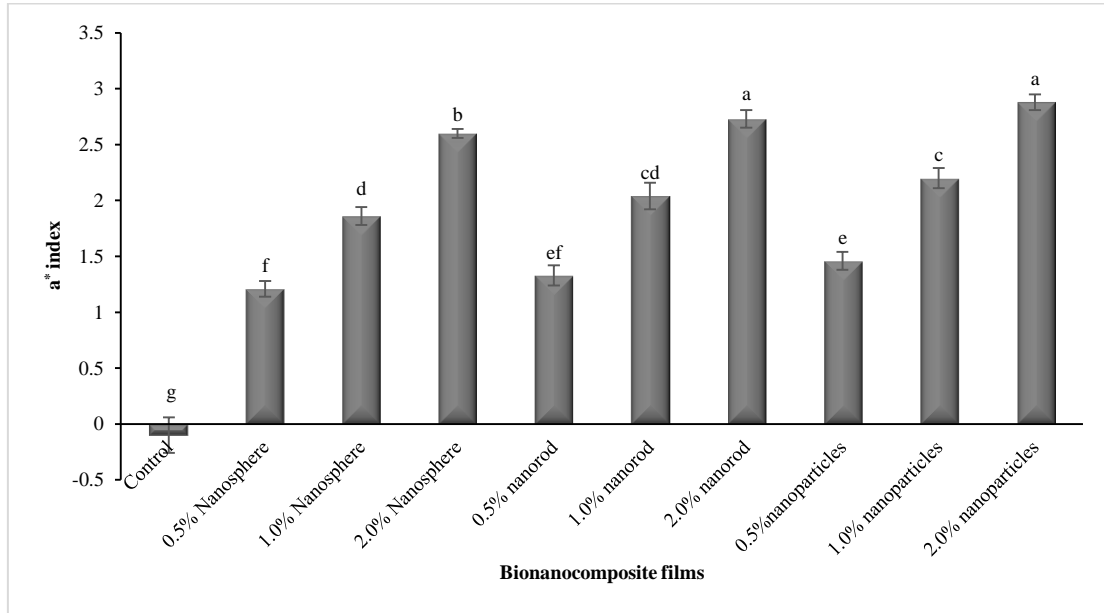
highest color lightness, and the addition of nano-ZnO led to a significant reduction in the L\* index (p<0.05). By increasing the concentration of ZnO nano-sphere, ZnO nano-rods, and ZnO nanoparticles from 0.5% to 2%, the amount of L\* decreased significantly (p<0.05). The L\* index values of the bionanocomposite films ranged from 76.89 to 88.20 (Figure 3).



**Figure 3.** Comparison of different concentration and different nano ZnO shape on the L\* values of tapioca starch films. Bars show mean ± standard deviation. Different small letters on the bars represent a significant difference at a 95% confidence level among bio nanocomposite films.

In terms of  $a^*$  index, the lowest value of this color index was related to the control sample (-0.10), and with adding different morphology of nano ZnO and increasing their concentration from 0.5% to 2%,  $a^*$  index

