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Pyruvic Acid-Modified Chitosan Nanocomposite Films Reinforced with ZnO and ZnO–MgO Nanoparticles: Structural, Thermal, Antimicrobial, and Active Packaging Properties

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Abstract

This study reports the preparation of pyruvic acid-modified chitosan films reinforced with ZnO and ZnO–MgO nanoparticles for active packaging applications. Chitosan was chemically modified through Schiff base formation with pyruvic acid to improve its structural functionality, and ZnO or ZnO–MgO nanoparticles were incorporated in situ to enhance the physicochemical, mechanical, thermal, barrier, and antimicrobial properties of the films. The prepared films were characterized by FTIR, XRD, SEM, TGA, UV–vis spectroscopy, contact angle analysis, and mechanical testing. The results confirmed successful chitosan modification and nanoparticle incorporation, leading to reduced crystallinity, improved thermal stability, enhanced tensile strength, better UV-blocking ability, and stronger antimicrobial activity compared with neat chitosan films. Among all formulations, the pyruvic acid-modified chitosan/ZnO–MgO film exhibited the best overall performance, showing the lowest moisture content, improved barrier properties, and the highest antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. In practical storage tests, strawberries coated with the nanocomposite films showed lower weight loss and delayed spoilage relative to the uncoated control. These findings suggest that pyruvic acid-modified chitosan films containing ZnO–MgO nanoparticles are promising sustainable materials for active food packaging.

Keywords: Chitosan. Pyruvic acid. ZnO nanoparticles. ZnO–MgO nanoparticles. Schiff base. Nanocomposite films

1 Introduction

Packaging serves as an important tool in food preservation, shelf-life extension, and food safety. However, traditional petroleum-based plastics have created significant environmental issues because they persist in the environment for long periods, contribute to microplastic pollution, emit greenhouse gases, and may leach chemical additives into food. This has led to growing interest in sustainable, biodegradable, and renewable packaging materials [1, 2].

Chitosan (CS) is a natural biopolymer that is highly promising for active food packaging because of its biocompatibility, film-forming ability, gas barrier properties, and intrinsic antimicrobial activity[3]. Despite these advantages, chitosan films still have important limitations, including brittleness, weak water vapor barrier properties, limited mechanical strength, and low solubility at neutral pH, which restrict their practical use in food packaging. To overcome these shortcomings, various strategies such as chemical modification, blending with other polymers, and incorporation of functional nanoparticles have been explored [4, 5].

Organic acids are commonly used to modify chitosan and improve its functionality for specific applications. Pyruvic acid (PY) can react with the amino groups of chitosan through Schiff base formation, resulting in improved solubility, a denser network structure, and enhanced physicochemical properties while retaining biodegradability and biocompatibility [6, 7]. At the same time, metal oxide nanoparticles such as ZnO and MgO are attractive additives because of their mechanical reinforcement, moisture-barrier, UV-shielding, and antimicrobial properties [8, 9]. However, one of the major challenges associated with nanoparticles is their limited dispersion in polymer matrices and their tendency to aggregate [10-12].

Chitosan-based materials have been extensively modified for functional use in food packaging, biomedical, and antimicrobial applications in recent studies [13]. Antibacterial and antioxidant properties have been enhanced by the addition of bioactive compounds, particularly plant extracts, in chitosan edible films and coatings applied to food products [14]. Similarly, chitosan edible coatings containing plant extracts have improved antimicrobial and antibiofilm activity against foodborne pathogens such as *Salmonella enteritidis* and *Escherichia coli* in chicken [15]. In another study, chitosan blend films with geraniol inhibited quorum sensing and biofilm formation, suggesting their potential as packaging materials for fresh fruit and vegetable preservation. The application of chitosan-based films containing flavonoids has also been reported to improve the preservation quality of chilled beef, further highlighting the potential of natural bioactive compounds in enhancing the biological properties of chitosan films. In addition to natural additives, chitosan has also been chemically modified by grafting and crosslinking to further improve its antimicrobial activity. For example, chitosan grafted with gentisic acid showed multi-target antibacterial activity against *Pseudomonas fluorescens*, while chitosan grafted with phytic acid exhibited excellent antibiofilm and antifungal properties [16].

Some studies have shown that the properties of chitosan-based biofilms can be improved by adding other biopolymers or proteins, such as *Camellia oleifera* residue protein [17], to obtain better hydration, permeability, mechanical strength, flexibility, antimicrobial activity, antioxidant activity [18], and biodegradability. Chitosan active films have also been developed for guacamole preservation, demonstrating their importance in active food packaging. Nanomaterials have likewise been used in a wide variety of systems; for instance, gelatin–chitosan biofilms loaded with ZnO nanoparticles have shown controlled release and anticancer properties, while green-synthesized MWCNTs/ZnO/chitosan nanocomposites exhibited antibacterial and antibiofilm activity against *Helicobacter pylori* [19]. In addition, edible starch-based biofilms modified with chitosan, seaweed, and sodium tripolyphosphate displayed improved water resistance and mechanical properties [20]. Overall, these studies show that chitosan is a versatile platform for

chemical modification, blending, crosslinking, and nanoparticle incorporation, making it a promising material for sustainable food packaging and biomedical applications.

