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Scientific Research

Lallemantia royleana seed mucilage-based active edible films: The effects of zinc oxide nanoparticles and zoulang plant's essential oil

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ABSTRACT

In this research, *Lallemantia royleana* seed mucilage was used as the base of the film, while essential oil derived from the zoulang plant's leaves and stems, along with zinc oxide nanoparticles (ZnO), were incorporated as additives to develop active edible films. Findings indicated that incorporating ZEO enhanced the films' antioxidant capabilities, permeability, thermal properties, tensile strength, and elongation of the film. FT-IR analysis confirmed the creation of new hydrogen-oxygen bonds between ZEO and the polysaccharide chains, contributing to a reinforced film structure. Conversely, the addition of ZnO nanoparticles was found to decrease the antioxidant properties, permeability, crystallinity, thermal resistance, moisture content, and tensile strength of the films. Additionally, ZnO nanoparticles contributed to enhanced plasticity, which increased the films' stretchability and resulted in greater thickness. SEM imaging verified the interaction between ZEO and ZnO nanoparticles, aligning with the findings from the FT-IR analysis. Overall, the study suggests that films developed using *Lallemantia royleana* mucilage, in combination with ZEO and ZnO nanoparticles, demonstrate strong potential for applications in food packaging and active films or coatings, particularly for materials vulnerable to oxidation.

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1- Introduction

Food products can be compromised by pests, insects, and microorganisms at various stages of production. To address this, food packaging technology has been developed to extend the shelf life of foods. Being ensure about food availability and maintaining product quality until consumption are also crucial factors [1].

Active packaging involves the inclusion of specific components designed to improve the functionality and effectiveness of packaging systems. These systems are typically categorized into two types. In the first category, the active elements are enclosed in separate sachets or pads placed within the package, and in the other, the active components are embedded directly into the packaging material itself [2].

In this study, the second type of packaging was employed, where active components were integrated into the matrix of an edible film. Driven by consumer demand for more sustainable and cost-effective solutions, researchers have focused on developing biodegradable and edible materials that enhance efficiency, food quality, freshness, and overall food safety [3]. The Production of edible films and coatings represents an innovative packaging approach aimed at preserving or even improving food quality. These covering materials must meet essential food preservation needs. For instance, coatings with low oxygen permeability are necessary to protect oxidation-sensitive products, while films with selective mass transfer capabilities help reduce moisture loss in fruits and vegetables during storage [4].

Nowadays, hydrocolloids employing has been increased in the food products as a thickening agent, food preservative, texturizer, gelling agents, film-forming agents, and foam stabilizer [5]. Hydrocolloids are extracted from various sources such as herbal seeds. *Lallemantia royleana* (LR) is an annual herb of the *Lamiaceae* family. This ilk is one of the largest and most distinctive plants in the world, growing widely in different parts of the Europe and the Middle East such as Iran. The antimicrobial and antioxidant properties of LR have been presented in some studies [6]. *Lallemantia royleana* seeds (LRS) are consist of carbohydrates (45.25%), crude fiber (30.67%), ash (3.63%), oil (18.27%), protein (25.60%) and it also contains linoleic, oleic, palmitic, stearic and beta-Sitosterol fatty acids [6]. LRS gum contains arabinose (37.88%), galactose (33.54%), rhamnose (18.44%), xylose (6.02%), and glucose (4.11%) [7].

Essential oils are one of the natural compounds in plants. They are aromatic substances that are naturally produced by the plant as secondary metabolites and their constituent ingredients are mainly terpenoids and phenolic compounds [8]. Essential oils would reduce the rate of oxidation in different ways. For example, it can reduce the initial reactions of fatty acid oxidation, absorb free radicals, and decrease metal ion binding [9]. Incorporating essential oils into the formulation of edible films can positively influence their mechanical characteristics, vapor barrier properties, surface texture, roughness, and color of the film [10-12]. Zoulang has healing and soothing properties. It is used to stimulate fertility, improve hemorrhoids and treat rheumatic diseases. This plant also participates in hematopoiesis due to its folic acid and iron content [13]. Terpenoids and phenolic compounds such as monoterpene, sesquiterpene, triterpenoids, flavonoids, coumarin, steroids, and acetylene are the main component of this plant [14].

Nanotechnology is employed increasingly in food industry. One of its important applications is in the field of edible films. Nanoparticles are used to enhance the

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performance and increase the bioavailability of biopolymer-based films [15]. Also, they are used to enhance the performance of films based on biopolymers [16]. Nanoparticles like titanium oxide, silica, zinc oxide and magnesium oxide are commonly used in food industry.

Considering tendency to use natural components instead of synthetic materials that are made by chemically changing, the purpose of the current research was to investigate the effects of zoulang essential oil (ZEO) addition on physicochemical and antioxidant characteristics of an edible films based on *Lallemantia royleana* seeds mucilage (LRSM) and feasibility of its application as a natural preservative.

2.Materials and methods

Materials

The Lallemantia royleana seeds and zoulang were bought from a traditional market in Babol, Mazandaran, Iran. Other materials, such as glycerol, Tween 80, calcium sulfate, calcium nitrate, and potassium sulfate, were procured from Merck Co. (Darmstadt, Germany). ZnO nanoparticles were supplied by US Research Nanomaterials, Inc., and 2,2-diphenyl-1-picrylhydrazyl was obtained from Sigma-Aldrich (MO, USA).

Extraction of Zoulang Essential Oil

Extraction of ZEO followed a three-step method. Initially, zoulang was dried in a chamber with a steady flow of dry air at 40°C. Following this, it was ground into a powder using a mill (DPA1, Moulinex, France). The essential oil was then extracted through hydrodistillation for 6 hours using a Clevenger apparatus.

Extraction of Lallemantia royleana seeds mucilage

First of all, LRS were washed thoroughly with ethanol 96% and placed in an oven (GPH-OV-50, JIM Engineering Ltd., United Kingdom) at 45 ˚C for 12 hours. Then, the seeds and water were mixed in a ratio of 1:120 and placed on a stirrer at 800rpm/min for 3h to absorb water. Then, the LRSM was separated from the seeds with a juicer (HR2820, Philips, Netherlands). To separate impurities from the mucilage, the mixture was centrifuged (VS4000D, Farzaneh Arman Co., Isfahan, Iran) at 4200 rpm for 15 minutes followed by drying in an oven (GPH-OV-50, JIM Engineering Ltd., United Kingdom) at 50 ˚C for 24h.

