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Green synthesis of multifunctional ZnO/chitosan nanocomposite film using wild Mentha pulegium extract for packaging applications

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ABSTRACT

Following the global corona virus pandemic and environmental contamination caused by chemical plastic packaging, awareness of the need for environmentally friendly biofilms and antibacterial coatings is increasing. In this study, a biodegradable hybrid film, comprising of green-synthesized zinc oxide nanoparticles (ZnO NPs) with a chitosan (CS) matrix, was fabricated using a simple casting procedure. The ZnO NPs were synthesized using wild Mentha pulegium extract, and the synthesized NPs and films were characterized using different approaches. The structural, morphological, mechanical, antibacterial, and optical properties, as well as the hydrophilicity, of the prepared samples were investigated using various techniques. Gas chromatography-mass spectrometry measurements revealed the presence of phenolic compounds in the M. pulegium extract. In addition, a strong coordination connection between Zn\(^{2+}\) and the chitosan matrix was confirmed, which resulted in a good dispersion of ZnO in the chitosan film. The surface of the composite films was transparent, smooth, and uniform, and the flexible bio-based hybrid films exhibited significant antibacterial and antioxidant characteristics, strong visible emission in the 480 nm region, and UV-blocking properties. The ZnO/CS films displayed a potential to extend the shelf life of fruits by up to eight days when stored at 23°C, and also acted as an acceptable barrier against oxygen and water. The biodegradable ZnO/CS film is expected to keep fruit fresher than general chemical plastic films and be used for the packaging of active ingredients.

1. Introduction

The COVID-19 outbreak resulted in millions of infections and deaths owing to its rapid spread. During this crisis, the use of healthy drugs and fruits increased considerably. To extend the shelf life and maintain the nutritional value of perishable fruits, non-toxic, eco-friendly, durable, and edible antibacterial nanostructured coatings are required. Most of our daily foodstuff and vegetables may become contaminated with microbes during processing, supplying, and transportation, as existing viruses in the environment can be simply transmitted through surfaces, thus spreading the infection between people [1, 2]. Moreover, owing to the non-biodegradable nature of petroleum-based plastic, environmental contamination by traditional plastic packaging has become increasingly severe in recent times. Accordingly, the search for a multifunctional alternative to reduce the use of conventional polymers in the plastics sector has emerged as a top priority. Particularly, the study and development of innovative biocompatible and biodegradable bio-based materials as alternatives to petroleum-based plastics are crucial [1–4]. Materials that can provide essential protection by absorbing harmful chemicals or compounds, such as ethylene, or releasing available elements, such as antimicrobials, antioxidants, or vitamins, have recently evolved into a system that can perform single or multiple functions regarding food quality maintenance and preservation [5]. Antimicrobial compounds in food packaging materials might limit...
or delay microbe development, thus extending the shelf life of the contained goods [6]. In addition, antibacterial coating techniques might continuously deliver active compounds to food surfaces, resulting in long-term bacterial inhibitory effects. Thus, the use of degradable anti-microbial packaging is a revolutionary technique that can address present and future food industry demands. Non-toxic and biodegradable bio-based packaging materials consisting of proteins, polysaccharides, and lipids can act as a protective barrier for food, thus extending its shelf life, while reducing the environmental impact of traditional packaging materials[5–10]. Owing to the combination of the benefits of the components, the design of new biopolymer-based inorganic–organic nano-composites that exhibit renewability, biocompatibility, biodegradability, enhanced mechanical property, and non-toxicity has recently received significant interest [11]. Natural materials, such as cellulose, chitosan, starch, agar, and gum, are examples of biopolymeric materials. The characteristics of these biopolymers can be enhanced by adding nano-sized additives or fillers to the polymer [12,13]. For example, chitosan (C6H12O6N), a non-toxic, biocompatible, biodegradable polymer, which can be obtained by the deacetylation of chitin, exhibits excellent intrinsic properties [14,15]. In addition, it is considered safe and known as FDA-approved material, and can be extracted from shrimp, fungi, and crabs [15]. Chitosan is widely used in medical, agricultural, textile, and wastewater treatment industries owing to its unique qualities [13–16]. Several antibacterial non-toxic inorganic solid compounds have been successfully used as additives in food-preserved coatings [16–19]. Particularly, the addition of nanofillers, such as TiO2, zinc oxide (ZnO), and silver nanoparticles (Ag NPs), into biopolymers enhances their mechanical characteristics and other activities, including antibacterial activity, biosensing, optical features, and oxygen scavenging [19–28].

Among several non-toxic and antibacterial compounds that have been used as additives in packaging, ZnO has been considered a safe agent and vital element for health in the food industry [9,10]. However, some studies have reported that ZnO NPs might induce specific risks in people, whereas other studies have reported the in vivo non-toxicity of ZnO NPs [20]. The antibacterial properties of ZnO is related to the generation of oxidative stress caused by its interaction with water or by the creation of photon energy in the UV band [9]. Although ZnO NPs have been synthesized using different physical-chemical methods, green methods involving the use of natural extracts for the synthesis of ZnO NPs for food packaging applications and health are considered the safest. Green synthesis is a facile, rapid, and environment-friendly method. Several studies have reported the use of ZnO NPs in biopolymer composites for active food packaging to kill harmful bacteria and reduce food degradation [21–24]. Furthermore, recent studies demonstrated the use of ZnO NPs as antibacterial agents in chitosan edible coatings using Psidium guajava L. fruit and Arabic gum to preserve postharvest fruits [25]. In addition, the use of medicinal plant extracts to synthesize NPs that can minimize cytotoxicity and improve therapeutic uses has been demonstrated. Various fruit and plant extracts have been investigated for the synthesis of metal NPs [26–32]. For example, a previous study demonstrated the synthesis of CuO and ZnO nanostructures using Mentha pulegium Flower/Leaf extract, and the nano-structures were observed to exhibit antibacterial properties against Escherichia coli and Staphylococcus aureus [32]. It should be noted that food quality degradation is also majorly caused by the oxidation of unsaturated lipids by free radicals and long-term exposure to the sun without appropriate covering.

