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Nano-based Biodegradable Food Packaging of *Vitis-Vinifera* Synthesized by PVA/ZnO Nanocomposites

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Biodegradable films for food packaging applications are one of the extensively researched and emerging materials. This is due to their low cost, easy processing, inertness, eco-friendly feature, and faster biodegradation. In this study, ZnO nanoparticles (ZnO-NPs) were selected (chemically stable) along with polyvinyl alcohol (PVA) matrix for biodegradable packaging. Two concentrations (0.5 wt% and 5.0 wt%) were considered for ZnO-NPs. The effect of packaging on quality of grapes (*Vitis-vinifera*) was studied. This was followed by characterization analysis using UV-Vis, SEM, XRD, and fruit quality. Results showed enhanced stability of biofilms upon the incorporation of ZnO-NPs into the PVA biofilms. Mechanical and physical properties were improved significantly. The *Vitis-Vinifera* (grapes) were packed into the biofilms and their quality in terms of physical appearance, freshness, taste, and antimicrobial activity were studied for 15 days. According to our results, the degradation of grapes was observed in terms of fruit color and decay rates within 3 days of exposure on controlled samples (without packaging at 6 °C). The quality of other grape samples remained excellent at the designated duration. The sample with concentration of 5.0 wt% showed the best quality among all other samples. We have provided extensive discussion on biofilm stability, associated properties, and fruit quality.

Keywords: Food packaging, Polyvinyl alcohol (PVA), Biodegradable, *Vitis-vinifera*, Fruit quality, Green nanotechnology

INTRODUCTION

Polymeric materials offer several valuable applications. People are accustomed to using disposable plastics, which has detrimental environmental consequences. The demands for mentioned products are due to their attractive properties such as low weight, high corrosion resistance (even in an aggressive environment), electrical and thermal resistance, and great mechanical properties [1]. In addition, these products have very low production costs compared to steel and other metals. Plastic is one of the widely used materials owing to its variety of applications in food packaging, agricultural, construction, and manufacturing sectors [2,3]. However, since the last decades, the extensive use of such products poses a serious threat to our ecological system; this increases the waste generation in the sea and on the earth ground [4,5]. On average, half of the all produced plastics are used only once and then are discarded in the environment accounting for waste generation [6]. A majority portion of the plastic bags contributes to around 1-2 BTs/Y (Billion Tons per annum) waste pollution [7], which is harmful to humans, sea mammals, and wildlife [8]. One of the important challenges in the food packaging field is plastic waste management. For instance, less than a quarter of packaging material waste generated in Europe is recycled and sent to Asia for further use [9]. Considering the harm of petroleum products, it is very important to replace the traditional petroleum-based products by eco-friendly materials [10]. Therefore, there are vast researches on the biodegradability of materials, among which bio-plastics and edible packaging are some of the most promising packaging [11]. Biodegradable packaging materials are renewable and

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sustainable materials that take from either natural source (*i.e.*, plants/animals and polysaccharides) or manmade sources (polyesters) [12].

In addition, green nanotechnology works as an important implement to overcome the problems related to food packaging. Food is affected by several environmental factors such as oxygen, moisture, microbes and bacteria [13]. If active nano-packaging is provided, food is protected from these factors; the latter enhances the food quality. Some of the limitations of the recent food packaging materials are lack of sustainability, insufficient mechanical strength, lack of recyclability, and poor shelf life [14]. The main objective of the enhanced food quality is to make sure about the health of environment and humankind. For those reasons, foods must be preserved from biological, chemical and physical pollution [15]. Processed food preservation is not possible without smart packaging, and that's why it has become one of the biggest industries. Smart packaging is a packaging system with embedded highly sensitive sensors to monitor the freshness, quality, and safety of food material [16,17]. From the number of experiments, researchers have found a very fast (within the week) microbial growth in food materials causing server problems for human health. Hence, nano-packaging is classified as food containment, food protection, and expediency [17]. Conventional food packaging is not efficient for active food preservation such as freezing, drying, sugaring, and curing. These are traditional methods applied to preserve foods [18]. The goal of modern societies is to replace the traditional food packaging by a new nano-active food packaging. According to literature, incorporating ZnO-NPs can improve the properties of a packaging film such as oxygen and moisture resistance, antimicrobial activity, stability with the shelf life, resistance from relative humidity or temperature, and the strength [19]. In active packaging, the role of nanocomposite is to interact directly and indirectly (surrounding) with food to allow proper protection [20].