In response to these limitations, the present study describes a new and sustainable active packaging platform based on pyruvic acid-modified chitosan (CSPY), reinforced with ZnO nanoparticles (CSPY/ZnONPs) and in situ synthesized bimetallic ZnO–MgO nanoparticles (CSPY/ZnO–MgONPs). In contrast to traditional methods that rely on acidic solvents or costly noble-metal nanoparticles, the CSPY matrix serves as a dual-purpose reagent: it covalently crosslinks chitosan through Schiff base formation, thereby improving insolubility at neutral pH, and it acts as a green stabilizing and reducing agent for the simultaneous formation of ZnO and MgO nanoparticles within the nanocomposite films. The resulting films were thoroughly characterized in terms of their structural, thermal, mechanical, and barrier properties. Their antimicrobial efficacy against *Escherichia coli* and *Staphylococcus aureus* was evaluated, and their practical packaging performance was assessed by monitoring postharvest quality parameters, including weight loss, microbial spoilage, and visual deterioration of fresh strawberries during storage. The results suggest that these modifications may be useful for

2 Experimental

2.1 Materials

Medium molecular weight chitosan (deacetylation degree 80%) was purchased from Sigma-Aldrich. Pyruvic acid, hydrochloric acid, and glacial acetic acid were also obtained from Sigma-Aldrich, while glycerol and absolute ethanol were supplied by Merck (Darmstadt, Germany). Zinc nitrate hexahydrate and magnesium nitrate hexahydrate were used as the precursors for nanoparticle synthesis. *Escherichia coli* (PTCC 1769) and *Staphylococcus aureus* (PTCC 1431) were obtained from the Persian Type Culture Collection, Iran. All chemicals were used as received without further purification.

2.2 Synthesis of chitosan-pyruvic acid (CSPY)

Chitosan-pyruvic acid conjugate (CSPY) was synthesized by dissolving 2.42 g of CS and 0.23 g of PY in 250 mL of 0.1 M HCl solution in a 500 mL round-bottom flask. The mixture was refluxed under magnetic stirring for 3 h [21]. After the reaction, the solution was transferred into a dialysis bag with a molecular weight cutoff of 3500 Da and dialyzed against distilled water for 48 h to remove unreacted small molecules and impurities. The dialyzed solution was then concentrated to one-fifth of its original volume by evaporation at 60 °C under reduced pressure. Finally, the product was freeze-dried to obtain a sponge-like solid [22]. The synthesis route is shown in (Fig. 1).

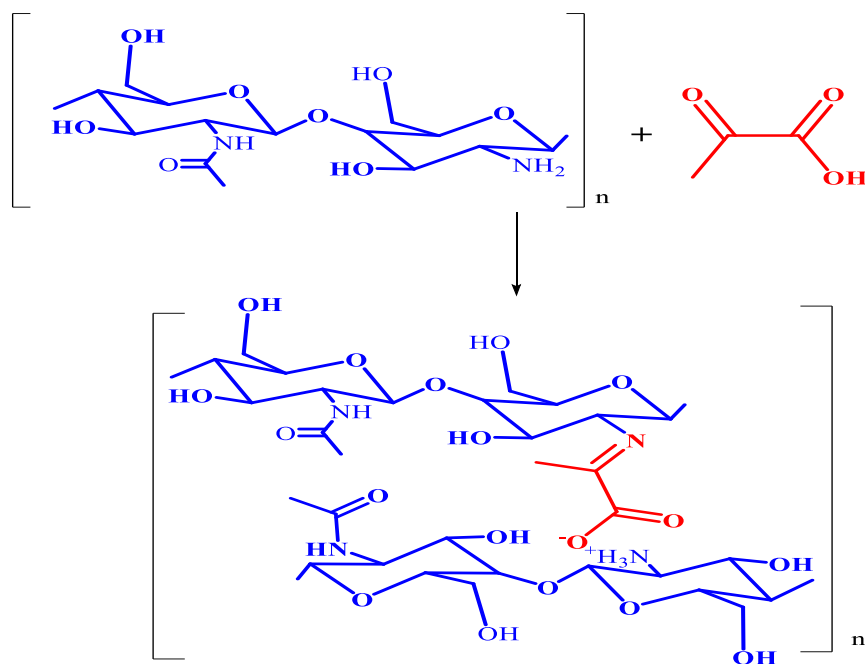


Fig.1. Synthesis of CSPY

2.3 Synthesis of CSPY/ZnONPs using CSPY

CSPY/ZnONPs were prepared by adding 20 mL of 0.50 M $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ solution to 20 mL of CSPY solution (0.2 mg/mL^{-1}) under magnetic stirring, followed by heating at 80°C for 8 h. After completion of the reaction, the resulting dispersion was dialyzed to remove unreacted precursors. The final product was obtained by freeze-drying [23, 24].