Film preparation

First, the dried mucilage was powdered with a mortar. Afterward, 1g of the powdered sample was added to 80 mL of distilled water and stirred on a magnetic stirrer for 2 hours to allow water absorption. It was then mixed with 0.375 mL of glycerol. Once the primary solution was prepared, ZnO nanoparticles were dissolved in 20 mL of distilled water at concentrations of 1.5%, 3%, and 4.5% based on the dried mucilage weight. The mixture was then homogenized using an ultrasonic homogenizer (FR-USC-22 LQ, Intellect, USA) at 400 W power and 40 kHz frequency for 10 minutes. This homogenized solution was subsequently added to the primary solution.

The ZEO was added in concentrations of 2%, 3.5% and 5% based on dried mucilage weight (v/w) to the solution. In the next step, 0.1 mL of tween 80 were added as emulsifier into the solution followed by ultrasonic degassing at 25 °C for 10 min. For film formation, 65 mL of each prepared solution was poured into 9 cm diameter petri dishes and placed in an oven at 30 ˚C for 48 hours. In the final step, dried films were peeled off from plates and conditioned in a desiccator with calcium nitrate (RH 55%) at 25 ˚C for 24 h.

Measuring the Thickness of the Films

The thickness of the films was measured using a digital micrometer (Mitutoyo, Japan) with a precision of 0.001 mm. Measurements were taken at 12 different locations on each

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film, and the average thickness was calculated and reported.

Moisture Content

To determine the moisture content, the samples were cut into 1 cm × 1 cm pieces and placed in an oven at 120°C until a constant weight was achieved. The moisture content was then calculated using equation (1) [17].

$$M\ (\%) = \frac{Wi - We}{Wi} \times 100 \quad (1)$$

Where M is the moisture content (%), W_i is the initial weight (g) and W_e is the weight of the film at the end of drying (g).

Water Solubility

The film samples were cut into 1 cm × 1 cm pieces and dried in an oven at 95°C for 24 hours. Their weights were recorded afterward. The samples were then immersed in 50 mL of distilled water and stirred on a magnetic stirrer at 25°C for 6 hours. After stirring, the samples were filtered and dried in an oven at 110°C until a constant weight was reached. The solubility of the films was then calculated using the following equation [18].

$$\text{Solubility}\ (\%) = \frac{Wi - Wf}{Wi} \times 100 \quad (2)$$

Where W_i is the initial weight (g) and W_f is the final weight (g).

Moisture absorption

The film samples (1 cm × 1 cm) were first conditioned in a desiccator with $CaSO_4$ for 24 hours to ensure complete drying, and their weights were recorded. Subsequently, the samples were transferred to a desiccator containing a saturated calcium nitrate solution (55% relative humidity) at 25°C until they reached a constant weight. The moisture absorption was then calculated using equation 3.[19].

$$\text{Water absorption}\ (\%) = \frac{Wt - Wo}{Wo} \times 100 \quad (3)$$

Where W_t is the final weight of the samples (g) and W_o is the initial dry weight (g).

Water Vapor Permeability

First of all, films were cut in to a circle shaped figure with 2 cm diameters. Then they sealed in glass vials with 2 cm diameter and 4.5 cm length. Paraffin wax was employed around the glass to insulate the inner-environment of it. Anhydrous calcium sulfate was used to decrease the RH of vials from its initial value to 0%.

The vials were then placed in a desiccator containing saturated potassium sulfate (RH 97%). The pressure gradient created by this setup was the driving force for water vapor transfer into the vials. The vials were weighed every 6 hours for 4 days using a scale with an accuracy of 0.001g. The data was recorded and plotted, and the slope of the resulting graph (weight vs. time) was determined using linear regression. The water vapor transfer rate (WVTR) was calculated by dividing the slope (S) by the surface area of the film (A) [20].

$$WVTR = \frac{S}{A} \quad (4)$$

Water vapor permeability (WVP) was calculated by equation 5:

$$WVP = \frac{WVTR \times X}{\Delta P} \quad (5)$$

Where X is the film thickness (mm) and ΔP is the water vapor pressure difference between the inside and outside of the vial (ΔP is 3115.42 Pa).

Antioxidant activity

To measure the antioxidant activity of various treatments, 25 mg of each sample was dissolved in 5 mL of distilled water. Then, 0.1 mL of the extract was added to 3.9

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mL of DPPH solution (0.04 g/L) in ethanol. The mixture was vortexed and incubated in a dark box at room temperature for 60 minutes. After incubation, the absorbance was measured using a UV-Vis spectrophotometer at 517 nm. The percentage of DPPH radical inhibition was then calculated using equation 6 [21].

$$\text{Radical scavenging activity (\%)} = 100 \times (1 - A_{sample}/A_{DPPH}) \quad (6)$$

where A_{Sample} indicates sample absorption and A_{DPPH} indicates pure DPPH solution absorption.

Fourier Transform Infrared Spectroscopy (FT-IR)

The samples were pre-conditioned at 55% relative humidity and 25°C for 24 hours. FT-IR measurements were conducted to obtain the film spectra using a THERMO NICOLET AVATAR 360 FT-IR ESP (Thermo Fisher Scientific, MA, USA). The samples were scanned across a wave number range of 600 cm^{-1} to 4000 cm^{-1} with automatic signal gain and a resolution of 4 cm^{-1}.

X-ray diffraction (XRD)

The samples were pre-conditioned at 55% relative humidity and 25°C for 24 hours. X-ray diffraction (XRD) analysis was performed using a Shimadzu XRD-6000 (Shimadzu, Japan) at 25°C, with a voltage of 40 kV and a current of 30 mA. The results were recorded over a diffraction angle (2θ) range from 0° to 60°, with a scanning rate of 3° per minute.

Scanning Electron Microscope (SEM)

The samples were conditioned for 24 hours at 25 °C and 55% relative humidity. Scanning electron microscopy (SEM) analysis of the film's surface morphology was performed using a FEI Quanta 200 SEM (Philips-FEI Co., Netherlands) with magnifications ranging from 10 to 100,000 times. To prepare the samples for imaging, they were coated with gold, and a heated tungsten cathode electron gun was employed.

Thermal Weight Analysis (TGA)

The samples were conditioned for 24 hours at 25 °C and 55% relative humidity. This analysis was conducted using a STA 504 device (BAHR, Germany), covering a temperature range from room temperature to 600 °C under nitrogen flow, with a heating rate of 10 °C/min.