Therefore, the search for new antioxidant and anti-UV ray coatings to resolve these highlighted issues is vital [33,34]. Natural antioxidant substances, including phenolic compounds derived from plants, can function as antioxidants by scavenging for lipid radicals and preventing food oxidation. M. pulegium, a member of the Labiatae family, is a valuable natural source of bioactive compounds and phenolic compounds that are widely used for various purposes and against several diseases. There are approximately six species of Mentha species in the flora of Asia [33]. Mentha species are generally known under the name ‘na na/pooneh’ in some countries in Asia and are commonly used as delicious herbal tea with promising medical properties [34]. To date, the anti-inflammatory, antispasmodic, carminative, antitussive, diaphoretic, astringent, analgesic, stimulant, and emmenagogue properties of this natural magic plant have been recognized [33–35]. Recently, gelatin/tapioca starch films reinforced with chitosan and ZnO NPs were fabricated, and the antibacterial activity of the films against gram-negative and gram-positive was investigated [36]. In addition, another recent study synthesized multifunctional packaging films composed of chitosan, nano-TiO2, and red apple pomace, and confirmed the novel antibacterial activity of the films against gram-positve than gram-negative bacteria [37]. Ghaseemzadeh et al. evaluated the antibacterial properties of prepared polysaccharide chitosan agaroase–Ag NPs nanocomposites using the disc diffusion approach. They found that the chitosan-agaroase films exhibited no antibacterial properties, whereas the Ag NP films exhibited antibacterial activity [38]. Shankar et al. recently investigated the antibacterial effect of chitosan–essential oils–Ag NPs nanocomposite films on the shelf life of strawberries. [39]. Hanafy et al. synthesized a variety of thin chitosan–TiO2 NPs–oleic acid nanocomposite films using casting processes. They found that the addition of TiO2 NPs to chitosan enhanced its antibacterial efficacy against Albicans compared to pure chitosan [40]. Zhang et al. investigated the influence of the size of ZnO NPs in the chitosan matrix on chitosan nanocomposite films with a thickness of 5–100 nm on the antibacterial properties of the film, and found that smaller ZnO particles exhibited better antibacterial action [41]. To the best of our knowledge, the use of ZnO NPs synthesized using wild M. pulegium extract as an additive in chitosan coatings with multiple properties, including flexibility, biodegradable, transparent, blue solid luminescence, antibacterial, UVA filtering properties, for the preservation of strawberry/red plum as model fruits have never been investigated. Therefore, in this study, ZnO NPs were synthesized via a facile, rapid, and environment-friendly green method using wild M. pulegium extract as an herbal capping agent, after which the NPs were embedded into the chitosan matrix as an antibacterial additive. The synthesized films were applied to several fruits, and their fruit preservation performance was evaluated by comparing the treated fruits to the control model fruits. In addition, this study further investigated the structural-optical, mechanical, and Physico-chemical properties, as well as the antioxidant and antibacterial activities, of the prepared samples.

2. Experimental works

2.1. Materials and synthesis of ZnO NPs, chitosan, and hybrid ZnO/ chitosan nanocomposite

Young leaves of wild M. pulegium were collected in May 2021 in Firoozkouh mountains, Alborz, Iran. Zinc acetate dihydrate (99.9%), sodium hydroxide, hydrochloric acid, and acetic acid were supplied by Merck, Germany. The standard strains used for the antibacterial tests were obtained from the Medical University of Tehran, Iran. Shrimp waste was purchased from Bandar Abbas, Hormozgan, Iran. To prepare the Mentha extract, approximately 10 g of dried wild M. pulegium leaves were properly crushed, added into 100 mL distilled (DI) water, and stirred at 90°C for 2 h. Subsequently, the mixture was filtered (Whatman No 1 filter paper) three times to obtain the Mentha extract, and the extract was stored at 4°C. The prepared extract was used directly in the green synthesis of ZnO. For the synthesis of ZnO, 0.5 M Zn (AC)2·2H2Ow was dissolved in 20 mL of DI water under continuous stirring for 30 min. Thereafter, approximately 10 (or 15 mL) of the prepared extract was added to the solution gradually for 2 h at 60°C, after which the resulting solution was centrifuged at 4,000 rpm for 10 min. Subsequently, the obtained precipitates were dried at 90°C in a hot air oven and calcined at 450°C for 2 h. To obtain chitosan, the shrimp wastes were washed several times, dried in an oven, and grounded to smaller
parts. Subsequently, the grounded shrimp waste (i.e., chitin) was subjected to demineralization, discoloration, deproteinization, and deacetylation steps to obtain chitosan [42]. Next, 4 g of the obtained chitosan was dissolved in 100 mL of 1% acetic acid for 3 h (SA). Subsequently, 5 wt.% of the green-synthesized ZnO NPs was added into 10 mL of DI water (SB) and mixed with the chitosan solution (SA) using a mechanical stirrer. Thereafter, 0.5 g of a plasticizer was dropped into the mixture, and the mixture was continuously stirred for 3 h. The obtained dough was placed on Petri dishes or dropped on aluminum foils and dried at room temperature for 24 h. Lastly, transparent and flexible ZnO/Chitosan nanocomposite films were obtained. A schematic representation of the experimental work is shown in Fig. 1.

2.2. Application of the fabricated bio-nano composite

To analyze the application of the prepared bio-nano composites, first, strawberries and red plums were washed and divided into different groups. Subsequently, they were coated with the prepared ZnO/chitosan (hereafter denoted as ZnO/CS) and neat CS flexible thin films and stored at room temperature. Each fruit was selected at a specific time to investigate its physical properties.

2.3. Weight loss

The red plums were weighed every day during the storage time using a digital balance. All the measurements of the two groups were conducted in duplicates to obtain the average value, and approximately 10 samples were measured per replication. The outcomes of these 10 samples were calculated by averaging them.

2.4. Biodegradability

The biodegradability of the prepared films was evaluated by investigating their soil degradation ability. To this end, their soil solubility was calculated using:

\[
WL(\%) = \frac{\text{weight loss}}{\text{initial weight}} \times 100
\]

Briefly, the dry films were scaled into little pieces and placed in an enriched soil. The final weights were measured after 50 days. The soil microorganisms were not changed to maintain a natural biodegradation environment.

2.5. Oxygen permeability

The oxygen permeability of the films was measured using a gas permeability tester (YY9909-China) in accordance with the ASTM D3985-05 standard. Oxygen permeability was calculated using the equation below:

\[
\text{Oxygen permeability} = \frac{(\text{oxygen transfer rate} \times l)}{\Delta P}
\]

\(\Delta P\) is the difference in oxygen partial pressure across the film, and \(l\) is the thickness of the film.