The 21st century is known as the century of technology. Foods are readily available in any season due to the availability of good packaging materials. Among fruits, grapes are consumed by Asian people; grapes are rich in biological benefits used for a variety of other products (such as jams, juices, wine, vinegar, and seed oil) [21]. Grapes are also beneficial for diabetic and cardiovascular patients [22]. Furthermore, grapes can also boost immunity due to their anti-inflammatory action [23]. Grapes contain a high number of vitamins and minerals and it is important to increase the shell life of grapes [24,25]. The incorporation of the cost-effective ZnO-NPs with biodegradable polymer increases its mechanical strength and stability. Such nano-packaging has the advantage of not only improving the food quality, but also enhancing mechanical strength and chemical stability [26,27]. This can be done when NPs are mixed with a solution of bridgeable polymer, which enhances gas and moisture barrier activity as well as humidity resistance in food packaging. On the other hand, grapes are highly reactive, which hinders long time storage for further use. They can be preserved in the refrigerator for between 3 to 6 days; after that, they become mussy and their color fades [28]. For such reasons, a great amount of fruit is thrown away due to spoilage and taste alteration in fruit markets and domestic domains. These wastes need urgent treatments.

In this research, an experiment was used to study the nano-based biodegradable food packaging by polyvinyl alcohol (PVA), modified through the utilization of zinc oxide nanoparticles (ZnO-NPs), for better quality and properties of food. A solution processing route (doctor blading) has been used for thin-film synthesis. Such an experimental setup is also successful for a variety of other food packaging systems (such as vegetables, fruits, meat, and fish); this is a low cost and straightforward method, and no inert atmosphere is required. It should be noted that only two concentration values were considered for the ZnO, 0.5 wt% (minimum) and 5.0 wt% (maximum). According to the literature, PEO/PVA (50/50 wt%), with incorporation of 0.5 wt% and 5.0 wt% ZnO as a filling factor in biofilm matrix, was effective. Higher concentration of ZnO-NPs results in agglomerations of particles and further deterioration of the film properties [29]. Therefore, only minimum and maximum concentrations have been selected for PVA. To the best of our knowledge, there were no such studies on PVA for food packaging applications, particularly on the preservation of grapes.

EXPERIMENTAL

Preparation of ZnO-NPs

ZnO-NPs were prepared from leaves extract of aloe barbadensis by using the solution preparation method [30]

Fig. 1. Design Process of ZnO-NPs preparations through green synthesis route.

shown in Fig. 1. A 0.01 M solution of $ZnNO_3$ was added to 55 ml of Aloe Barbadensis leave extract solution and stirred at 80 °C until boiling when the mixture was converted into deep yellow color. The solution was then dissolved in acetone for 30 min to achieve complete dispersion. The solution was then sent for centrifugation at a constant speed of 20 min. After filtration, the ultrafine powder was grounded using agate mortar till nanoparticles were obtained. These NPs were then characterized by UV-Vis and the absorption peak showed the appearance of ZnO.

Preparation of PVA/ZnO-NPs Biofilm

The films were prepared through the solution processing route by the doctor blading method. A 15 wt% of PVA was mixed with distilled water at 80 °C with constant stirring for 3 h until PVA is completely dissolved with distilled water. The ZnO-NPs at different concentrations (0.5 and 5 wt%) were then dissolved in the above solution and stirred again for 3 h at 80 °C under constant speed. The solution of PVA/ZnO-NPs was then doctor bladed on A4 transparent substrate for thin-film coatings. A uniform coating thickness of 20 µm was developed by automatic doctor blading equipment. The films were then heat-treated at 70 °C for 05mins for improved properties.