Mechanical Properties

Mechanical testing was conducted using a TA.XTPlus texture analyzer (Stable Micro Systems Co., UK) in accordance with ASTM standard methods. The film samples were first cut into a dumbbell shape measuring 5.5 × 1 cm^2 and then conditioned for 48 hours in a desiccator with calcium nitrate to maintain a relative humidity of 55% at 25 °C. The initial gap between the jaws was set at 30 mm, with a movement speed of 0.83 mm/sec. Following the mechanical tests, tensile strength (TS) and elongation at break (EAB) were determined using Equations 7 and 8 [19].

$$TS = F_{max} / A \quad (7)$$

$$EAB = (L_{max} / L_0) \times 100 \quad (8)$$

Where, F_{max} is maximum load (N), A is cross section area (m^2), L_{max} is extension at the moment of rupture (m) and L_0 is initial length of the film samples (m).

Statistical Analysis

Statistical analysis of the results was performed using Microsoft Excel 2013 and SPSS Statistics 23 software (IBM Corporation, Armonk, NY, USA). Analysis of variance (ANOVA) was conducted at a significance level of 95% using Duncan's test to evaluate differences between the means. The results are presented as mean ± standard deviation.

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3.Results and Discussion

Film thickness

Table 1 shows the results of thickness measurements. As it can be seen from the table, the film's thickness varied between 0.133 mm and 0.166 mm. The results indicated that the incorporation of essential oil did not significantly affect the thickness of the edible films. However, the addition of nanoparticles resulted in an increase in film thickness. This increase can be attributed to the higher dry matter content of the film [20]. An increase in thickness can significantly impact mechanical strength, water vapor permeability, light transmission, and the color of the film. [22]. Other researchers reported similar findings regarding the impact of zinc oxide nanoparticles on the thickness of pectin/alginate films [23].

TABLE 1 The thickness, moisture content, water solubility, water absorption and water vapor permeability of film samples.

sample	Thickness (mm)	Moisture content (%)	Water solubility (%)	Water absorption (%)	Water vapor permeability ($\times 10^{-7}$ g/m.h.Pa)
control	0.133 ± 0.009^{a}	28.07 ± 0.13^{a}	40.74 ± 0.73^{b}	16.66 ± 0.03^{a}	11.87 ± 0.33^{a}
A	0.165 ± 0.005^{d}	26.53 ± 0.35^{b}	37.93 ± 0.76^{a}	10.52 ± 0.18^{c}	10.40 ± 0.46^{c}
B	0.153 ± 0.007^{c}	24.61 ± 0.15^{c}	41.93 ± 1.24^{b}	9.67 ± 0.24^{c}	10.34 ± 0.24^{c}
C	0.145 ± 0.005^{b}	25.26 ± 0.18^{d}	42.85 ± 1.07^{b}	13.15 ± 0.28^{b}	11.22 ± 0.18^{b}
D	0.142 ± 0.004^{b}	24.47 ± 0.27^{c}	50 ± 0.45^{d}	8.1 ± 0.74^{d}	10.77 ± 0.34^{c}
E	0.166 ± 0.008^{d}	23.35 ± 0.54^{e}	46.66 ± 1.08^{c}	2.5 ± 0.1^{e}	7.50 ± 0.26^{d}

(A) EO= 2% N.P= 4.5%, (B) EO= 3.5% N.P= 3%, (C) EO= 2% N.P= 1.5%, (D) EO= 5% N.P= 1.5 %, (E) EO= 5% N.P= 4.5%

Moisture content

The results of the moisture content test are presented in Table 1. As the percentages of nanoparticles and essential oil increased, the moisture content of the films decreased. Due to the hydrophobic properties of zoulang essential oil, samples containing 3.5% and 5% essential oil exhibited the greatest reduction in moisture content. Similar results have been reported by other researchers. They stated that adding essential oil to the film matrix reduced the moisture content of the films [24, 25]. There was a significant difference between all groups ($P<0.05$), except for groups B and D, which did not differ significantly ($P>0.05$). In another study, it has been reported that adding nanoparticles to film can reduce the moisture content, which might be due to the ability of nanoparticles to create pores on the film surface [26].

Water solubility

Insolubility, or water resistance, is a key property of edible films that makes them suitable for food packaging. This characteristic is particularly important when the water activity of the product is high or when the edible film comes into contact with moisture during food processing [27]. As indicated in Table 1, the solubility of the control film was approximately 40%. The solubility of the films increased significantly with higher concentrations of ZEO ($p < 0.05$). This increase may be attributed to the detrimental effect of ZEO on the strength of the polysaccharide-polysaccharide bonds within the film [28]. Conversely, an increase in the concentration of nanoparticles within

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the film structure leads to a reduction in solubility. This effect may be attributed to the formation of a stronger structure within the film matrix, as well as the dimensional characteristics of the nanoparticles [29].

Water absorption

The results of the water absorption test are shown in Table 1. The water absorption for the control sample was about 17%, with increasing the percentage of essential oils and nanoparticles, the water absorption decreased ($p<0.05$). There was no significant difference between groups A and B ($p> 0.05$) but the other groups had a significant difference ($p <0.05$). The samples with 5% essential oil and 4.5% nanoparticles had the lowest water absorption (2-3%). This reduction may be due to the hydrophobic nature of nanoparticles and essential oils. In addition, the formation of a lattice structure by ZEO and ZnO nanoparticles can increase water resistance [26-30]. Similar results have been reported by Jamróz et al. (2018). They observed a decrease in water absorption by adding lavender essential oil to the film composition [31]. Vaezi et al. (2018) also stated that the addition of ZnO NPs in the film matrix has reduced water absorption [32].

Water vapor permeability

Water vapor permeability is a critical functional property of packaging films, as it helps preserve food quality by minimizing moisture transfer between the food and the surrounding environment. [33], therefore, using a hydrophobic film or coating can prevent undesirable changes in the water activity of packaged food [34]. As shown in the Table 1, control samples had the highest permeability and adding ZEO and ZnO nanoparticles to the film structure, reduced its WVP significantly ($p<0.05$). Samples with the highest concentrations of ZEO and ZnO nanoparticles had the lowest permeability. Researchers have stated that the WVP depends on hydrophilic-lipophilic balance of the film [35]. So, this decrease might be due to the dominant hydrophobic properties of the ZnO nanoparticles and also ZEOs [35]. In addition, uniform distribution of small-sized ZnO nanoparticles and filling the small pores can reduce the amount of free pathways for water vapor transfer [35]. Similar results were reported by other researchers who studied the development of starch films with zinc oxide nanoparticles [15]. Moreover, the existence of lipid drops can change the vapor penetration pathways and increase their length [36]. Other researchers have reported comparable findings, investigating the effects of oregano essential oil on basil seed gum films. [37].