2.4 Synthesis of CSPY/ZnO-MgONPs using CSPY

CSPY/ZnO-MgONPs were prepared by mixing 20.0 mL of $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ solution (0.50 M) and 20.0 mL of $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ solution (0.50 M) to achieve a Zn: Mg molar ratio of 1:1. Subsequently, the mixed metal salt solution was added to 20.0 mL of CSPY solution (0.2 mg/mL^{-1}), and the mixture was stirred under magnetic agitation at 80°C for 8 h. Upon completion of the reaction, the resulting dispersion was dialyzed to remove excess precursors and unreacted species. Finally, the product was collected by freeze-drying to yield CSPY/ZnO-MgONPs [25].

2.5 Preparation of films

The films were prepared according to the formulations shown in (Fig.2, Table 1). Briefly, 2 g of each powder sample, including CS, CSPY, CSPY/ZnONPs, or CSPY/ZnO-MgONPs, was dissolved in 100 mL of 1% (v/v) acetic acid solution at 90°C under stirring at 150 rpm for 20 min. Then, glycerol (1% w/v) and gelatin (1% w/v) were added to the solution, and the mixture was homogenized by magnetic stirring at 5000 rpm for 4 h. The homogeneous suspension was cast into 140 mm Petri dishes and dried in an oven at 40°C for 24 h. After drying, the films were carefully

peeled off and conditioned at 23 ± 2 °C and $50 \pm 5\%$ relative humidity before testing. Four replicates were prepared for each formulation [26, 27].

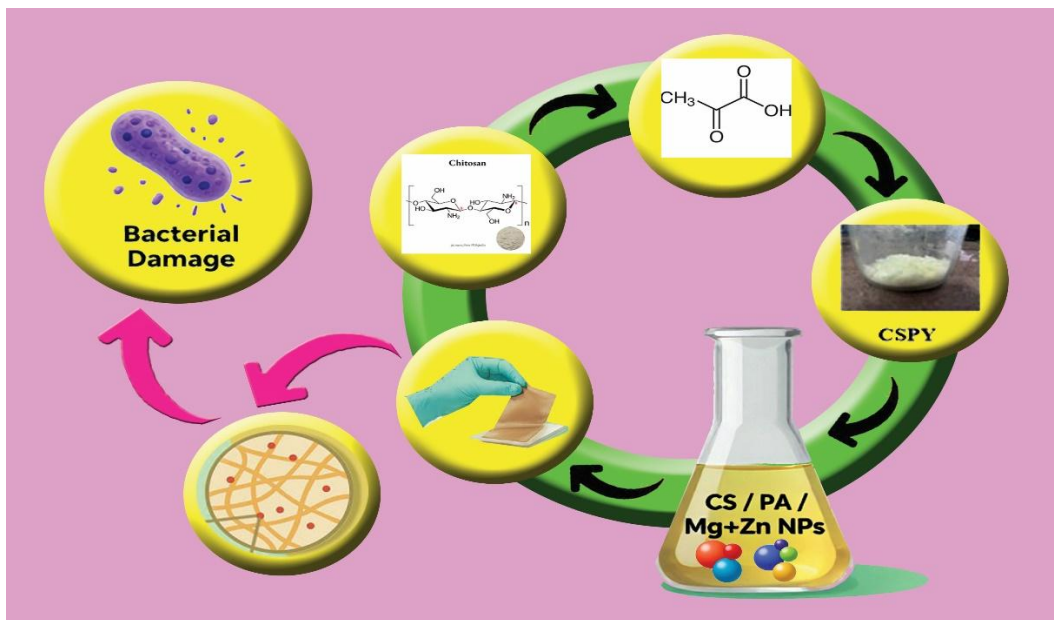


Fig. 2. Schematic diagram of the Preparation of biofilms by CSPY/ZnONPs and CSPY/ZnO-MgONPs fruit preservation.

Table 1 Composition of Films Formulations

Designation of films	CS	PY	CSPY/ZnONPs	CSPY/ZnO-MgONPs	Glycerol/Gylatin
CS	2	-	-	--	1:1
CSPY	-	2	-	-	1:1
CSPY/ZnONPs	-	-	2	-	1:1
CSPY/ZnO-MgONPs	-	-	-	2	1:1

2.6 Thickness analysis

The thickness of the films was measured at five random points using a digital dial thickness caliper (Neoteck Co., Canada) [28].