Preparation of Fruit Samples

Locally purchased fresh grapes *(Viti-Vinifera)* fruits having almost uniform size, shape and weight were selected for packaging characterizations. To investigate the fruit quality, fruit samples were classified into three different categories such as control sample (without packaging) in refrigeration (at 6 °C) and samples packed with PVA/ZnO-NPs (0.5 to 5 wt%). The selected samples were then sanitized in a chlorine bath and washed with distilled water. The dried samples were then packed with PVA/ZnO-NPs biodegradable films in a controlled atmosphere (6 °C). The packed fruits were then stored at ambient temperature for 14 days to characterize the food quality. The oxygen transmission (OT) and water vapor transmission (WVT) rate, anti-microbial activity, and CO_2 activity were characterized for grapes (*Vitis-Vinifera*) before and after packaging.

Characterization of Packaging Film (PVA/ZnO-NPs)

Mechanical testing. The mechanical braking power, elongation, and tensile strength of packaging film were determined by Beijing Universal Testing Machine (*WDW T-50*). ASTM (*D/882-10*) standard was followed for sample preparations. The mechanical strength was calculated by using the following equation (Eq. (1)):

$$Tensile\ strength = \frac{Breaking\ force}{Film\ area} \qquad (1)\ [31]$$

Surface morphology. The morphological information of bio-nano composite (PVA/ZnO-NPs) and particle size orientation of ZnO-NPs were determined by Scanning Electron Microscope using (*JEOL JSM-6480*) model.

X-Rays diffraction. XRD for different samples has been done for structural analysis and for determining the particle size of ZnO-NPs using Scherr's Equation.

UV-Vis spectrometer. UV-Vis (*PerkinElmer LAMBDA-365*) spectrometer was used to observe the absorption peak of ZnO-NPs embedded in biodegradable polymer films. The absorption peaks for such films lay in the range of ZnO that justifies the appearance of ZnO-NPs in the biofilm matrix.

Characterization of Food Quality

Fruit decay. The weight loss of the fruit sample is an important parameter for determining fruit decay. The decay rates were calculated during storage time. Fruit samples were observed every day and weight loss was determined. The rate of decay of fruit samples was calculated by following equation (Eq. (2)).

$$\% \, fruit \; decay = \frac{weig \quad of \; the \; decayed}{total \, weigh \quad of \; fruits} \times 100 \qquad (2)$$

Fruit color. DIM (Digital Imaging Method) was used to observe the color of fruit samples. The fruit samples were exposed to photo-light and sample images were taken. These images helped analyze the color change in the fruit samples. The total color change can be calculated by following Eq. (3).

$$\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2)]^{1/2} \qquad (3)$$

Here, ΔE represents the total color change while, ΔL shows the change in lightness, Δa represents the redness changes, and Δb shows the changes in yellowness.

Enzymatic Browning index. The Browning index was calculated by using the standard method. A 15 ml of fruit sample was centrifuged at a constant speed for 10 min, 7 ml of ethyl alcohol was added and again centrifugation for more than 10 min. When the centrifugation was completed, UV-Vis spectroscopy was used to determine the absorption peak.

Antimicrobial activity of PVA/ZnO film. Several different experiments were performed with nano-food packaging to prevent the bacterial growth among fruits. Due to this reason, antimicrobial agents were incorporated into the PVA biodegradable film. The efficiencies of the biodegradable film with different concentrations of ZnO-NPs were evaluated and compared with the control samples. Two common bacterial strains, S. aureus and *E. coli,* were considered.

RESULTS AND DISCUSSION

Characterization of Packaging Film and ZnO-NPs

The surface morphology and distribution over PVA film were characterized by SEM (*JEOL JSM-6480*). UV-Vis was used to observe the absorption peak of ZnO. XRD was used to characterize the crystallinity of biodegradable biofilms. Generally, PVA has an amorphous structure, but crystalline pure PVA was observed in XRD patterns due to heat treatment of the films.