Antioxidant activity

The results of the DPPH radical scavenging activity are illustrated in Figure 1. A significant difference ($p < 0.05$) was observed between all film samples and the pure DPPH sample, indicating that LRSM possesses antioxidant properties as well. These results align with findings from other researchers who studied LRSM, noting that the observed antioxidant properties are attributed to the phenolic compounds present in mucilage [38].

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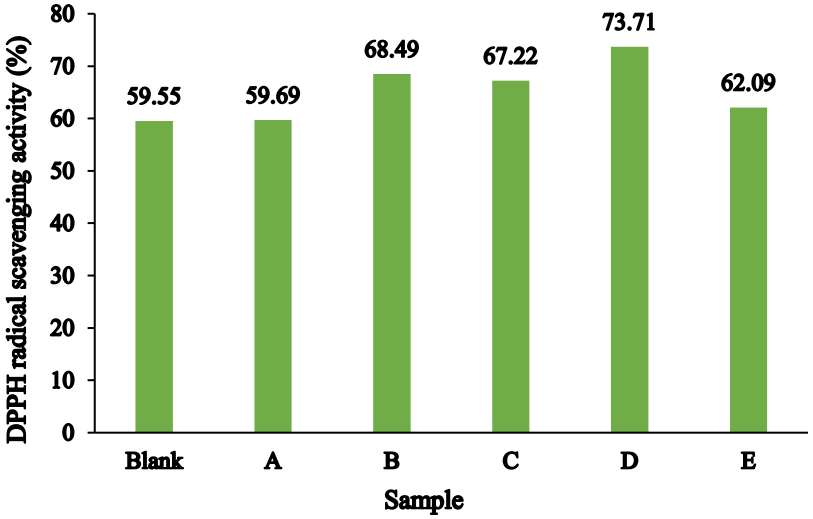

Fig 1: Antioxidant activity. (A) EO= 2% N.P= 4.5%, (B) EO= 3.5% N.P= 3%, (C) EO= 2% N.P= 1.5%, (D) EO= 5% N.P= 1.5 %, (E) EO= 5% N.P= 4.5%

There was no significant difference between sample A and the control sample ($p > 0.05$), but the other samples had a significant difference with each other ($p<0.05$). The addition of 1.5% ZnO nanoparticles to the film appears to have marginally enhanced its antioxidant properties. This slight improvement may be attributed to the transfer of electron density from the oxygen to the unpaired electron on the nitrogen atom in DPPH [39]. These findings are consistent with those of other researchers who investigated the antioxidant and antimicrobial properties of active packaging films made from carboxymethyl cellulose combined with curcumin and zinc oxide [40]. However, with increasing the percentage of nanoparticles from 1.5% to 4.5%, the antioxidant properties decreased. Other researchers have reported similar observations regarding the reduction of antioxidant properties by adding ZnO nanoparticles to the film structure [41, 42]. As demonstrated in Figure 1, the antioxidant properties of the film improve with higher concentrations of essential oil. ZEO exhibits antioxidant capabilities attributed to its phenolic content. Other researchers also confirmed the antioxidant properties of ZEO [43-45].

X-ray diffraction (XRD)

The results of the X-ray diffraction test are shown in Figure 2. The diffractogram of control film showed a peak at 2θ of 14.54°, 17.38° and 26.01° which illustrated the semi-crystalline structure of sample. As can be seen in the nanoparticle containing samples, slight peaks were observed in 2θ of 31.75°, 34.56°, 36.25°, 47.49° and 56.55°. The height of these peaks increases with increasing the percentage of ZnO nanoparticles while the position of the peaks did not change. The same results were reported in another study [46]. A similar trend was noted with the addition of ZEO to the samples, which resulted in an increase in the crystallinity of the edible films [47]. Constant position of the peaks even after adding the ZEO illustrated the compatibility of essential oil with the polymer part of the film.

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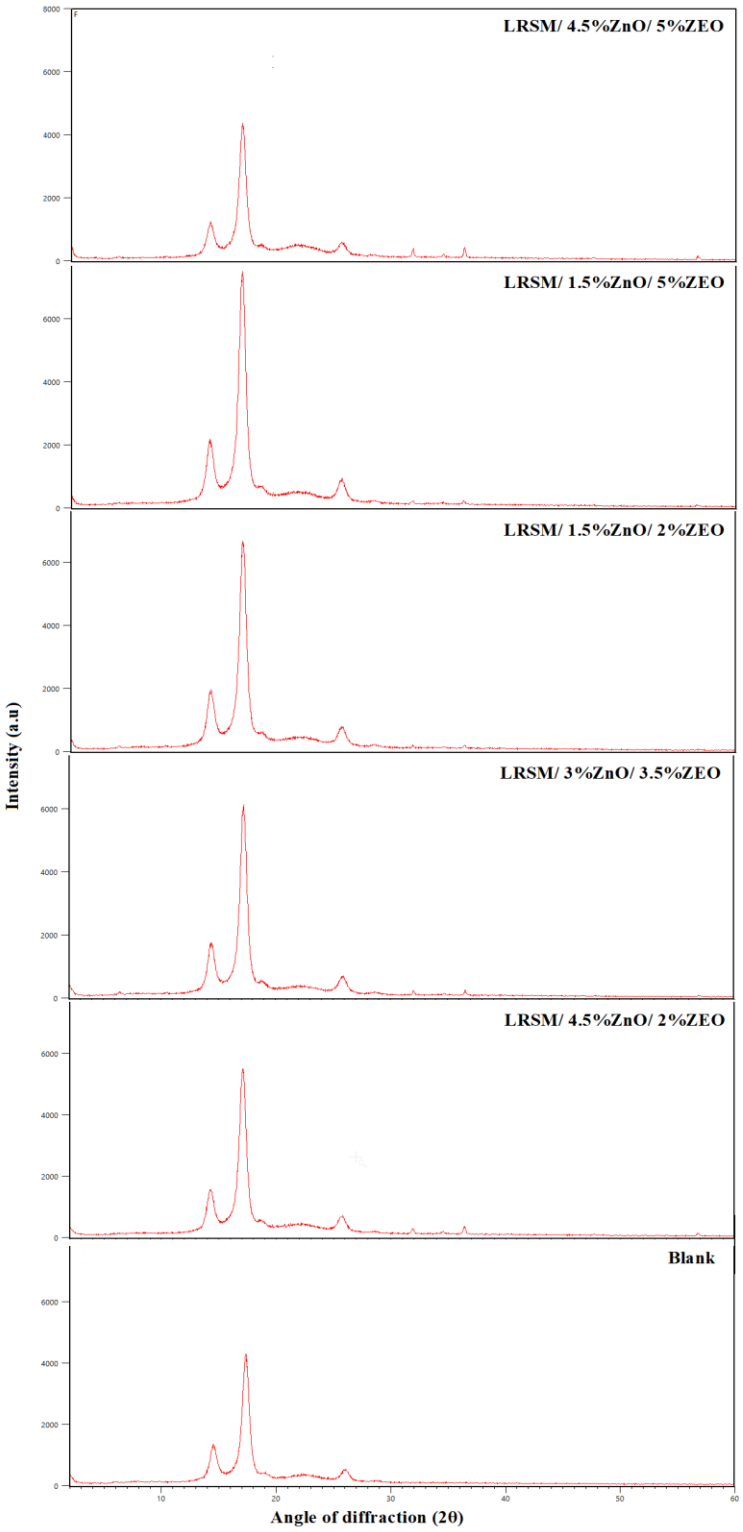