2.6. Sensing investigations

We used two panels of 15 experts each, one for each of the five senses. The red plums were stored at room temperature for 10 days as part of the sensory evaluation. All aspects of the appearance and overall acceptability of the product were scored on a five-point hedonic scale ranging from 1 (very unappealing) to 5 (very appealing)) (1 = strong dislike, 2 = dislike, 3 = neither like nor dislike, 4 = like, and 5 = like a lot). The data was evaluated using ANOVA and Duncan tests. The possibility-value was set to < 0.05, which is the significance threshold.

2.7. Antibacterial activity test

The antibacterial activity of the biosynthesized ZnO NPs (prepared using different concentrations (5, 10, and 15 mg/mL) of the extract, and prepared ZnO/CS hybrid films against pathogenic microorganisms, namely gram-positive \(S.\) \(aureus\) ATCC 6538 and gram-negative \(E.\) \(coli\) ATCC 25922, was investigated using the colony counting and agar diffusion method [43–45]. Standard bacterial cultures were procured from the Tehran University of medical science, Tehran, Iran. The bacteria cultures were grown and maintained using trypticase soy agar. The density of bacteria suspension was adjusted to a density of 0.5 McFarland standards \((1.5 \times 10^8 \text{ CFU/mL}). The bacterial suspension was diluted three times to achieve the desired cell number. Based on the concentration of the samples, a specific volume of the bacterial suspension was added to the sample and placed in a liquid culture medium in an incubator shaker. Thereafter, 0.1 cc \((1.5 \times 10^8)\) of the bacteria was spread on solid culture medium plates containing the ZnO NPs fabricated using the prepared extract. Subsequently, they were placed in an

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Fig. 1. Schematic illustration of the preparation route of the hybrid zinc oxide (ZnO)/Chitosan nanocomposite including its multifunction features and characterizations.
incubator at 30 °C. The bacteria colonies formed were counted after 24 h using a Colony Count device, and the percentage of reduction compared to the blank sample was calculated. The number of colonies per unit volume was calculated as follows [45]:

\[
\text{number of log reductions} = \log B - \log A
\]

where B and A are the mean number of bacteria (CFU/mL) in the blank tubes and treated samples after 24 h incubation, respectively. The antibacterial activity of the hybrid films was evaluated using the agar diffusion test [44–46]. Approximately 0.1 cc of the activated bacterial growth solution (10^5 CFU/mL) was spread on the surface of the agar plate and maintained at room temperature for 1 h. Hybrid films were cut into small pieces using a surgical scissor. Reluctant pieces of the prepared films (Neat CS film:1, Commercial synthesized ZnO/CS:2 and our green synthesized ZnO/CS film:3) were placed on the agar surface, after which the Petri dishes were incubated at 30 °C for 24 h and screened for the inhibition zone by measuring the corresponding diameter. The antibacterial experiments (powder and composite samples) were repeated two to three times. Neat CS film and ZnO/CS film prepared using commercial ZnO nanopowders were used as control.

2.8. Antioxidant activity

The antioxidant activity of the hybrid films was determined using 2,2'-diphenyl-1-picrylhydrazyl (DPPH) and 2, 2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging assay [36]. Briefly, the prepared hybrid film samples were immersed in 1% acetic acid to obtain a film solution. Subsequently, 3 cc of the prepared film solution was added into 2 cc of DPPH ethanol solution (100 μM) and was incubated for 3 h under an ink condition. Subsequently, the absorbance of the mixture at 517 nm was investigated using a UV–vis spectrophotometer. The DPPH radical scavenging ability of the films was calculated using the following formula:[46]

\[
\text{DPPH scavenging ability} = \left(1 - \frac{A_{DPPH}}{A_{blank}}\right) \times 100
\]

where \(A_{DPPH}\) is the absorbance of DPPH solution and \(A_{b}\) is the absorbance of the film sample in DPPH solution.

2.9. Cell viability

To analyze the cell viability of the prepared samples, L929 cells (8104 cells per well) were planted on the surface of sterilized films and grown in 1 mL of Dulbecco’s Modified Eagle Medium (DMEM) containing 10% Fetal Bovine Serum (FBS) at 37 °C under 5% CO₂. The cytotoxicity of the L929 cells cultured on various surfaces for 24, 48, and 72 h was investigated using MTT assay. MTT (5 mg/mL in PBS) was applied until each well contained 1 mg/mL MTT. After 3 h, the medium was changed to 500 mL DMSO. For measurement, 100 L of each well’s solution was pipette mixed. At 570 nm, the absorbance levels were read in triplicate against a blank reagent. The viability was calculated using:

\[
\text{Cell viability} (\%) = \left(\frac{A_{test}}{A_{control}}\right) \times 100
\]

where \(A_{test}\) and \(A_{control}\) are the absorption values of the test and control groups, respectively.

2.10. Gas chromatography and mass spectrometry analysis

Gas chromatography-mass spectrometry (GC-MS) analysis was performed (Agilent GC, 6890, USA) on the prepared samples. Briefly, 20 μL of the extract was diluted using hexane. The helium carrier gas flow rate was set to 1.23 mL/min. After injection, the oven temperature was maintained at 40°C for 2 min, after which the temperature was increased to 210°C at 3°C/min for 10 min. The split ratio was 1:10, and the electron ionization of the mass detector was 70 eV. The volatile chemicals were identified using mass spectra library search data.

2.11. Mechanical properties

According to industry standards (ASTM D-882-97), mechanical characteristics were determined using a texture analyzer (Stable Micro Systems Ltd., UK) Before testing, films were cut into 6 cm × 2 cm slices, and the crosshead speed was 0.005 mm/s.

2.12. Analytical methods (Structural and Optical characterization)

The X-ray diffraction (XRD) pattern of the ZnO NPs was obtained using a PANalytical PW3050/60 X-ray diffractometer with a Cu-Kα radiation source (λ = 0.15418 nm). The samples were scanned at a scanning rate of 0.5/minute in the 2θ range of 10–70°. The morphology and topography of the samples, as well as elemental chemical surface analysis, were investigated using transmission electron microscopy (TEM; Philips CM120), field emission scanning electron microscopy (FE-SEM; MIRA3 TESCAN), energy-dispersive X-ray spectroscopy (EDX; MIRA3 TESCAN), and X-ray photoelectron spectroscopy (XPS; BESTEC (EA 101)). For the XPS measurement, a monochromatic Al Kα, X-ray source (20 W) surveyed the region of samples. The angle between the source and analyzer was fixed at 45°. In the XPS main chamber, samples were exposed to X-rays under ultra-high vacuum (UHV) in the dark. X-ray exposure was performed under chamber pressures of -10 millibar. The calibration procedure was based on the C 1s peak of the adventitious carbon. The optical characteristics, functional groups, and structural flaws of the produced NPs were investigated using UV Fourier transform infrared (FTIR), and photoluminescence (PL) spectroscopies (UV-visible spectrophotometer, PerkinElmer, and Cary fluorescence spectrophotometer, respectively). The PL spectrum of the samples was obtained at room temperature at an excitation wavelength of 290 nm.