2.7 Mechanical Properties

The mechanical properties of the films were evaluated according to ASTM D882 using a universal testing machine. Film strips were conditioned at 50% relative humidity for 48 h before testing, and tensile strength and elongation at break were determined at a crosshead speed of 60 mm min^{-1} . At least five replicates were analyzed for each sample to ensure reproducibility. Tensile strength (TS)

and elongation at break (EAB) were calculated. At least five replicates were carried out for each sample [29].

2.8 Moisture content

Films were cut into 2×2 cm pieces, weighed (initial weight, M_1), and placed in glass Petri dishes. The samples were dried in an oven at 105°C for 24 h until constant weight was achieved (dry weight, M_2). Moisture content was calculated using three replicates per film according to the following equation [30].

Moisture content (%)

$$= \frac{m_1 - m_2}{m_1} \times 100$$

2.9 Water solubility

The water solubility of the films was determined by immersing preconditioned film samples in 50 mL deionized water for 24 h at room temperature, followed by filtration, drying, and reweighing to calculate the percentage of soluble matter. Three replicates were analyzed for each sample to ensure reliability [29].

Solubility (%)

$$= \frac{\text{initial dry weight} - \text{final dry weight}}{\text{initial dry weight}} \times 100$$

2.10 Chemical characterization of films

The fracture surface morphology of the films was examined by field emission scanning electron microscopy (FESEM) after tensile testing. The samples were mounted on stubs, coated with carbon and gold to minimize static charge, and observed using a TE-SCAN microscope at 20 kV and 8 mA [31]. Fourier transform infrared (FT-IR) spectra of the films and the pure chitosan control were recorded using an AVATAR Thermo spectrometer over the range of 4000–650 cm^{-1} , and measurements were taken from three different locations for each sample [29]. X-ray diffraction (XRD) patterns were obtained with a PHILIPS PW 1800 diffractometer at 25 °C, 40 kV, and 30 mA over a 2θ range of 3° to 40° [32]. Thermogravimetric analysis (TGA) and differential thermogravimetric analysis (DTG) were performed using a TGA/SDTQ600 analyzer on approximately 5 mg of sample heated to 650 °C at 10 °C min^{-1} under nitrogen atmosphere [33]. UV–visible transmission spectra of film samples (2 × 2 cm) were recorded from 200 to 1100 nm using a Jenway UV-7315 spectrophotometer with air as the reference [34]. Finally, the surface hydrophobicity of the films was determined by contact angle measurements using a JIKAN CAG-20 system, where 5 μL of ultrapure water was placed on the film surface and analyzed by the associated software [35].

2.11 Antimicrobial activity evaluation

The antimicrobial activity of the films was evaluated using the disc diffusion method. Briefly, bacterial suspensions standardized to the 0.5 McFarland scale were uniformly spread on Mueller-Hinton agar plates. Film samples were then placed on the agar surface, along with positive and negative controls. The plates were incubated at 37 °C for 18–24 h, depending on the bacterial strain. After incubation, the inhibition zones were measured using calipers. All tests were performed in triplicate to ensure reproducibility [35].

2.12 Weight loss

The weight loss of strawberries was calculated during storage to evaluate the effectiveness of the films in reducing moisture loss and preserving fruit quality. Fruit samples were weighed at each storage interval, and the percentage of weight loss was determined using the initial and final masses. This parameter is commonly used in fresh-produce packaging studies because lower weight loss generally indicates a better moisture barrier and improved shelf-life performance.

Weight loss (%) =

$$(M1 - M2)/M1 \times 100$$

where M1 represents the initial mass and M2 denotes the mass after storage.

2.13 Statistical analyses

The statistical analyses were performed using one-way analysis of variance (ANOVA), followed by Duncan's multiple range test to determine significant differences among samples at $p < 0.05$. All figures were created using ORIGINPRO 2022 software (Stat-Ease Inc., Minneapolis, USA).

3 Results and discussions

3.1 FT-IR analysis of chitosan–pyruvic acid system

The FTIR spectra showed (Fig.3) shifts and intensity changes in the characteristic bands of chitosan after reaction with pyruvic acid. The spectrum of pristine chitosan exhibits a broad absorption band in the range of 3200–3400 cm^{-1} , attributed to the overlapping stretching vibrations of –OH and –NH₂ groups, which indicates extensive intra and intermolecular hydrogen bonding within the polymer chains. Absorption bands at 2870–2920 cm^{-1} correspond to the C–H stretching vibrations of the chitosan backbone. The characteristic peak at approximately 1650 cm^{-1} is assigned to the C=O stretching vibration, while the band near 1580 cm^{-1} is associated with the –NH₂ bending vibration of chitosan.