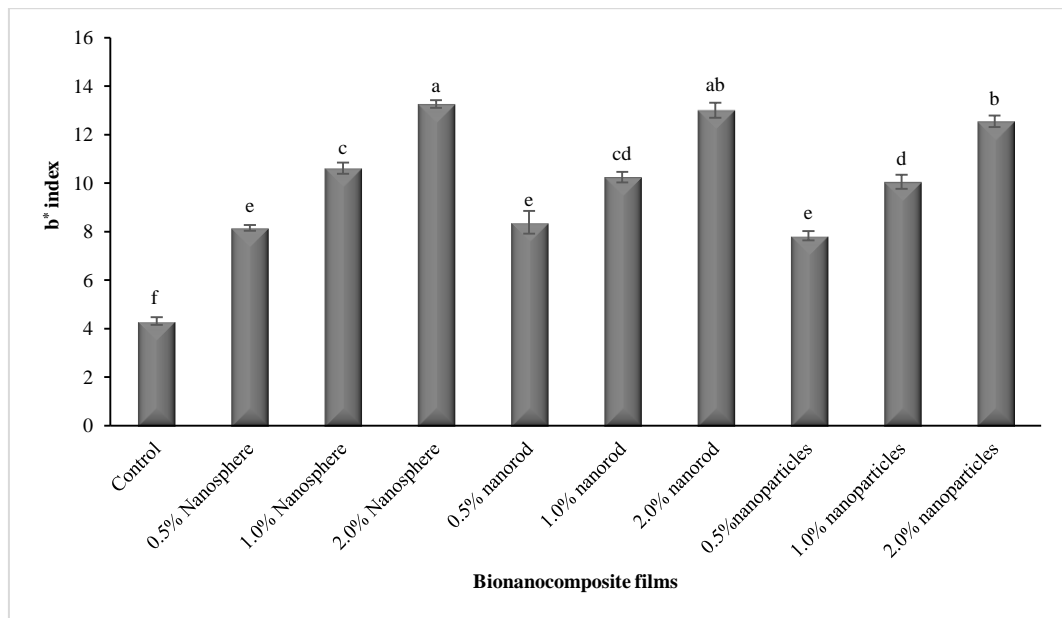
significantly increased ( $p < 0.05$ ). The highest amount of  $a^*$  index was observed in the films containing 2% ZnO nanorods (2.73) and 2% mixture of ZnO nanoparticles (2.88) (Figure 4).



**Figure 4.** Comparison of different concentration and different nano ZnO shape on the  $a^*$  values of tapioca starch. Bars show mean  $\pm$  standard deviation. Different small letters on the bars represent a significant difference at a 95% confidence level among bio nanocomposite films.

In terms of  $b^*$  index, the lowest amount was observed in the control sample. Adding different morphology of nano ZnO and increasing their concentration from 0.5% to 2%

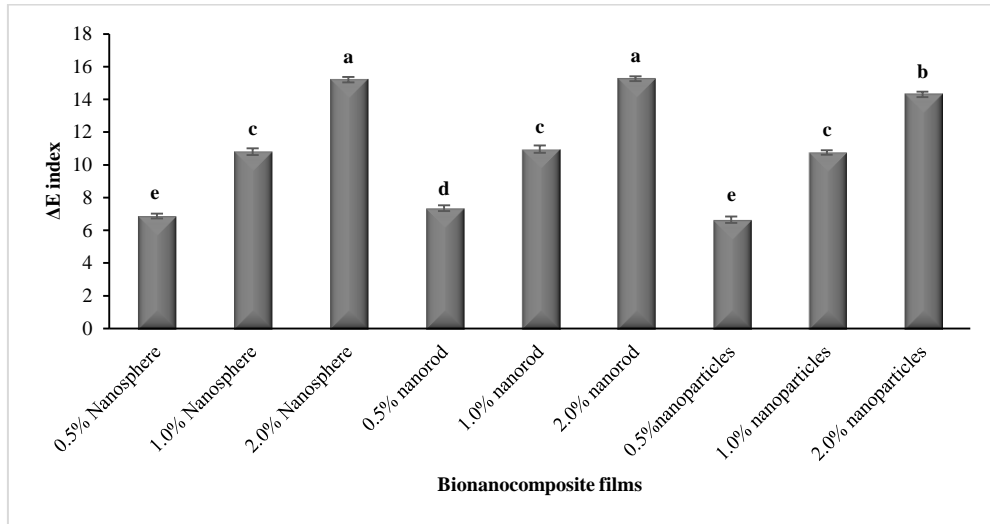
led to an increase in the  $b^*$  index. The  $b^*$  index values of the starch-based bionanocomposite films ranged from 4.31 to 13.26 (Figure 5).



**Figure 5.** Comparison of different concentration and different nano ZnO shape on the  $b^*$  values of tapioca starch films. Bars show mean  $\pm$  standard deviation. Different small letters on the bars represent a significant difference at a 95% confidence level among bio nanocomposite films.