Surface morphology of packaging flim and ZnO-NPs. The surface morphology of pure PVA film, pure ZnO-NPs, and PVA/ZnO-NPS bio-film with (0.5 and 5 wt%) has been investigated by SEM. A smooth surface with a trace amount of agglomeration of pure PVA film can be seen in Fig. 2a. The agglomerated and flake-like structure of ZnO-NPs is shown in Fig. 2b. Figure 2c and Fig. 2d show the bio-nanocomposite films of PVA/ZnO-NPs with 0.5 and 5 wt% ZnO, respectively, where the random distribution of ZnO-NPs was observed. According to Fig. 2c and Fig. 2d, the distribution of particles increased with an increase in the concentration of ZnO-NPs in the biopolymer matrix [32].

Moreover, the distribution of ZnO-NPs in the PVA matrix improved the mechanical strength and physical properties such as density and hardness of the bioflim. The flake spots in the SEM images in Fig. 2b show the ZnO-NPs which are randomly distributed. Besides, the agglomeration of ZnO-NPs was observed at the fracture surface of the PVA showing a higher concentration of PVA. It is concluded from SEM images that the ZnO^+ surface interacted with the unpaired electron of carbonyl of PVA, which increases the surface area for the interaction between ZnO and PVA. This case was observed in the PVA with ZnO-NPs (0.5 wt%) film. On the other hand, in the film with ZnO-NPs (5 wt%), a random distribution was observed with a very small agglomerated cluster due to reactions [33].

Absorption spectrum of packaging film. The presence of ZnO-NPs in the PVA film matrix was justified by the UV-Vis spectrometer. Figure 3 illustrates the absorption peaks of PVA/ZnO-NPs (0.5 wt%- a black peak) and PVA/ZnO-NPs (5 wt%- a red peak). As ZnO is a transparent material and its absorption peak lies in the region of (~370-390 nm) [34], the presence of the ZnO compound in the PVA matrix was confirmed. A significant increase in absorption intensity of PVA/ZnO-NPS (5 wt%) was observed by an increase in the concentration.

Crystallinity of packaging Film and ZnO-NPs. The diffraction pattern of pure ZnO (which was synthesized by leaves extract of aloe barbadensis; a green synthesis route) is shown in Fig. 4 representing the characteristics peaks of ZnO. The highest peak was observed at 36 at an angle of (2θ) which corresponds to (101) planes of ZnO with the JCPDS Card no. 01-079-2205.

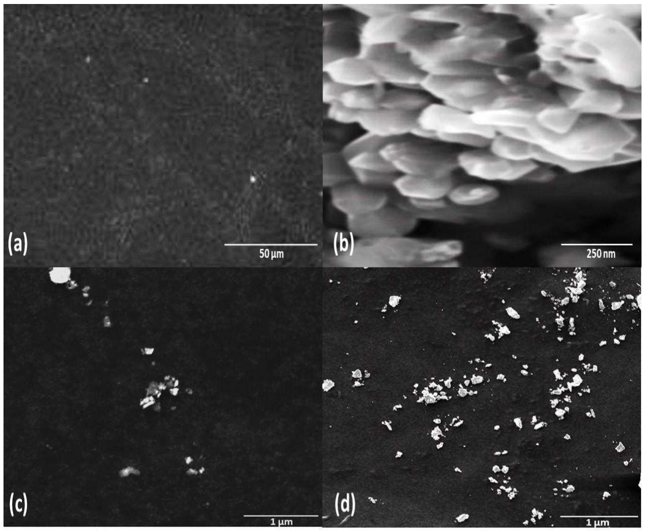

Fig. 2. SEM images of (a) pure polyvinyl alcohol (PVA) film with 25 m thickness, (b) ZnO-NPs prepared by aloe barbadensis leaves extract (a green synthesis route), (c) 0.5 wt% of PVA/ZnO-NPs nanocomposite film with 25 µm thickness and (d) 5 wt% of PVA/ZnO-NPs nanocomposite film with 25 µm thickness.