Pyruvic acid displays a broad –OH stretching band between 2500–3300 cm^{-1} , along with a strong absorption at 1710–1740 cm^{-1} corresponding to the C=O stretching of the carboxylic acid group, and a distinct band at 1680–1700 cm^{-1} associated with the keto carbonyl group. Following the reaction between chitosan and pyruvic acid, notable changes are observed in the FT-IR spectrum of CS/PY. The broad –OH/–NH₂ band decreases in intensity and undergoes a slight shift, indicating enhanced hydrogen bonding between the chitosan matrix and pyruvic acid. Importantly, the characteristic carbonyl absorption bands of pyruvic acid in the 1710–1740 cm^{-1} and 1680–1700 cm^{-1} regions significantly diminish, while a new band with increased intensity emerges in

the 1630–1650 cm^{-1} region. This newly intensified band is attributed to the formation of imine ($\text{C}=\text{N}$) linkages via the Schiff base reaction between the primary amino groups of chitosan and the carbonyl group of pyruvic acid. Concurrently, the reduction in intensity of the $\text{N}-\text{H}$ bending band at $\sim 1580 \text{ cm}^{-1}$ consistent with imine formation the partial consumption of free amino groups during chemical modification.

Furthermore, spectral changes in the 1200–1300 cm^{-1} region correspond to $\text{C}-\text{N}$ and $\text{C}-\text{O}$ stretching vibrations, supporting the formation of new covalent bonds. The characteristic bands associated with the glycosidic structure of chitosan in the 1000–1150 cm^{-1} region remain intact, confirming that the polysaccharide backbone is preserved. Overall, the FTIR results support the successful modification of chitosan with pyruvic acid and are consistent with the formation of Schiff base linkages.

Table 2 FT-IR absorption bands of chitosan, pyruvic acid, and their mixture.

Interpretation	Mixture (CS/PY)	Pyruvic acid (PY)	Chitosan (CS)	Assignment	Wavenumber (cm^{-1})
Enhanced hydrogen bonding between CS and PY	Broader, reduced intensity	Broad	Broad, Strong	OH and $-\text{NH}_2$ stretching	3200–3400
Polysaccharide backbone retained	Present	Weak	Present	$\text{C}-\text{H}$ stretching ($-\text{CH}_2$, $-\text{CH}_3$)	2870–2920
PY of carboxyl group in CS–PY interaction	Decreased/shifted	Strong	–	$\text{C}=\text{O}$ stretching (COOH)	1710–1740
Involvement of carbonyl group in Schiff base reaction	Reduced	Strong	-	$\text{C}=\text{O}$ stretching (keto group of PY)	1680–1700
Formation of imine (Schiff base) linkage	Stronger/shifted	–	Weak	$\text{C}=\text{N}$ stretching / amide I region	1630–1650
Consumption of free amino groups of chitosan	Reduced	–	Strong	NH_2 bending	1580
Formation of new $\text{C}-\text{N}$ / $\text{C}-\text{O}$ bonds	Altered	Present	Present	$\text{C}-\text{N}$ and $\text{C}-\text{O}$ stretching	1200–1300
Preservation of chitosan backbone structure	Present	Weak	present	$\text{C}-\text{O}-\text{C}$ and $\text{C}-\text{O}$ stretching (glycosidic bonds)	1000–1150