Fig 2: XRD patterns of pure LRSM and modified samples

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Another study also reported an increase in crystallinity and peak intensity, with no change in peak location, when oregano essential oil was added to chitosan/gelatin films. [48]. As the concentration of ZEO increased, the peak heights also rose. However, at higher concentrations of ZnO nanoparticles, a decrease in peak height was observed compared to lower percentages of ZnO nanoparticles. This reduction may be due to the bonding interactions between the ZnO nanoparticles and the ZEO.

Scanning Electron Microscope (SEM)

The results of SEM have been demonstrated in Figure 3. Addition of ZEO into the film composition is usually followed by homogenization or adding emulsifiers to the mixture. When the film dried (as seen in the Figure 4) the ZEO can change the film structure.

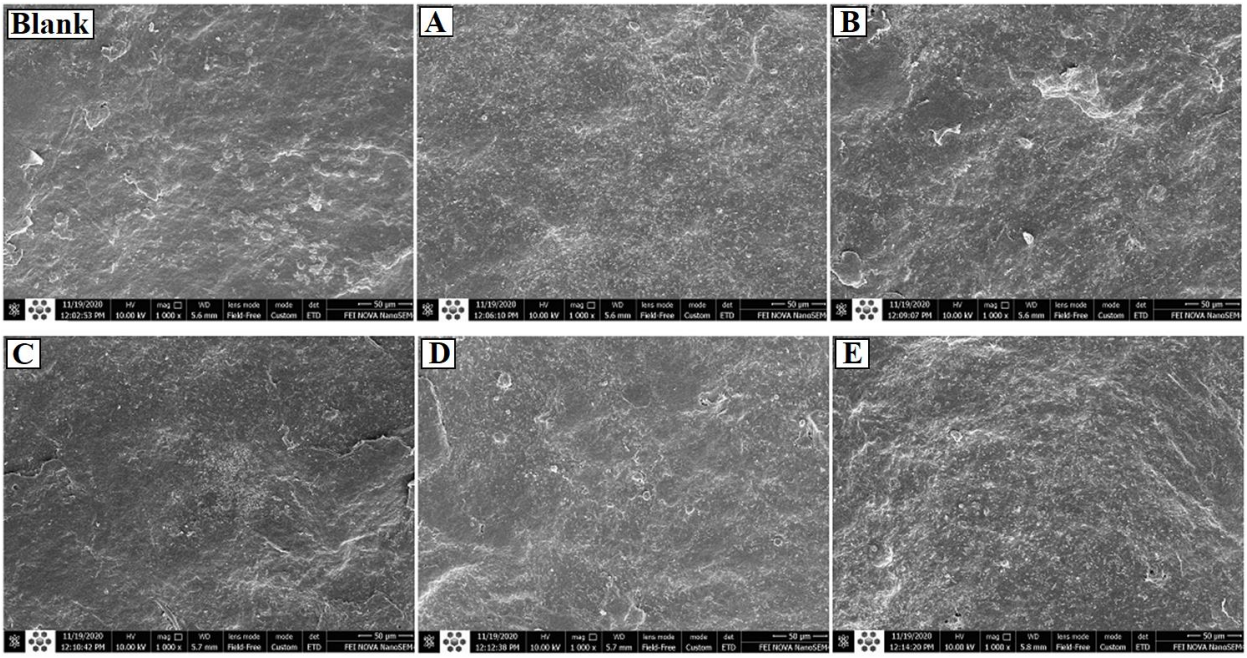

Fig 3: SEM of the surface of film samples (A) EO= 2% N.P= 4.5%, (B) EO= 3.5% N.P= 3%, (C) EO= 2% N.P= 1.5%, (D) EO= 5% N.P= 1.5 %, (E) EO= 5% N.P= 4.5%

The morphology and structural features of films affect its mechanical and gas barrier properties [49]. As shown in Figure 3, control samples have smoother and more homogenous surface compared with the other films which have ZEO and/or ZnO nanoparticles in their formulation. In addition, all samples have a wrinkled surface, which could be formed during drying process [40]. The amount of this wrinkles seems to decrease in samples with higher amounts of ZnO nanoparticles. Although Zinc oxide nanoparticles were distributed well on the film surface, increasing the percentage of these nanoparticles caused rougher and more heterogeneous film surface. Similar observations were made in another study examining starch films with zinc nanoparticles, where an increase in surface roughness was noted [15].