By increasing the nano ZnO concentration in tapioca starch films also significantly increased the  $\Delta E$  value of the films ( $p < 0.05$ ), so that at a concentration of 0.5% ZnO nano-sphere, ZnO nanorods, and ZnO

nanoparticles, the  $\Delta E$  were 6.88, 7.35, and 6.65, respectively, and at a concentration of 2% reached to 15.21, 15.26 and 14.31, respectively (Figure 6).

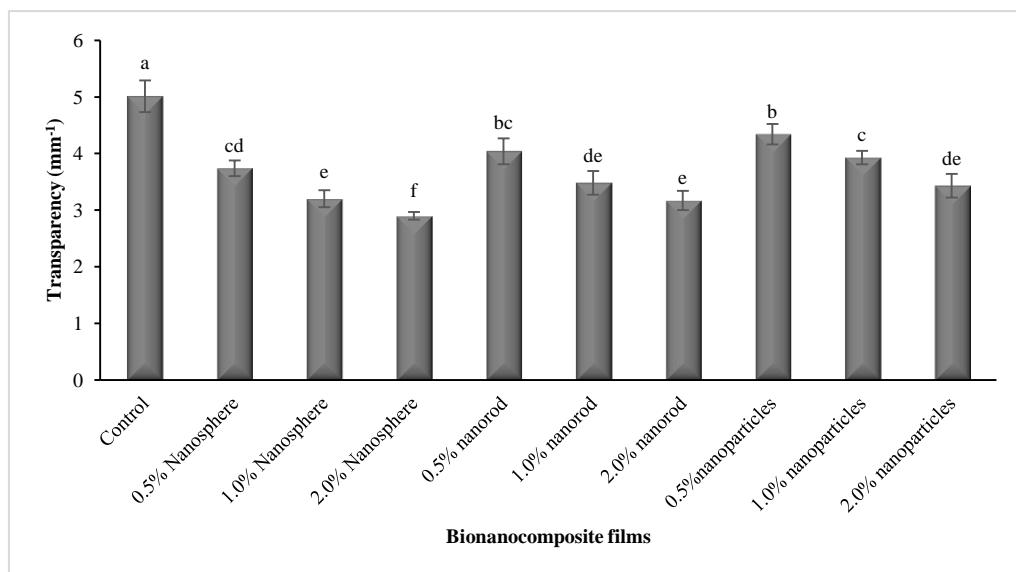


**Figure 6.** Comparison of different concentration and different nano ZnO shape on the  $\Delta E$  values of tapioca starch films. Bars show mean  $\pm$  standard deviation. Different small letters on the bars represent a significant difference at a 95% confidence level among bio nanocomposite films.

### The film transparency

Figure 7 compares the transparency of tapioca starch bionanocomposites containing different forms and levels of nano-ZnO. The Figure shows that the control sample had higher transparency compared to films containing nano-ZnO (5.01  $\text{mm}^{-1}$ ) and by adding ZnO nano-sphere, ZnO nanorods, and ZnO nanoparticles to films and

increasing their concentration from 0.5% to 2%, the transparency of the starch films significantly reduced ( $p < 0.05$ ). The lowest amount of transparency was observed in the film sample containing a 2% ZnO nano-sphere (2.90  $\text{mm}^{-1}$ ).



**Figure 7.** Comparison of different concentration and different nano ZnO shape on the transparency ( $\text{mm}^{-1}$ ) of tapioca starch films. Bars show mean ( $n = 5$ )  $\pm$  standard deviation. Different small letters on the bars represent a significant difference at a 95% confidence level among bio nanocomposite films.

### The antibacterial activity of bionanocomposite films

#### against *E. coli* by dynamic method

The microbial growth curves of *E. coli* against tapioca bionanocomposite containing different morphology and concentration of nano ZnO are shown in Figure 8. The Figure demonstrated that the starch films containing different shape of nano ZnO both increased the lag phase and significantly reduced the log phase. Increasing the concentration of nano-sphere, ZnO nanorods, and ZnO nanoparticles also caused a remarkable reduction in microbial growth kinetics. The more downward

microbial growth kinetics, the higher its inhibition because the lag phase increases, as well as the log phase, decreases. In other words, nano-ZnO leads to the destruction of microorganisms by reducing the growth rate and prolonging the lag phase of microorganisms or inactivating them. The antibacterial activity of nano-sphere morphology was higher than nanorods and a mixture of nanoparticles.