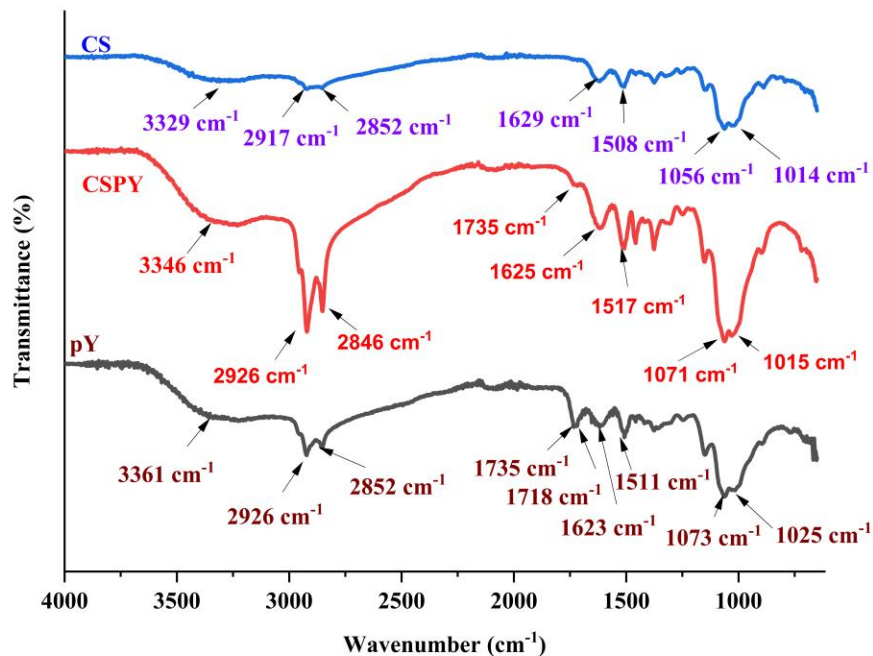


Fig. 3. FT-IR spectra of CS/PY

3.2 Thickness, Moisture content and water solubility

The incorporation of PY into CS resulted in a significant increase in both moisture content (from 12.54% to 18.69%) and water solubility (from 35.29% to 58.75%). This enhancement is consistent with the introduction of hydrophilic carboxyl and imine ($C=N$) functional groups via Schiff base formation. Such structural modifications strengthen polymer–water interactions, as corroborated by recent studies on acid-functionalized chitosan derivatives (Table.3) [36].

Notably, the incorporation of bimetallic ZnO and MgO nanoparticles into the CSPY matrix (CSPY/ZnO-MgONPs) reduced the moisture content to 11.45%, the lowest value among all formulations. This suggests a synergistic packing effect induced by the dual-metal oxide system, where Zn^{2+} and Mg^{2+} ions likely coordinate with chitosan functional groups to increase network density and reduce hydrophilicity [37]. However, the water solubility of this nanocomposite (37.09%) remained slightly higher than that of pristine CS (35.29%). This can be attributed to residual hydrophilic pyruvate moieties or the surface polarity of the nanofillers, aligning with the moderate hydrophilicity typically observed in metal oxide–chitosan hybrids. Regarding thickness, a steady increase was observed from pristine CS (0.12.00 mm) to CSPY/ZnO-MgONPs (0.15. mm). The increase in film thickness and solution viscosity correlates with higher solid content and nanofiller loading. This moderate thickening is advantageous as it improves mechanical handling and processability without compromising elasticity, a critical factor for practical food packaging applications. Overall, the CSPY/ZnO-MgONPs nanocomposite offers an optimal balance:

minimal moisture content (enhancing barrier properties), moderate water solubility (ensuring edibility and controlled disintegration), and superior structural integrity. These combined attributes directly contribute to the primary objective of this work: extending the postharvest shelf life of perishable fruits such as strawberries [29].

Table 3 Summary of Moisture absorption, water solubility, swelling content and Thickness for films.

Chitosan blend film	Moisture content (%)	Water solubility (%)	Thickness (mm)
CS	12.54 ± 0.729 ^c	35.29 ± 0.701 ^{cd}	0.12.0 ± 0.80 ^a
CS/PY	18.69 ± 0.450 ^a	58.75 ± 0.543 ^a	0.13.0 ± 1.54 ^b
CSPY/ZnONPs	14.89 ± 0.810 ^b	42.36 ± 0.250 ^b	0.14.0 ± 1.32 ^c
CSPY/ZnO-MgONPs	11.45 ± 0.243 ^{cd}	37.09 ± 0.216 ^c	0.15.0 ± 0.98 ^d

3.3 Mechanical properties of films

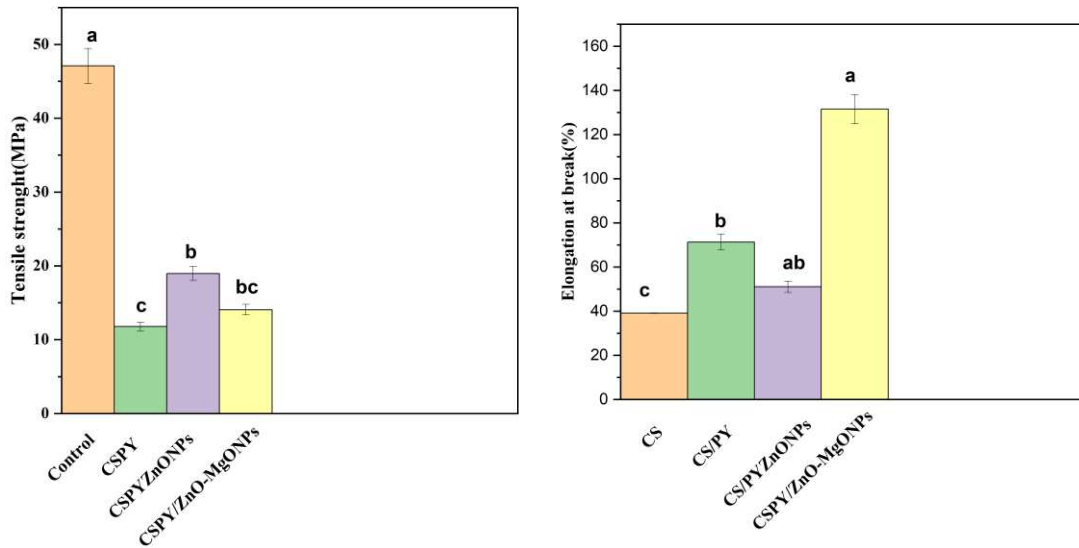


Fig. 4. TS and EAB spectra of composite films;