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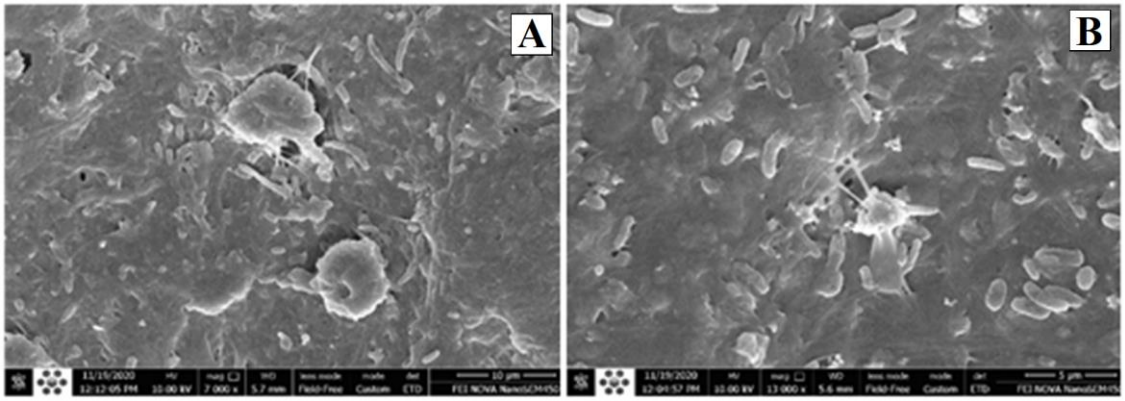

Fig 4: Surface microstructure of film sample

Adding the ZEO does a noticeable difference at the film surface. In general, it seems that the essential oil mixes well with the polymer base and is homogeneously distributed on the film surface. As the percentage of ZEO increased, the surface roughness of the film as well as their diameter increased. Also, in some places (few locations) it caused pores on the surface of the film (Figure 4A), which may be due to the lack of proper bonding between the ZEO and the film matrix due to its different nature (hydrophilic-hydrophobic).

A similar result was reported by other researchers in the development of fish gelatin film with cinnamon essential oil. They observed an increase in pores at the film surface with an increase in the percentage of essential oil, which was due to the different nature of the polymer base and the essential oil [50]. This defect may be remedied by increasing the emulsifier percentage. Due to its phenolic content, the essential oil can form new O-H bonds to some extent, which creates a bond between the essential oil and the polymer base, as a result, improves its mechanical and inhibitory properties. The essential oil also forms bonds with nanoparticles, especially at high concentrations which can increase mechanical strength as well as improve inhibitory properties (Figure 4B).

Fourier Transform Infrared Spectroscopy (FT-IR)

This method is based on the absorption of infrared light by molecular vibrations. The FT-IR spectra of the various samples are shown in Figure 5. The spectrum of film samples had specific peaks in the region of 3200-3500, 2852-2915, 1312-1573 and 800-1200 cm^{-1}. The peaks in the wavenumbers between 3200 cm^{-1} and 3500 cm^{-1} are related to O-H bonds. The addition of essential oils, attributed to their phenolic content, resulted in the emergence of strong and broad peaks in this region. It was also noted that films containing higher concentrations of essential oils exhibited broader and more intense peaks compared to those with lower concentrations [26]. Furthermore, phenols can form new O-H bonds, which may enhance the integration of ZEO with the film's polysaccharide matrix. This interaction is supported by the XRD analysis, which shows an increase in peak height without any displacement of the peaks. On the other hand, the incorporation of ZnO nanoparticles resulted in a peak shift from 3274 cm^{-1} to 3222 cm^{-1}, indicating the formation of new bonds between the nanoparticles and the essential oil. The absorption intensity at region of 2853 cm^{-1} to

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2918 cm^{-1} are related to the C-H bonds, which includes the bond vibrations of C-H, $C-H_2$, $C-H_3$ and bending vibrations, symmetric, asymmetric and occasionally doubles overlapping with O-H. In addition, the peaks in regions 1312 and 1573 are related to symmetric and asymmetric C-OO carboxyl bonds, respectively.