3. Results and discussion

3.1. Structural properties (XRD, FTIR, XPS, FESEM, TEM, and EDX—elemental mapping results)

The XRD patterns of the neat CS, commercial/green-synthesized ZnO, and prepared ZnO/CS biopolymer are shown in Fig. 2. The neat CS exhibited two peaks at ~2θ = 10° and 2θ = 20°, which corresponded to the hydrated and anhydrous structure of the CS film [47]. Peaks corresponding to the hexagonal wurtzite structure of ZnO (JCPDS 01-075-0576) belonging to the P63mc space group were observed in the XRD patterns of the biosynthesized ZnO NPs, and no extra peaks related to their oxidation forms were observed in the sample [48]; Compared to that of the commercial ZnO, the intensity of the XRD peaks of the green-synthesized ZnO decreased slightly without a shift in position or the appearance of extra peaks. The crystallite size of the green-synthesized sample (according to the Debye-Sherrer equation) was determined by XRD analysis as 45 nm. The incorporation of ZnO into the hydrated chitosan matrix resulted in decreased peak intensities.

The FTIR spectra of pure CS, M. pulegium extract, commercial/green-synthesized ZnO NPs, and ZnO/CS films are shown in Fig. 3. The characteristic peaks of chitosan were observed at 3430–3455 cm⁻¹, which is related to the OH stretching vibrations. Additional peaks were observed at 2950 and 2878 cm⁻¹ owing to the symmetric and symmetric stretching vibrations of CH₂ groups, and the peaks located at 1656 and 1599 cm⁻¹ were assigned to the C-O stretching vibration of amides (-NH₂) [49-51]. The peaks of ZnO were observed at 400–600 cm⁻¹, which corresponded to the presence of Zn-O [51]. The ZnO/CS film exhibited intense bands in the range of 1250–1500 cm⁻¹, which shifted compared to those of other samples. This region corresponded to Zn–OH bending, implying the interaction of CS and ZnO NPs. In addition, peaks were observed in the range 896–1154 cm⁻¹, which could be attributed to the saccharide
structure. The peak at 1725 cm$^{-1}$ was ascribed to C=C stretching (aromatic) owing to the aromatic ring system, whereas the band between 3440 and 2890 cm$^{-1}$ is related to the stretching vibrations of the main and secondary amines, OH stretching, and CH stretching of alkanes. The vibrations observed at 1180 cm$^{-1}$ corresponded to CN stretching [6]. The presence of a band at 495 cm$^{-1}$ in the FTIR spectra of the green-synthesized ZnO NPs is a hallmark indication of Zn–O bond, confirming that the substance is zinc oxide. Furthermore, the FTIR results indicated that the soluble components in the M. pulegium leaf extract may have acted as capping agents, and prevented aggregation of the NPs in solution, while playing a role in their extracellular production and shape. [12,51]. It is known that plant extracts consist of active biomolecules that decrease and stabilize ZnO NPs. Polyphenols can function as reducing agents in the production of NPs, preventing harmful secondary products. Moreover, functional groups (–C–O–C–, –C–O–, –C=O, and –C=O) in flavones, alkaloids, phenols, and anthracenes can create metallic NPs. The FTIR data (Fig. 3) revealed the presence of functional groups, including –C–O–C–, –C–O–, –C–C–, and –C=O in the green-synthesized ZnO, whereas these peaks were not observed in the FTIR spectra of the commercial ZnO, but a strong notable peak was observed at ~500 cm$^{-1}$, which corresponded to Zn–O band. These capping ligands can be stabilized the NPs, thus inhibiting growth and aggregation. FTIR results indicated that the –OH groups of the extract vanished during the green synthesis of the ZnO NPs owing to the structural changes in the extract during bioreduction and the formation of some C=C functional groups in the green-synthesized ZnO NPs. Generally, the synthesis of metallic NPs from plant extracts involves three phases: (1) activation (bioreduction of metal ions and nucleation), (2) growth, and (3) termination (defining the final shape of the NPs) (Fig. 4).

The presence of ZnO NPs in the chitosan nanocomposite film was confirmed using XPS measurement. The XPS profiles of the pure ZnO, chitosan, and ZnO/CS nanocomposite are shown in Fig. 5. In addition to the broad spectrum of the prepared nanocomposite in Fig. 5(b), the binding energies of C 1s, N 1s, O 1s, and Zn 2p are shown in Fig. 5(c). The prominent carbon peak at 286 eV in the XPS profile of the chitosan matrix corresponded to the carbon bound to hydroxyl and nitrogen groups [52]. Two more notable peaks were observed in the carbon-related spectra, which can be attributed to C=O or O=C–O bonding, and the resulting value is consistent with those of known studies [53]. A peak was observed in the XPS profile of the chitosan matrix at 460 eV, which corresponded to the assignments of the -NH2 and -NH- groups. Peaks corresponding to lattice and surface oxygen or related vacancies were observed in the O 1s spectrum of the nanocomposite [52,53]. In addition, Zn peaks were observed owing to the presence of ZnO NPs in composite with (+2) oxidation state (Fig. 5(c)).
Furthermore, the FTIR and XPS results suggested the formation of molecular connections between peptide chains, such as ionic and hydrogen bonds, Van der Waals forces, hydrophobic interactions, covalent polar and nonpolar bonds, and crosslinking, in the protein structure of chitosan and synthesized ZnO. These connections involved Van der Waals and electrostatic forces, hydrophobic and hydrogen bonds, and excluded volume effects, or chemical binding.

The morphology and surface of the commercial/green-synthesized ZnO NPs, and hybrid nanocomposites were investigated using FESEM and TEM (Fig. 6). The FESEM images of the biosynthesized ZnO NPs revealed that they exhibited spherical/cubic and hexagonal shapes with an average size of 320 nm (Fig. 6(a, c)). Compared to the commercial ZnO NPs (Fig. 6(b)), the green-synthesized ZnO NPs exhibited a more uniform and regular morphology; Commercial ZnO NPs exhibited an irregular mixture of spherical/cubic and rod morphologies with an average particle size of ~90 nm. The surface morphology of pure chitosan powder is shown in Fig. 6(d).