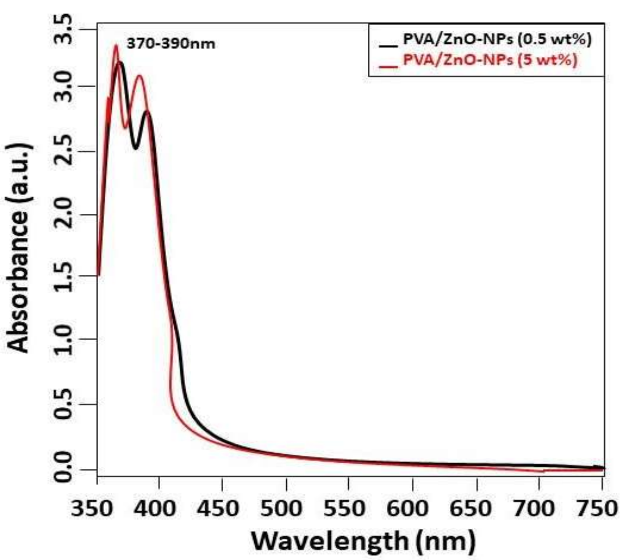

Fig. 3. UV-Vis absorption spectrum of biodegradable packaging film with 25 µm thickness for PVA/ZnO-NPs (0.5 wt%, black peak) and PVA/ZnO-NPs (0.5 wt%, red peak), showing the absorption peak of the Zn-NPs at 37 0 and 390 nm, respectively.

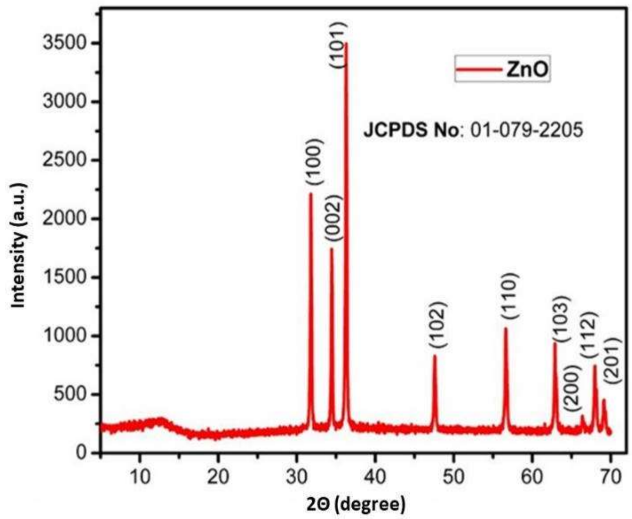

Fig. 4. XRD analysis of ZnO-NPs through nano-green synthesis route from leaves extract of aloe barbadensis.

In Fig. 4, crystalline planes were justified [35] and the result indicates the successful formation of ZnO. A highly crystalline pattern of pure ZnO-NPs was confirmed by the XRD pattern. The most common method was used to calculate the average crystalline size of ZnO-NPS using the Scherrer equation; [$D = (0.9 \times \lambda)/\beta Cos\theta$]. The average powder size of ZnO-NPs was about 62.5 nm.

On the other hand, it can be observed from XRD (Fig. 5) that the specific diffraction peak, appeared at 20 in the pure PVA film, was due to the heat treatment of the pure PVA film. Moreover, the small specific peak appeared in 37 in PVA/ZnO-NPs (0.5 wt% and 5 wt%) confirms the crystalline form of ZnO. Figure 5 shows ZnO-NPs, as filling factor, was well intact with the film [33].

Quality Evaluation of Packaging Film

Mechanical properties. As the concentration of ZnO-NPs increased, the tensile strength and Young's modulus increased, which led to further increase in the elongation of the sample. This is due to the higher concentration of ZnO nano-powders leading to a high brittleness. From the experiment results, it was found that the tensile strength of packaging film having ZnO-NPs (0.5 wt%) was 13 MPa. The latter value for the packaging film having ZnO-NPs (5 wt%) was 19 MPa.