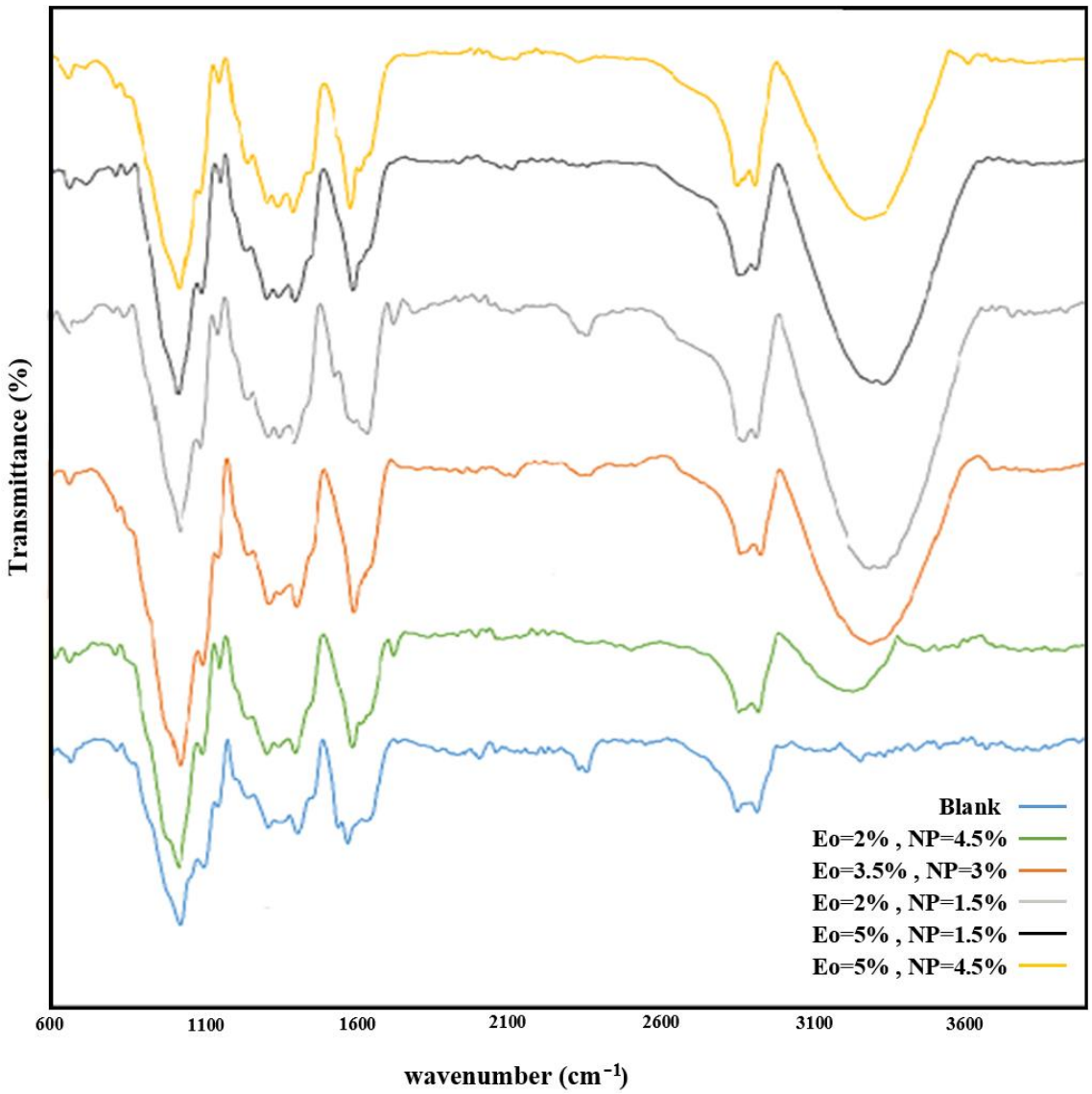

Fig 5: FT-IR spectra of film samples

The presence of these groups can indicate binding sites for ions, which can play an important role in the structure of film lattice [7]. A special peak in wavenumber 1406 cm^{-1} in all samples is due to the addition of glycerol in the structure of the films [51]. On the other hand, poor absorption in wavenumber 1250 cm^{-1} and 1300 cm^{-1} can be due to low amine percentage. Also, the strong and broad peaks that appear in the range of 800 cm^{-1} to 1200 cm^{-1}, which known as the "fingerprint" range for carbohydrates, are related to the vibrations of the C-O, C–O–C glycosidic and C–O–H stretching vibrations

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of the polymer chain structure. Addition of ZnO nanoparticles to the film matrix due to the creation of new bonds between oxygen and carbon in the polymer chain, shifted these peaks from 1022 cm^{-1} and 1115 cm^{-1} to 1027 cm^{-1} and 1102 cm^{-1}, respectively. In addition, absorption at wavenumber 814 cm^{-1} and 889 cm^{-1} is probably due to the presence of β-D-manopyranose units [7].

Thermal Weight Analysis (TGA)

TGA analysis is utilized to examine the chemical and physical changes in materials in response to temperature variations. The thermograms of the film samples are presented in Figure 6. The differential thermal gravimetry (DTG) curves revealed four distinct weight loss steps across all samples. The initial weight loss, occurring between 90-110 °C, was attributed to the evaporation of water absorbed by the polysaccharide chains and glycerol, as well as the loss of low molecular weight compounds [52]. The second weight loss around 190-230 °C could be associated with water attached to ZnO NPs and decomposition of glycerol [53, 54]. The third weight loss around 300 °C was attributed to thermal decomposition of polysaccharides and also it was due to degradation of ZnO NPs [55, 56]. And the fourth stage, at about 370-410 ºC, may be due to thermal decomposition of ZEO. The aromatic structures present in the ZEO are highly stable due to the resonance of the benzene ring [52]. Additionally, this reduction may be attributed to the degradation of saccharide rings and the disintegration of the macromolecular chains in LRSM [57]. The presence of a low amount of tween 80 can also be effective in this peak [53].

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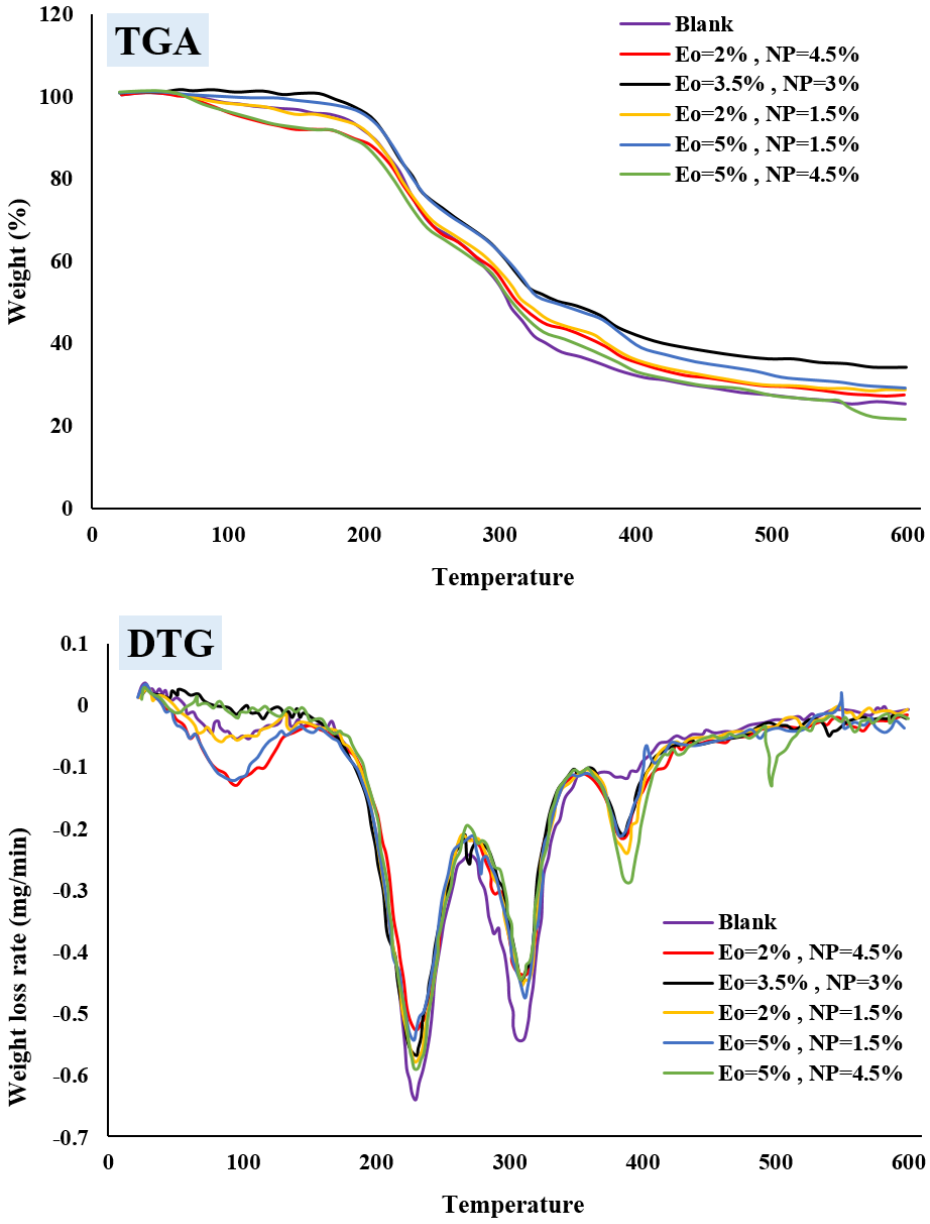

Fig 6: TGA and DTG thermograms of samples

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The addition of ZnO nanoparticles decreased the thermal stability of the edible films. Although the impact of ZnO nanoparticles on thermal stability was minimal, they did contribute to a slight reduction in the films' overall stability. Consequently, samples with the highest nanoparticle content exhibited the lowest thermal stability. This decrease may be attributed to the plasticizing effects of ZnO nanoparticles and the resultant modifications to the structure of the film samples [46]. The addition of essential oil had minimal impact on the thermal properties of the films. However, it seems to have slightly enhanced these properties. This improvement may be attributed to the rearrangement of the polymer structure, particularly following the incorporation of higher concentrations of ZEO [53]. Additionally, the bioactive compounds found in essential oils, such as phenolic content, can enhance the thermal stability of edible films [58]. The results were in good agreement with the results of the XRD test, showing that with increasing the degree of crystallinity, the thermal stability was also increased, which might be due to the more energy required to break the higher crystalline structure.