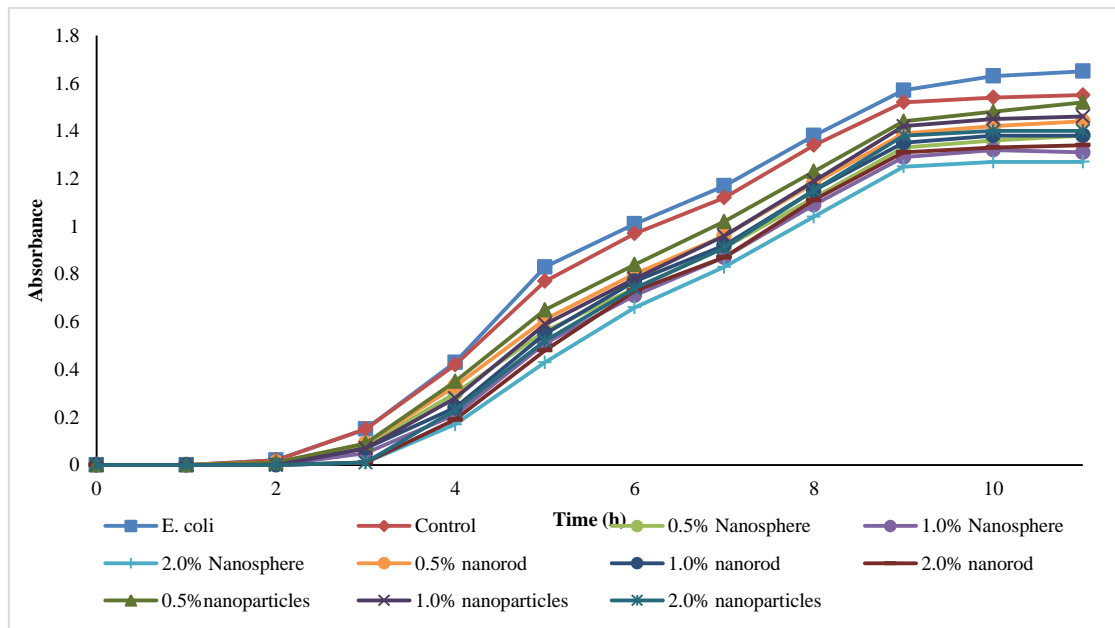


Figure 8. The *Escherichia. coli* growth against tapioca based bionanocomposite containing different zinc oxide nano shapes.

### DISCUSSION

The thickness of the films is an important factor that affects the calculation of the final product price, so that the thinner the film, the lower the cost of production. The films' thickness depends on the film solution volume in the mold and the mold area [27]. The thickness of the films also affects their mechanical and barrier properties [28]. The addition of different forms of nano-ZnO didn't significantly affect the thickness of tapioca starch-based films, which were similar to the results reported by some other researchers [29-31].

The film's color and appearance in food packaging is a major and effective parameter in the consumer's selection and acceptance of the food product. Most packaging films are colorless and clear, but in some cases, due to

the food product sensitivity to light and likely loss of its nutrients due to light oxidation, as well as the color matching of the contents with the packaging material to attract the consumer, the use of light-inhibiting and color-forming compounds in the packaging films seems necessary [32]. Therefore, in this study, the effect of incorporating different morphology and concentrations of nano-ZnO on the color indexes of tapioca starch-based bionanocomposites was investigated, and the results showed that tapioca starch film was colorless and the incorporation of different shapes of nano ZnO led to a decrease in lightness and an increase in the yellowness and redness of the bionanocomposite film and with increasing the nano-ZnO concentration, the color change

of the films also increased. Similarly, Marvizadeh et al. [33] observed that increasing the ZnO nanorods in the combined films based on tapioca starch and gelatin significantly reduced the lightness and increased the  $a^*$  and  $b^*$  values of the produced films. Other researchers have also shown the darker colors of bionanocomposite films due to the incorporation of metal oxide nanoparticles [34-36].