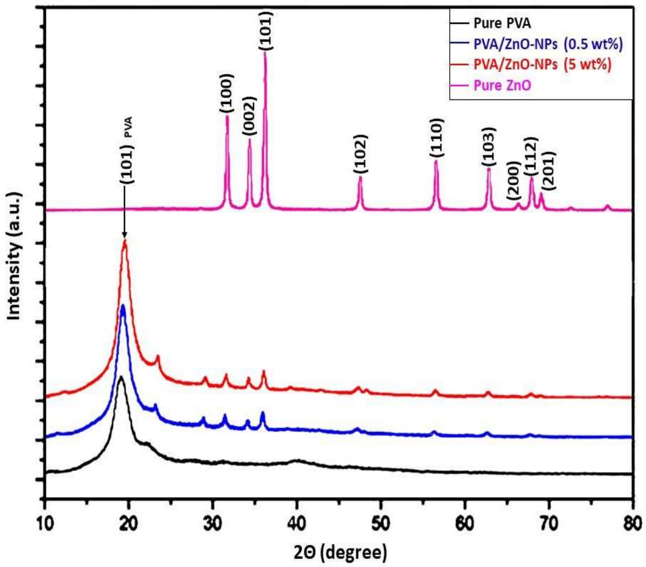

Fig. 5. XRD analysis of pure PVA (black peak), PVA with 0.5 wt% ZnO-NPs (blue peak), PVA with 5 wt% ZnO-NPs (red peak), and ZnO (pink). The film thickness for pure PVA and nanocomposite remained constant (25 nm).

Table 1. Quality Evaluation of Fabricated Nanofilm of PVA/ZnO-NPs

Quality evaluation of the film	PVA	0.5 wt% ZnO	5 wt% ZnO
Thickness (μm)	25	25	25
Breaking power (mm)	92	90	82
Tensile strength (MPa)	12	13	19
Elongation (%)	42	41	36

Antimicrobial analysis. For very good quality food preservation, it is important to consider the antimicrobial activity of that packaging material, which helps microbial preservation by pathogenicity. Therefore, the antimicrobial activity of any synthesized film or product used for food quality protection must be evaluated for the potential microbes. Fabricated films of PVA/ZnO-NPs were tested for quality assurance of food considering antimicrobial assay concerning escherichia coli (*E. coli*) and staphylococcus aureus (*S.aureus*), which are the most common pathogens of many food species. The optical density (OD) values measured using UV-Vis (*PerkinElmer LAMBDA-365*) increased with an augmenting bacterial growth. The reduction in the microbial activity of PVA/ZnO-NPs is shown in Fig. 6 (left) and Fig. 6 (right). It was observed from Fig. 6 (left) and Fig. 5 (right) that the Zn^{2+} ion in the film formed an oxidizing agent when passes through the bacterial cell wall. Compared to the 0.5% of ZnO-NPs embedded in the film matrix, the 5% ZnO-NPs showed greater antimicrobial activity, which increases the food quality for a longer time [36].

The antimicrobial activity (%R) is based on the type of bacteria, microbial growth, and ZnO-NPs concentrations as discussed in Fig. 7. It was observed from several experiments that antimicrobial activity increases with an increase in the number of ZnO-NPs embedded in the PVA polymer matrix. Moreover, biodegradable films have a greater %R for *E. coli* than *S. aureus.* This is because the discharged metallic ion (Zn^{2+}) passing through the bacterial membrane cell produces oxidizing agent that is responsible for damaging the wall of bacterial cell. During the synthesis of ZnO-NPs, a cation of the surfactant induced a positive charge on the surface of the nanoparticle. This positive charge on the surface of the nanoparticles showed an excellent R%. It was also found that *S. aureus* has a thick layer in the bacterial cell membrane while *E. coli* has a thin layer with a negative charge which enhanced the interaction with Zn^{+} ion. Hence, the thinner layer of peptidoglycan has a greater %R. Compared to 0.5% concentration of NPs in the film, NPS having 5% in films had greater antimicrobetrical activity for S. Aureus (90%) and *E. coli* (98%) [36].

Fruit quality analysis. To ensure the *Vitis-Vinifera* quality, the grapes were kept in three different environments: the open atmosphere (open sample), and PVA/ZnO-NPs (0.5 and 5 wt% ZnO-NPS). The Grapes were then analyzed for fruit decay rate, atmospheric gas transmission rate, aromatization alteration, browning extent, and metal ions releasing (Table 2).