Mechanical Properties

Mechanical properties are crucial characteristics of edible films. Biodegradable films need to possess adequate strength during production and transportation. Consequently, parameters such as tensile strength (TS) and elongation at break (EAB) were assessed to evaluate the films' strength. The results of the mechanical tests are presented in Table 2. Tensile strength (TS) refers to a material's capacity to withstand tensile stress until rupture. The TS value for the control sample was 17.09±0.08 MPa. Generally, the TS of the samples increased with the incorporation of ZnO nanoparticles (NPs) and ZEO. However, unexpectedly, the addition of ZnO NPs significantly decreased the TS of the film samples ($p<0.05$). The edible film containing the highest concentration of ZnO NPs exhibited the lowest TS at 17.55±0.11 MPa. This reduction in TS may be attributed to weak interfacial interactions between the ZnO nanoparticles and the film matrix. Additionally, ZnO NPs have plasticizing effects that can enhance the mobility of polymer chains, while the incorporation of nanoparticles may also diminish the electrostatic bonding among the polymer chains [46]. Unlike ZnO NPs, the addition of ZEO significantly ($p<0.05$) increased the TS of the films. The sample with 5% ZEO had the highest TS (20.63±0.05 MPa). Addition of essential oil probably increases TS due to the formation of hydroxyl bonds between the polyphenolic content and polymer chains. The formation of strong bonds between the ZEO and the carbohydrate polymer reduces the mobility of the chains, which in turn increases the TS [58]. The results of FT-IR analysis confirm the increase of hydroxyl bonds by adding ZEO.

EAB is the film's stretch ability prior to ruptured. All samples had higher EAB than the control one. According to the results, in general, adding ZnO NPs to edible films did not have a significant effect on EAB of films ($p>0.05$), but it improved the EAB slightly. This slight increase may be due to the plasticizing properties of the nanoparticles, as well could attribute to no interruption effect of ZnO NPs in the movement of polymer chains [46].

Adding ZEO to the film matrix significantly increased EAB ($p<0.05$). As a result, the EAB increased from 4.60±0.07% for the control sample to 7.22±0.11% and 6.92±0.09% for the samples with the highest concentrations of ZEO. The incorporation of essential oil into polysaccharide-based films can enhance cohesiveness and flexibility by disrupting the inter-chain links of the polymers. Overall, adding essential oils tends to reduce the rigidity of the films, thereby improving the flexibility of the polymer

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chains and increasing their stretchability [58]. Other researchers achieved similar results and reported an increase in stretchability of films by adding essential oil to the film matrix [46, 52, 59].

Conclusion

In this study, it was observed that incorporating zoulang essential oil and ZnO nanoparticles into the film structure resulted in significant changes to the physicochemical properties of the films. The addition of ZEO enhanced solubility, antioxidant properties, crystallinity, thermal properties, tensile strength, and elongation at break (EAB) in the film samples. Conversely, the moisture content, water absorption, and water vapor permeability (WVP) of the films decreased. The introduction of nanoparticles led to an increase in thickness while reducing moisture content, solubility, water absorption, WVP, antioxidant properties, crystallinity, and tensile strength. Notably, significant insights were gained regarding the new interactions within the film matrix, such as the increase in O-H bonds resulting from the addition of ZEO, which contributed to improved mechanical properties. Ultimately, zoulang essential oil shows promising potential for use in food packaging films, although the performance of ZnO nanoparticles did not meet expectations.

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Conflict of interest The authors confirm that they have no conflict of interest to declare for this publication.

Ethical approval This article does not report any studies involving human participants or animals conducted by any of the authors.

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فیلم‌های خوراکی فعال مبتنی بر موسیلاژ دانه بالنگو: بررسی اثرات نانوذرات اکسید روی و اسانس گیاه زولانگ

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چکیده

در این تحقیق از موسیلاژ دانه بالنگو به عنوان پایه فیلم استفاده شد، در حالی که اسانس حاصل از برگ و ساقه گیاه زولانگ به همراه نانوذرات اکسید روی (ZnO) به عنوان افزودنی برای تولید فیلم‌های خوراکی فعال ترکیب شدند. یافته‌ها نشان داد که ترکیب اسانس حاصل از برگ و ساقه گیاه زولانگ (ZEO) قابلیت‌های آنتی اکسیدانی، نفوذپذیری، خواص حرارتی، استحکام کششی و ازدیاد طول فیلم را افزایش می‌دهد. تجزیه و تحلیل FT-IR ایجاد پیوندهای هیدروژن-اکسیژن جدید بین ZEO و زنجیره های پلی ساکارید را تایید کرد که به تقویت ساختار فیلم کمک می کند. درحالیکه افزودن نانوذرات اکسید روی باعث کاهش خواص آنتی اکسیدانی، نفوذپذیری، کریستالینیتی، مقاومت حرارتی، رطوبت و استحکام کششی لایه‌ها شد. علاوه بر این، نانوذرات ZnO به افزایش پلاستیسیته کمک کردند که باعث افزایش کشش پذیری لایه ها و ضخامت بیشتر آنها شد. تصویربرداری SEM برهمکنش بین نانوذرات ZEO و ZnOرا تأیید کرد و با یافته‌های آنالیز FT-IR همسو بود. به طور کلی، این مطالعه نشان می‌دهد که فیلم‌هایی که با استفاده از موسیلاژ دانه بالنگو در ترکیب با نانوذرات ZEO و ZnO ساخته شده‌اند، پتانسیل بالایی برای کاربرد در بسته‌بندی مواد غذایی و فیلم‌ها یا پوشش‌های فعال، به‌ویژه برای مواد آسیب‌پذیر در برابر اکسیداسیون دارند.

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