The surface and cross-section FESEM images of the green-
and polymer substrates to preserve the integrity of their phys
investigations on the synthesis and characteristics of absorbers [10] .
strong emission peak at a wavelength of 480 nm, in which the major
of the hybrid CS and ZnO/CS films excited at a wavelength of 290 nm at
revealed that clove essential oil-loaded Chitosan
protect organic materials from UV radiation. In addition, a study
sunburns. ZnO as UV absorbers is frequently employed as coatings to
–
ical
These absorbers have most commonly been applied to wooden, glass,
materi
material that has been exposed to sun radiation has triggered numerous
ations. The need to employ organic and inorganic UV absorbers to shield
3.2. Optical properties (UV-vis and PL spectroscopies)
Fig. 6. Field emission scanning electron microscopy (FESEM) images of (a and c) green-synthesized and (b) commercial ZnO NPs, (d) neat CS powder, (e) surface and (f) cross-section image of the green-synthesized ZnO/CS nanocomposite film, (g, h) transmission electron microscopy (TEM) image and (h) selected area diffraction pattern of the green-synthesized ZnO nanopowders.
synthesized ZnO/CS film are shown in Fig. 6(e, f). The film exhibited a
smooth, continuous, and compact surface; however, aggregates were
observed on the CS/ZnO film, which were attributed to the larger ZnO
particles. The TEM image of the green-synthesized ZnO NPs revealed
that the synthesized particles were mostly spherical in shape (Fig. 6(g)).
The selected area electron diffraction pattern of the green-synthesized
ZnO NPs is shown in Fig. 6(b). The bright diffraction spots confirmed
the high crystallinity of ZnO with a wurtzite structure.
EDX and elemental mapping technique were performed to analyze
the chemical composition of the prepared hybrid nanocomposite. The
elemental mapping results revealed the uniform distribution of O and Zn
elements in the green-synthesized ZnO NPs (Fig. 7(a)). Fig. 7(b) and (c)
show the EDX profiles of the prepared nanocomposites. The sample
was mainly composed of C, O, Zn, Ca, and N elements, which were also
present in the hybrid film. These results suggest that chitosan is a good
substrate for dispersing ZnO NPs.
3.3. GC-MS result
The components of the M. Pulegium extract were analyzed using GC-
MS. The GC-MS result revealed that the essential compounds in the
extract were piperitenone oxide and piperitenone, which are phenolic
compounds (Fig. 9 and Table 1 ). The other main compounds in the
extract included Eicosane (4.8%), Caryophyllene oxide (2.39%), Alpha
glycerol linoleate (0.86%), and Borneol (0.7%).
3.4. Antimicrobial property, antioxidant activity, and cell viability of the
samples
First, this section presents the investigation of the antibacterial
properties of the extract and green-synthesized ZnO containing two
different extract concentrations (10 and 15 mL), namely ZnO-G′
and ZnO-G′′, respectively. Subsequently, the investigation of the antibacte-
rial activity of the prepared neat CS and ZnO/CS composite films
is presented. For better investigation, the prepared ZnO/CS composite film
(green-synthesized) was compared to another ZnO/CS film containing a
differently chemically-synthesized ZnO filler (commercial ZnO sample). The anti-
bacterial activity of the green-synthesized ZnO NPs was evaluated using
standard colony counting. The ZnO NPs exhibited antibacterial activity
against two bacteria, but the antibacterial activity for gram-positive
bacteria was higher than that for gram-negative bacteria (Fig. 10(a)).
With an increase in the exposure time and extract concentration in the
green-synthesized ZnO, the antibacterial activity of NPs increased
significantly. After 6 h treatment with the green-synthesized ZnO NPs,
the population of S. aureus and E. coli reduced by 99.9 and 97.9%,
respectively, indicating the good antibacterial property of the green-
synthesized ZnO NPs (Table 2, Fig. 10(b) and (c)). In contrast, the
pure extract exhibited less antibacterial activity with a reduction per-
centage of 10% for both bacteria after 6 h treatment. The antibacterial
action of neat CS and ZnO/CS films was tested against S. aureus and
E. coli bacteria, and the A film was used as control, and the results are
shown in Fig. 10(d) and (e). A dense population of both bacterial cells
was observed with the neat CS (namely 1) and the ZnO/CS film
containing chemically-synthesized NPs as a filler (namely 2). In contrast, a clear inhibition zone was observed on the piece of our green-synthesized ZnO/CS film. This demonstrated the practical antibacterial activity of the green-synthesized ZnO/CS. These results suggest the potential of the prepared film to inhibit a wide range of bacterial colonies. In a previous study, the antibacterial activities of ZnO NPs prepared from *Dysphania ambrosioides* extract were compared to those of commercial ZnO, and the inhibition zone of the prepared NPs against bacteria was similar to that of commercial ZnO [55]. In this study, the antibacterial activity of the prepared ZnO/CS film was slightly higher for gram-positive bacteria than for *E. Coli*. It has been reported that gram-negative bacteria are generally more resistant to ZnO NPs than gram-positive bacteria [56]. The slightly higher resilience of gram-negative bacteria can be attributed to the features of their cell wall construction. Compared to gram-positive bacteria, the cell wall of gram-negative bacteria contains an extra lipopolysaccharides (LPS)-containing outer membrane [56]. LPS has been proven to strengthen the barrier characteristics of the outer membrane, thus enhancing the bacterial resistance [56]. Moreover, as ZnO is photocatalytic, light, which can induce a charge separation in metal oxide materials, triggers the generation of electrons and holes in the NPs. The solid oxidative ability of holes can result in the formation of hydroxyl radicals when they react with water. In addition, electrons can reduce oxygen to superoxide anion (reactive oxygen species: ROS). Biologically, ROS can peroxidize the membrane lipids of germs, break DNA strands, inactivate proteins, and eventually kill germs. Metal ions have an impact on the respiratory chain and block some enzymes. Consequently, this results in the formation and accumulation of singlet oxygen, hydroxyl radical, hydrogen peroxide, superoxide anions, and other ROS. Moreover, the internal components of bacteria, such as proteins and DNA, can be damaged by ROS. Thus, the production of ROS on the surface of ZnO NP plays a role in its antibacterial activity. Akhil Krishnan et al. (2020) synthesized chitosan-ZNP nanocomposites using an environmentally friendly chemical reduction process, and tested the antibacterial activity of the nanocomposites films against *E. coli* using the agar disc diffusion technique. They found that the ZNP-loaded films exhibited a marked inhibition zone, which may be related to the biophysical events that occur at the sample–bacteria interface, such as NPs attachment to the bacterial surface, which results in tension and permanent membrane damage. In this study, it was believed that the negative surface potential of the ZnO/CS film exhibited higher antimicrobial properties against gram-positive bacteria. In addition, ROS and Zn\(^{2+}\) ions are released.