The CSPY/ZnO–MgONPs film exhibited superior mechanical performance compared to both pristine CS and unmodified CSPY films (Fig.4). This improvement can be attributed to several synergistic mechanisms: (i) covalent crosslinking between CS amino groups and PY carbonyls, which increases network density and restricts polymer chain mobility; (ii) strong interfacial interactions between the CSPY matrix and the bimetallic oxide nanoparticles, which act as multifunctional physical crosslinkers that dissipate mechanical stress; and (iii) the synergistic reinforcement effect of ZnO and MgO, which enhances load-transfer efficiency and minimizes polymer chain slippage. These findings align with recent studies demonstrating that dual-metal-oxide nanocomposite biofilms achieve an optimal balance of high tensile strength and reduced brittleness, making them highly suitable for practical food packaging applications [38].

Moreover, the observed thickness increase reflects not merely a geometric expansion but also the development of a more compact and homogeneous microstructure. As evidenced by SEM analysis (Fig. 5), the CSPY/ZnO–MgONPs films exhibit a dense, uniformly rough surface with well-dispersed nanoparticles. This refined morphology enhances film durability and creates an effective barrier against moisture and gas transmission, which is critical for preserving the postharvest quality of highly perishable fruits like strawberries. Consequently, the strategic combination of chemical modification (via PY) and bimetallic nonreinforcement (ZnO–MgO) yields chitosan-based films with optimized thickness and mechanical robustness. These attributes are essential for sustainable active packaging, enabling the material to withstand mechanical handling, maintain structural integrity, and effectively extend food shelf life.

3.4 Effects of films on surface morphology of CS

The unmodified chitosan matrix is characterized by strong intermolecular hydrogen bonding and tight chain packing, resulting in a smooth, compact, and homogeneous surface without cracks, pores, or phase separation (Fig. 5A). In contrast, the CS/PY film (Fig. 5B) exhibits a significantly rougher and more porous morphology. This change is attributed to the formation of Schiff bases between the amine groups of chitosan and the carbonyl groups of pyruvic acid, which introduces steric hindrance and disrupts polymer chain organization. Consequently, surface irregularity increases, enhancing hydrophilicity and providing more active sites for interaction with water or antimicrobial agents. The presence of bright, well-dispersed spherical nanoparticles within the matrix of the CSPY/ZnONPs film (Fig. 5C–D) confirms the in-situ synthesis and uniform distribution of ZnO nanoparticles. These particles act as physical crosslinkers, increasing surface roughness while reinforcing the structural integrity of the film, consistent with previous reports on ZnO–chitosan nanocomposites [39]. Interestingly, the CSPY/ZnO–MgONPs film (Fig. 5E–F) displays a highly disordered and heterogeneous topography, characterized by particles of varying sizes and a dense surface coverage. This observation suggests the synergistic co-deposition of ZnO and MgO nanoparticles, which enhances surface area and nano-roughness, thereby improving antimicrobial interaction and barrier performance. Overall, SEM micrographs confirm that the film morphology is significantly altered by chemical modification with pyruvic acid and the incorporation of dual metal oxide nanoparticles, leading to improvements in mechanical properties, water barrier performance, and antimicrobial activity.