Table 2 shows the quality of the grapes in different environment (open atmosphere, 0.5 wt% and 5 wt% of ZnO in PVA film). Open sample had higher decay rate compared to grapes packed with biofilms. This is due to direct environment interaction with fruit samples. The 5 wt% ZnO-NPs in biofilms showed excellent properties in terms of

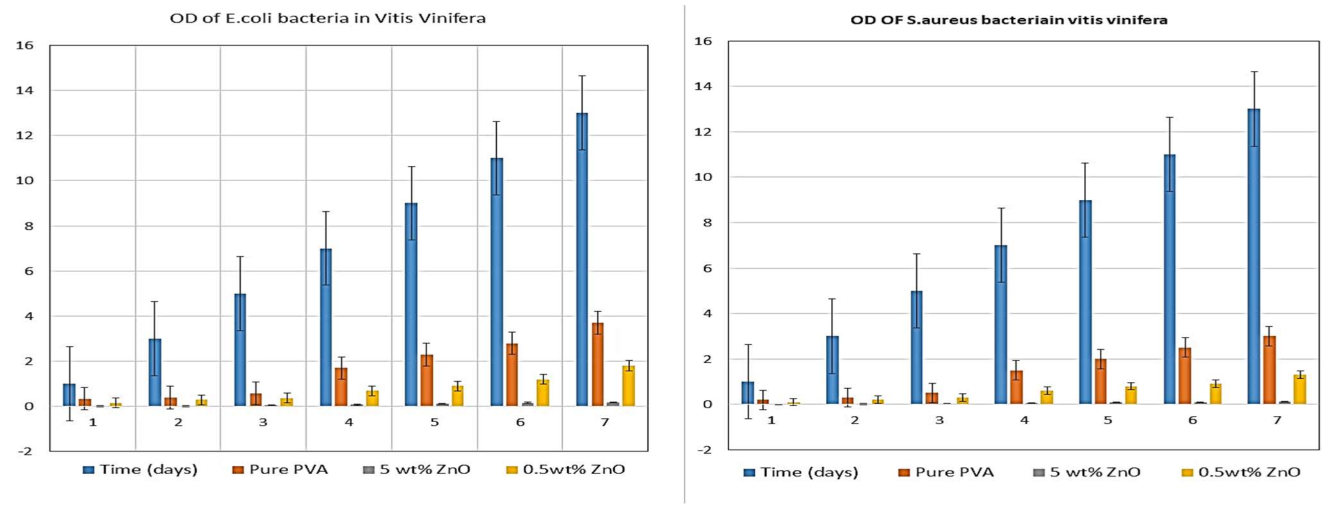

Fig. 6. XRD analysis of pure PVA (black peak), PVA with 0.5 wt% ZnO-NPs (blue peak) and), PVA with 0.5 wt% ZnO-NPs (red peak). The film thickness for pure PVA and nanocomposite remained constant (25 nm).