![Fig. 7. (a, c) Energy-dispersive X-ray spectroscopy (EDX) profile and (b, d) Elemental mapping of prepared ZnO NPs and ZnO/CS nanocomposite film.](image-url)
differently by surface-modifying reagent molecules (ROS). Moreover, numerous polyphenolic and flavonoid groups were observed in the extract. In addition to their anti-oxidant, anti-inflammatory, and anti-cancer properties, these chemicals are beneficial for human health. In addition, the abundance of hydroxyl and carbonyl groups in these compounds makes them effective antibacterial agents, as they can trigger the release of Zn$^{2+}$ and generation of ROS [57,58]. Polyphenols can act as reducing agents to prevent the creation of hazardous byproducts. These capping ligands stabilize NPs to prevent growth and aggregation. Thus, these features can be affected by the size, morphology, zeta potential, and final surface/shape of the NPs, and even after calcination, the NPs exhibited enhanced antibacterial due to
Viable cell numbers of S. aureus and E. coli.

Table 2

| Time   | Item       | S. aureus |        |        | E. coli |        |        |
|--------|------------|-----------|--------|--------|---------|--------|--------|
|        | Blank      | Viable (CFU/ml) | 1 × 10⁸ | 9 × 10⁷ | 5 × 10⁷ | 5 × 10⁷ | 1 × 10⁷ |
|        | Extract    | 10        | 50     | 80     | -       | 40     | 60     |
| 1 h    | Reduction (%) | -        | -      | -      | -       | -      | -      |
| 6 h    | Viable (CFU/ml) | 1 × 10⁸ | 9 × 10⁷ | 1 × 10⁷ | <1 × 10⁷ | 1 × 10⁷ | 9 × 10⁷ |
|        | Reduction (%) | -        | 98     | 99.9   | -       | 96     | 97.9   |

3.5. Mechanical properties, visual appearance, and water contact angle of the films

One of the most significant factors that affects the nutritional quality and consumer attractiveness of food items is the appearance and color of food coatings. The CS and ZnO/CS films appeared transparent, glossy, smooth, and free of defects (Fig. 12(a)). The CS and ZnO/CS samples appeared pale yellow and light brown in hue, respectively. Typically, according to literature, the addition of plant extracts or ZnO fillers to chitosan matrix affect the shade of the film [61]. However, the films synthesized in this study were smooth and free of wrinkles, pores, and bubbles.

It is essential to evaluate the mechanical properties of biomaterials to understand their behavior during practical applications, such as packing and transportation. Table 3 shows the mechanical characteristics of the prepared films. The films exhibited an average thickness of ~80 μm. In addition, the addition of the green-synthesized ZnO NPs to the chitosan matrix enhanced the mechanical properties of the biopolymer-based

Fig. 10. (a-c) Antibacterial activity of the green-synthesized ZnO containing two different extract concentrations (10 and 15 mL), namely ZnO-G′ and ZnO-G″, respectively, obtained via standard colony counting assay and (d, e) Antibacterial activity of the prepared neat CS and ZnO/CS composite films against E. coli and S. aureus obtained via the disk diffusion method.

Table 3

| Time   | Item       | S. aureus |        |        | E. coli |        |        |
|--------|------------|-----------|--------|--------|---------|--------|--------|
|        | Blank      | Viable (CFU/ml) | 1 × 10⁸ | 9 × 10⁷ | 5 × 10⁷ | 5 × 10⁷ | 1 × 10⁷ |
|        | Extract    | 10        | 50     | 80     | -       | 40     | 60     |
|        | Reduction (%) | -        | -      | -      | -       | -      | -      |
| 1 h    | Viable (CFU/ml) | 1 × 10⁸ | 9 × 10⁷ | 1 × 10⁷ | <1 × 10⁷ | 1 × 10⁷ | 9 × 10⁷ |
|        | Reduction (%) | -        | 98     | 99.9   | -       | 96     | 97.9   |

reductive oxygen species generation [59,60]. The oxidizing and reducing properties of hydrogen in these compounds facilitated the formation of numerous connections with other molecules. The molecules of the extract affected on the formation of the final NPs, thus increasing their reactivity and ability to attach to the surface of the bacterium, thus enhancing their penetration into the bacteria. For enhanced ion transport or penetration, plant extract chemicals adhere to the surface of the bacterium and generate new routes near the surface of the bacteria. Numerous of these compounds can readily form several bonds with other molecules because of the dual role of hydrogen as both an oxidizing and reducing agent. Chitosan and ZnO possesses a variety of hydrogen bonds, Van der Waals forces, hydrophobic interactions between molecules, covalent polar and non-polar bonds, and crosslinking for various application. Table 6 shows a comparison of previously obtained results and the results obtained in this study. For example, recently, Peng et al. reported the synthesis of ternary Ag/Ag₂O/ZnO heterostructures embellishing cellulose/chitosan sheets, which functioned as an excellent support for ZnO and Ag NPs. Using biominalization, the hydroxyl groups of the cellulose and chitosan chains were exposed and used to create Ag and ZnO NPs. The Ag/Ag₂O/ZnO decorating cellulose/chitosan (AZ@CC) films displayed outstanding antibacterial action against S. aureus and E. coli. Additionally, AZ@CC films demonstrated vigorous photocatalytic activity against methyl orange.