Increased opacity and decreased transparency of food packaging films are suitable ways to protect food products from light [37]. In this study, the transparency was significantly reduced by incorporating different shapes of nano ZnO and increasing their concentrations in starch-based films. Oleyaei et al. [38] also reported similar results in investigating the effect of nano-TiO<sub>2</sub> on the opacity of potato starch-based films. The results reported by Vaezi et al. [39] and Guz et al. [40] were also consistent with the results of this study.

A general microbial growth curve has 4 phases: lag, log, stationary, and death. Generally, the higher the antimicrobial activity of an additive, the more the lag phase is delayed, and the maximum microorganism population in the log phase is reduced. This study investigated the effect of different forms and concentrations of nano-ZnO on the microbial growth kinetics of *E. coli*. The obtained results showed that by adding different shapes of nano-ZnO and increasing their levels in starch films, the antibacterial activity of films against *E. coli* increased. The ZnO nano-sphere showed higher antibacterial activity than ZnO nano-rods and ZnO nanoparticles.

So far, several mechanisms have been proposed for the antibacterial activity of ZnO nanoparticles, which are in the form of physical, chemical, and physicochemical interactions between nanoparticles and bacteria. These interactions include chemical reactions between Zn<sup>2+</sup> ions and cell membrane compounds. Zn<sup>2+</sup> ions, after passing through the membrane and being transported into the cells, can react chemically with intracellular compounds and damage them. ZnO nanoparticles can also produce H<sub>2</sub>O<sub>2</sub> in the environment, which, after production, can react with cell membrane compounds. Increasing surface groups as active sites for producing reactive oxygen species (ROS), including hydrogen peroxide, superoxide, and hydroxyl radicals, leads to

oxidative stress [41]. These radicals cause various changes and damage in the cell, including increased permeability to nanomaterials, lipid peroxidation, disruption of ion transport, changes in the structure of proteins and DNA, and ultimately cell death [21, 42]. ZnO nanoparticles can also damage cell membranes by physically blocking cell membrane conduction channels, interrupting electron transmissions, and physically damaging cell membranes through wear, corrosion, and rupture [43].

Similarly, in research conducted by Raza et al. [44], the effect of nanoparticle shape on antimicrobial activity was observed. These researchers reported that the antimicrobial action of spherical silver nanoparticles was higher than silver nanoparticles with a triangular shape. However, Van Dong et al. [45] and Pal et al. [46] showed that the antibacterial activity of silver nanoparticles with a triangular shape was higher than the spherical shape. These researchers attributed the higher activity of triangular nanoparticles in relation to their geometric structure and crystalline plates. Park et al. [47] reported higher antimicrobial activity of silver nano-plates than that of spherical and cubic nanoparticles. The research on the effect of the nano-ZnO shape on the antimicrobial action against *E. coli* found that ZnO nano-spheres and ZnO nano-flakes had higher antimicrobial activity than other shapes [48]. Da Silva et al. [49] stated that nano-ZnO with a higher surface area and a smaller size had better antimicrobial activity. Rezaei et al. [50] found that pure gluten film (control sample) had no antimicrobial activity on bacteria and fungi. Still, films containing nano-ZnO demonstrated a significant inhibitory activity on these microorganisms. Nafchi et al. [22] showed that the bovine gelatin and sago starch bionanocomposites films containing ZnO nanorods exhibited excellent antibacterial activity against *E. coli*.

## CONCLUSIONS

This research demonstrated that incorporating different nano-ZnO morphology into the tapioca starch-based bionanocomposite films caused darkening and increased opacity of the produced films. The starch bionanocomposites containing Nano-ZnO showed significant antibacterial activity against *Escherichia coli*. The antibacterial activity of these nanoparticles depended



on their morphology, so most of this activity was observed in the ZnO nano-sphere. Future research must be done on other nanoparticles to find a solid theory on morphological effects on bionanocomposite properties.

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#### Conflict of interests

There is no conflict of interest in the study.

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