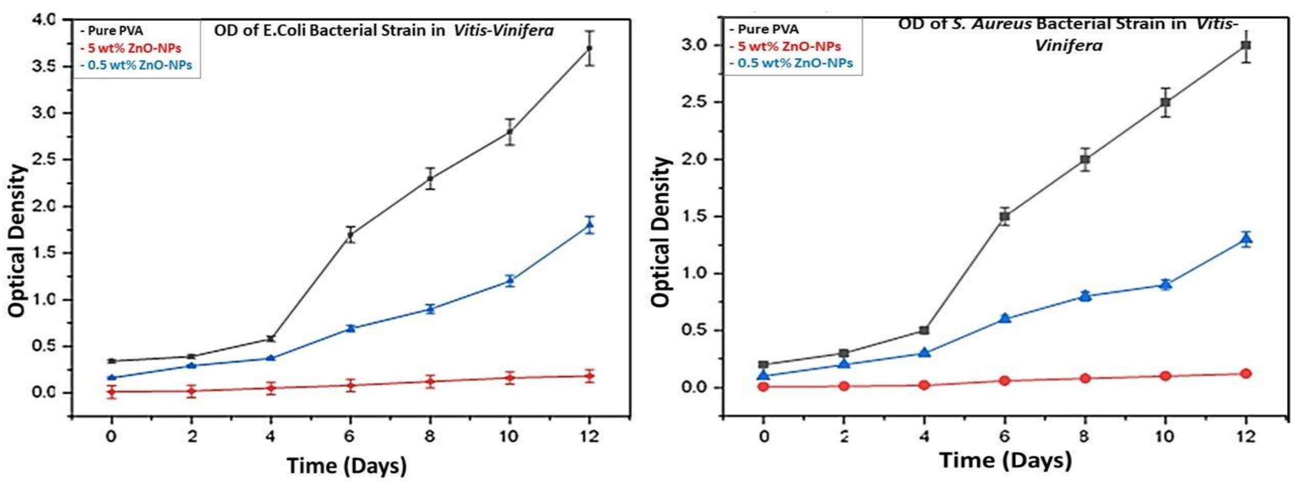

Fig. 7. Optical density curve for *E. coli*bacterial strain growth (b) Optical density curve for S. aureus bacterial strain growth.

Table 2. *Vitis-Vinifera* Fruit Quality Parameter of Control and PVAstic Embedded and Loaded ZnO-NPs

Parameter	Open sample	ZnO-NPs 0.5 wt%	ZnO-NPs 5 wt%
Browning index	3.5	2	1.7
Zn metal accumulation (ppm)	Not observed	0.004	0.058
Rigidity	8	13	18.4
Decay rate%	14.5	9	8.8
O_2%	1.8	8.9	9.1
CO_2%	27	15	12

browning index and rigidity, however, the Zn metallic ion accumulation in 5 wt% was higher compared to 0.5 wt% and open sample, due to higher concentration of ZnO in biofilms.

Visual and physical taste inspection was done for fruit decay as shown in Fig. 8. It was observed that the transmission rate of the atmospheric gases such as O_2 and N_2 in the fruit sample was greatly reduced by using packaging film. This can be justified by the color change and browning index for fruit that was kept in untreated packaging bags. Moreover, the high concentration of ZnO-NPs (5 wt% ZnO-NPs) released the metallic ions from the treated samples and had longer life than the control sample.

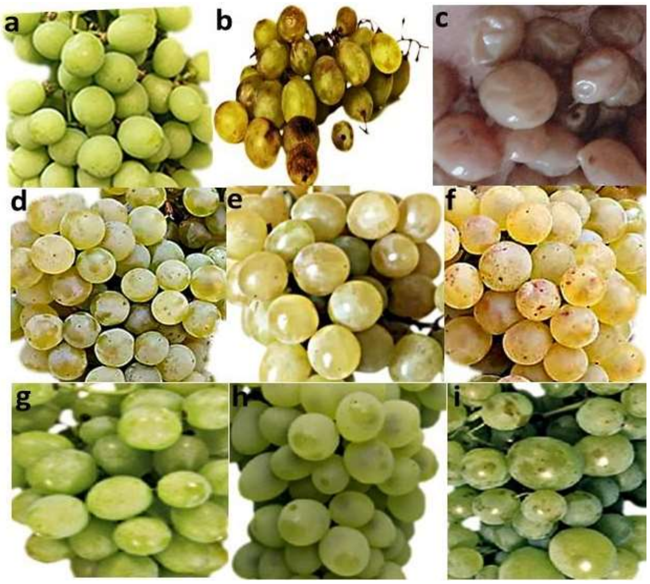

Fig. 8. Physical condition of fruit samples; (a-c) control samples (with Day 1, Day 7, and Day, 15 respectively) at 6 °C, (d-f) PVA/ZnO-NPs 0.5 wt% (Day 1, Day 7, and Day 15, respectively) at 6 °C and (g-i) PVA/ZnO-NPs 5 wt% (with Day 1, Day 7 and Day 15 respectively) at 6 °C.

CONCLUSIONS

In this study, ZnO-NPs were incorporated into PVA for the synthesis of biodegradable films for food packaging applications. Results showed excellent antibacterial activity for the sample made by incorporation of 5 wt% ZnO-NPs into the PVA; which was better than that obtained for other samples (0.5 wt% and controlled ones). Furthermore, the thickness and breakage power of biofilms were also excellent. The other factors such as fruit color, decay rates, and ZnO-NPs retention were outstanding among all other samples.

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