The radical scavenging activities of the CS and ZnO/CS film evaluated using the DPPH test were 37.21 and 90.2 %, respectively. This was attributed to the intense antioxidant activity and high phenolic content of the extract. This biocompatibility of materials is essential for their biomaterial application. The morphology of L929 cells on the ZnO/CS composite film after 72 h of incubation is shown in Fig. 11(a). The image revealed that the L929 cells were well propagated and proliferated on the film. Next, L929 cells were used to determine the cytotoxicity of the prepared films using the MTT assay, and the results are shown in Fig. 11 (b). The cell survival percentage on the ZnO/CS sample was greater than 74% at different intervals, demonstrating its suitable biocompatibility with L929 cells. This could be attributed to the fact that the prepared sample is a harmless and biocompatible biopolymer.
composite films by enhancing the Tensile strength (TS), and Young’s modulus (YM) of the neat chitosan film by increasing the molecular interaction between chitosan and ZnO, which enhanced the interfacial interactions. The shape and size of fillers, dispersion of particles in the matrix, and matrix–particle interaction is believed to affect the mechanical characteristics of particle-filled polymer composites. The ZnO/CS sample was observed to exhibit enhanced flexibility, as demonstrated by the improved elongation at break. In addition, the high polyphenolic content of the leaf extract enhanced the flexibility of the chitosan film. Similar behavior was reported by S. Palatsingh et al. who reported that the incorporation of ZnO NPs into CS matrix enhanced the strength of the composite film compared to that of pure CS film [62].

Figure 12(b) and (c) show the water contact angles of the CS and ZnO/CS composite films, respectively. The composite films exhibited a hydrophilic surface. The contact angle of the neat CS film was 73.8°, whereas that of the ZnO/CS film was 78°, which increased its hydrophobicity compared to that of the neat film. The increase in the water contact angle of the ZnO/CS film may be attributed to the hydrophobicity of the metal-semiconductor ZnO, cross-linking, or interaction between chitosan and produced NPs, which resulted in a reduction in the free hydroxyl group on the surface of the film.

3.6. Effectiveness of the developed film for the packaging of fruits, and sensory investigation

The efficiency of the composite films as a strawberry and red plum packaging material was investigated. Fig. 13 shows the results of the visual observations. The inside appearance of strawberry is essential for determining its freshness. After eight days, the inside appearance of the uncoated strawberry was black with white patches and nearly rotten (the inset of Fig. 13(a)). In contrast, the strawberry packaged with a ZnO/CS composite covering exhibited a far better internal appearance. After ten days of storage, mildew, as well as several moldy areas, were observed on the surface of the red plums covered with plastic (chemical) film. In addition, the juice of the fruit was sticky and released a foul odor (Fig. 13(b)). After 10 days of storage, mildew was observed just on the upper end of the red plums covered with CS film, with a few black patches on the surface (Fig. 13(b)). In contrast, the fruits wrapped in ZnO/CS nanocomposite films remained fresh and had a pleasing look (Fig. 13(b)). The weight loss of fruit is crucial for determining the ability of the coating to extend the shelf life of the fruit. The weight reduction of the uncoated red plum and red plum coated with the neat CS and ZnO/CS composite films under ambient conditions is shown in Fig. 13(c). The use of the film on the red plums prevented the weight loss of the samples, as shown in the graph. After 10 days of storage, the weight of the uncoated fruit reduced by more than 25%. After the application of the coating, the weight loss percentage reduced. When the ZnO/CS film was
utilized as the coating, it exhibited the best performance in minimizing the weight loss of the red plum. These results demonstrated the potential application of the synthesized composite film as a packaging material for strawberry and red plum, and this should be further investigated in future studies. Chitosan can indeed be used in food packaging owing to its semi-permeable qualities. In addition, it exhibits a low permeability to water vapor and is an excellent barrier to oxygen gas.

Furthermore, it limits the diameter of the pores on the surface of

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**Fig. 13.** Images of (a) the strawberries wrapped in the prepared CS and ZnO/CS coating, (b) Red plums wrapped in polyethylene (chemical) film and our prepared coatings after 10 days storage at room temperature, (c) Weight loss of uncoated red plum and red plum coated with CS and ZnO/CS coatings.

**Fig. 14.** Images of (a) schematic of the protecting features, such as antibacterial activity mechanism/UV-shielding, of the prepared ZnO/CS sample and (b) Barrier efficiency and oxygen permeability.
fruits, as well as its permeability to oxygen and water, which reduces the enzymatic activity of the fruit and delays decomposition (See Fig. 14 (a)). The shorter shelf-life of perishable fruits is due to the fast respiration rate after harvesting, oxygen level inside fruits, sugar content change, rapid metabolic metabolism of nutrients, and microbial growth. It has been reported that composites coatings and virgin edible fruit coatings (hydrocolloid/lipid/metallic nanoparticle combinations) could help in prolonging the shelf life of fruits: Functional groups and cross-linking structures that can regulate respiratory gas exchange, as well as microbiological safety and antioxidant activity of the fruit coverings, are all features of edible hybrid coatings. In addition, fruits with a high polyphenol content have been discovered to exhibit a longer shelf life when coated with edible antimicrobial coatings generated from agro industrial waste.

Polyphenolic fruits should be ingested regularly by COVID-19 patients to aid recuperation. When using nanomaterials in the real world, toxicology is a severe concern. Particularly, it essential to conduct significantly more research on the toxicity of nanomaterial when consumed in large quantities with edible coatings to protect human health and the environment. These findings of this study can be used to develop a highly effective coating for preserving fruit. In addition, most fruit coating studies focused on bacterial infection protection, and there are limited studies on antiviral fruit coatings. Farm produce is contaminated with viruses from “farm” to “table.” Therefore, studies on antibacterial and antiviral fruit coverings are recommended to reduce virus transmission. COVID-19 carriers may spread coronavirus to produce. More research is needed to determine the effectiveness of multifunctional antimicrobial customized coatings against coronavirus and other viruses.

Figure 14(b) shows the O₂ permeability of the neat CS and ZnO/CS samples, which was investigated to determine the influence of fillers on polymer for packaging applications. Compared to pure CS, the ZnO/CS film exhibited decreased O₂ permeability. The decrease in the O₂ permeability of the composite corresponded to the very crystalline obstructive potential of ZnO. Interfacial adhesion and uniform ZnO NPs diffusion in the biopolymer promoted chain immobilization, which resulted in improved barrier characteristics. The results revealed that ZnO/CS was the most effective barrier to H₂O/O₂ penetration. Table 4 presents the evolution of the sensory characteristics (such as color, texture, and appearance) of red plum during the 10 days of storage. During storage, the red plums were packaged using neat CS, ZnO/CS biopolymers, and polyethylene (chemical) polymer. The control and chemical packed samples exhibited distinct differences in look and texture. For comparison, the identical unwrapped red plum was investigated. Among the samples, the sample packed using the ZnO/CS sample exhibited the most appealing and acceptable features. It is important to synthesize nanocomposite films that have a low chance of transferring NPs to real food. As a real food model, fresh chicken breast meat was stored using the composite films, and the amount of Zn²⁺ ions before and after storage was measured (Table 4). Equal pieces of chicken breast flesh (which were purchased from a nearby butcher shop) were placed in several bags prepared using the nanocomposite samples for the contact testing to measure the quantity of Zn transfer. Subsequently, the sealed samples were stored at 4°C for four days. Thereafter, the samples were dissolved in nitric acid and hydrogen peroxide in a microwave, after which the ZnO content of the residual ashes was determined using atomic absorption spectrophotometry. The amount of Zn²⁺ in unwrapped meats was also measured, and was found to be approximately 0.014 mg per 100 g of meat. The same amount of Zn²⁺ was measured in the meats wrapped in pure CS films. In the samples wrapped with ZnO/CS films, the Zn²⁺ levels increased slightly after storage, indicating the release of ZnO from the nanocomposite film. Nevertheless, the amount of Zn²⁺ ions transferred from the nanocomposite film to the fillet samples was still significantly below the migration limit of daily zinc consumption (40 mg/day) set by the National Institute of Health for food contact materials [63]. However, there is still no specific standard for ZnO NPs migration.

Table 5

| Sample          | Zn concentration (mg/100 g meat) |
|-----------------|----------------------------------|
|                 | Day 0                            |
|                 | 0.280 ± 0.010                    |
| Control         | 0.236 ± 0.002                    |
| ZnO/CS          | 0.230 ± 0.001                    |
|                 | Day 4                            |
| Control (Neat chicken breast flesh) | 2.581 ± 0.020 |

3.7. Soil degradation

The soil degradation test revealed that the weight of the film decreased over time. The weight loss and soil degradation of the three films in soil are shown in Fig. 15(a) and (b). The films were broken down by microorganisms into carbon dioxide, water, inorganic chemicals, and biomass at the same rate as other organic materials in the compost pile, leaving no hazardous material behind. Bioplastics may be degraded by microorganisms from a variety of taxa, including Firmicutes, Proteobacteria, Ascomycetes, and Basidiomycetes. These bacteria may be found in a variety of environments, including terrestrial and marine soil, composting facilities, and even the stomachs of insects. Photo-degradation is the first step of the decomposition of plastic in nature, followed by hydrolysis and humic-oxidation. These activities result in the breakdown of plastic waste into low molecular weight chemicals, which can be metabolized by microbial activity. This process, however, is extremely sluggish and can take decades to complete [64]. Biofilm disintegration has recently been discovered to have beneficial impacts on soil fertility and plant nutrition, and results in increased plant growth. [65]. Briefly, as mentioned previously, COVID-19 caused millions of infections and deaths owing to its rapid spread. During this crisis, the use of healthy drugs and fruits increased considerably. Although there is no proof that COVID can be transmitted via foods (vegetables and fruits), food can be contaminated by the unsanitized hands of asymptomatic people. For example, people may touch a product to check for damage or ripeness, after which they return it to the shelf or shopping cart. Owing to the long-term stability of coronavirus on diverse surfaces, the contaminated hands of either the seller or buyer could be a possible source of indirect transmission of severe coronavirus or other diseases. Thus, it is highly recommended to use hand sanitizers to prevent virus infection. However, it is challenging to wash some foods, such as strawberries and crinkly lettuce [66-70]. Environmentally friendly edible films and coatings are becoming increasingly important for human safety and fruit shelf life. By coating berries with edible antiviral coatings made of alginate/oleic acid and green tea extract, Falco et al. (2019) demonstrated the control of viral infection and increase in berry safety [64-68]. Particularly, they found that the coatings exhibited high antibacterial activity against foodborne viruses, murine norovirus, and human norovirus. The authors suggested the development of edible antiviral coatings for fresh and lightly processed fruits. Fruits are vital in the rehabilitation of COVID-19 patients, and the presence of edible coating on them will help prolong their shelf life.
Therefore, if you are looking for a way to boost your polyphenol intake, these fruits may prove helpful. Polyphenols are highly beneficial to our health. The immunological and antiviral efficacy of polyphenols, such as herbaceous and rhoifolin, effectively increases immune and antiviral effectiveness against coronavirus. In addition, cytokine storm is caused by coronaviruses. When cytokine storm hits, the afflicted victims die. A cytokine storm caused by a coronavirus can be prevented by several naturally occurring polyphenols, such as curcumin, apigenin, resveratrol, and kaempferol [70–74]. These findings suggest that edible antimicrobial coatings on fruits can help prevent virus transmission before infection, while also extending the shelf-life of polyphenolic fruits, which are vital for the recovery of COVID-19 patients.

4. Conclusion

In this study, chitosan hybrid nanocomposite films containing 5 wt.% of ZnO, synthesized using wild M. pulegium extract through a simple casting process, with multifunction properties were prepared and characterized using different analytical methods. The flexible, biodegradable, and transparent films exhibited excellent antibacterial/antioxidant activity. In addition, physical, mechanical, and antibacterial properties were investigated to assess antibacterial ability and photocatalytic performance. The green-synthesized ZnO NPs exhibited a more uniform, spherical, and regular morphology and stronger antibacterial activity compared to commercial ZnO NPs. In contrast, the prepared ZnO/CS composite could function as a UV-shielding coating as it could absorb UV radiation with wavelengths of less than 400 nm. The resulting ZnO/CS film improved the shelf life of...
some model fruits by up to 10 days at 23 °C, whereas uncoated fruits started to deteriorate after only four days. Based on obtained results, the fabricated ZnO/CS biofilm could be proposed as a feasible alternative to synthetic polymers in the packaging industries to enhance product freshness during storage.

Data availability statement

All data included in this study are available upon request by contact with the corresponding author.

CRediT authorship contribution statement

Sanaz Alamdari: Investigation, Writing – original draft, Conceptualization. Ozid Mirzadeh: Writing – review & editing, Fatemeh Nasiri Jahroodi: Methodology. Majid Jafar Tafreshi: Conceptualization, Validation. Morteza Sasan Ghamarsi: Formal analysis, Writing – review & editing. Somayeh Salmani Shik: Investigation. Mohammad Hossein Majles Arz: Funding acquisition, Validation. Kyu-Yeon Lee: Data curation. Hyung-Ho Park: Supervision, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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