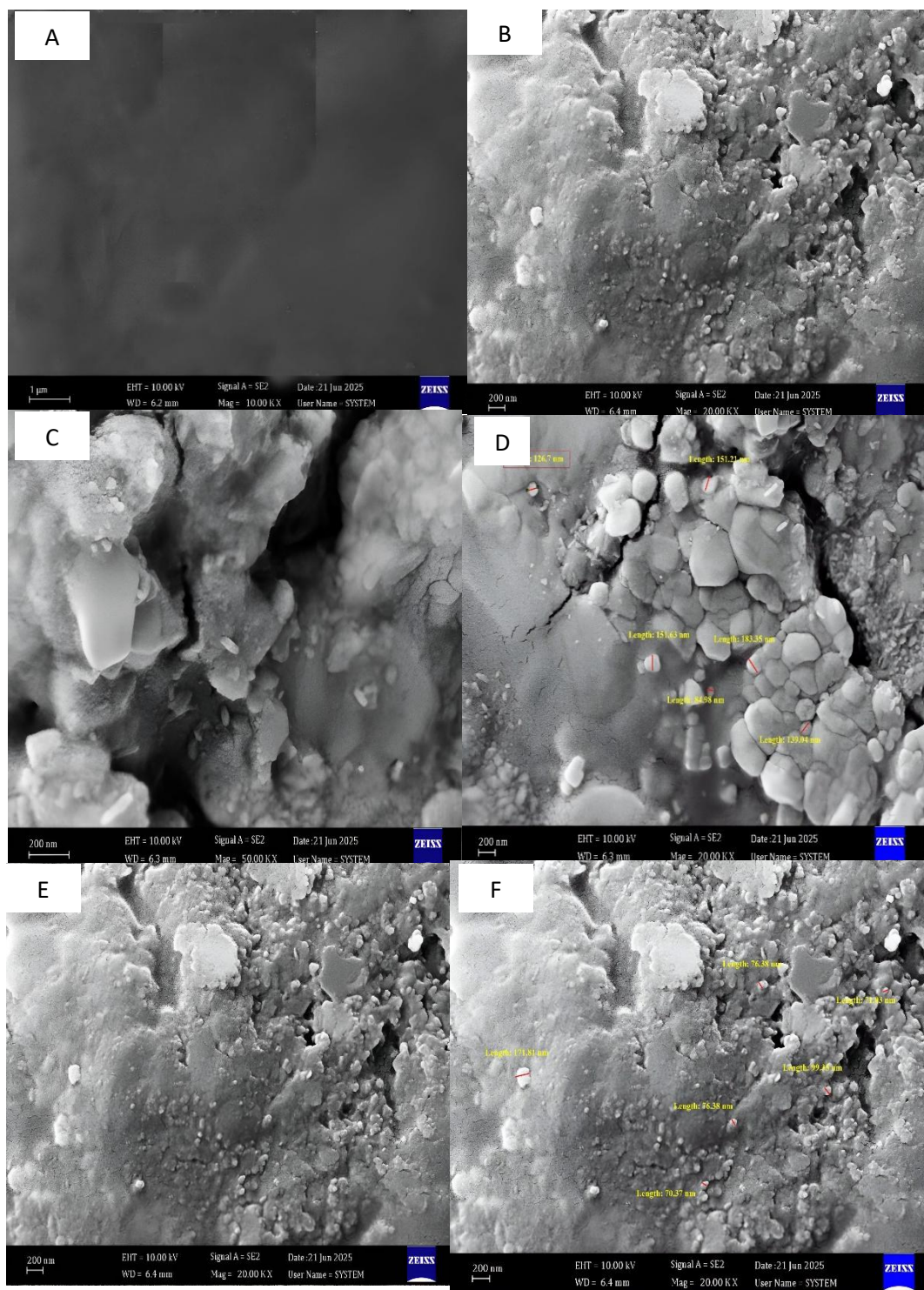


Fig. 5. (a) Photographs of CS (A), CSPY(B), CSPY/ZnONPs (C, D) and CSPY/ZnO-MgONPs (E, F) films.

3.5 ATR-FT-IR spectra analysis

The ATR-FTIR spectra of the prepared chitosan-based films (Fig. 6) reveal key molecular interactions and confirm the successful chemical modification and incorporation of nanoparticles.

A broad absorption band at 3300–3400 cm^{-1} , corresponding to O–H stretching and N–H bending vibrations, indicates the presence of extensive hydrogen bonding within the chitosan matrix. The slight broadening and shift of this band in CSPY and nanocomposite films suggest enhanced intermolecular interactions due to Schiff base formation and polymer–nanoparticle interactions [40]. The peak at 2920 cm^{-1} , attributed to C–H stretching of the chitosan backbone, remains largely unchanged across all formulations, indicating that the primary polysaccharide structure is preserved after modification. A significant change is observed in the amide I region (1650 cm^{-1}). In pure CS, this band corresponds to C=O stretching of residual N-acetyl groups. In CSPY and nanocomposite films, this peak shifts to approximately 1640–1655 cm^{-1} , accompanied by the appearance of new bands at 1560 cm^{-1} (N–H bending of protonated amines) and 1410 cm^{-1} (asymmetric COO^- stretching). These changes indicate the conversion of $-\text{NH}_2$ groups into imine and carboxylate functionalities, enhancing pH-responsive solubility. Notably, the emergence of a distinct band at 1030 cm^{-1} in CSPY/ZnO–MgONPs films is attributed to metal–oxygen (M–O) stretching vibrations (Zn–O and Mg–O), providing direct evidence for the successful in situ synthesis and incorporation of ZnO and MgO nanoparticles within the polymer matrix. Collectively, these spectral changes confirm: (1) covalent CS/PY conjugation via Schiff base formation, (2) electrostatic and polar interactions between the modified polymer and metal oxide nanoparticles [41].

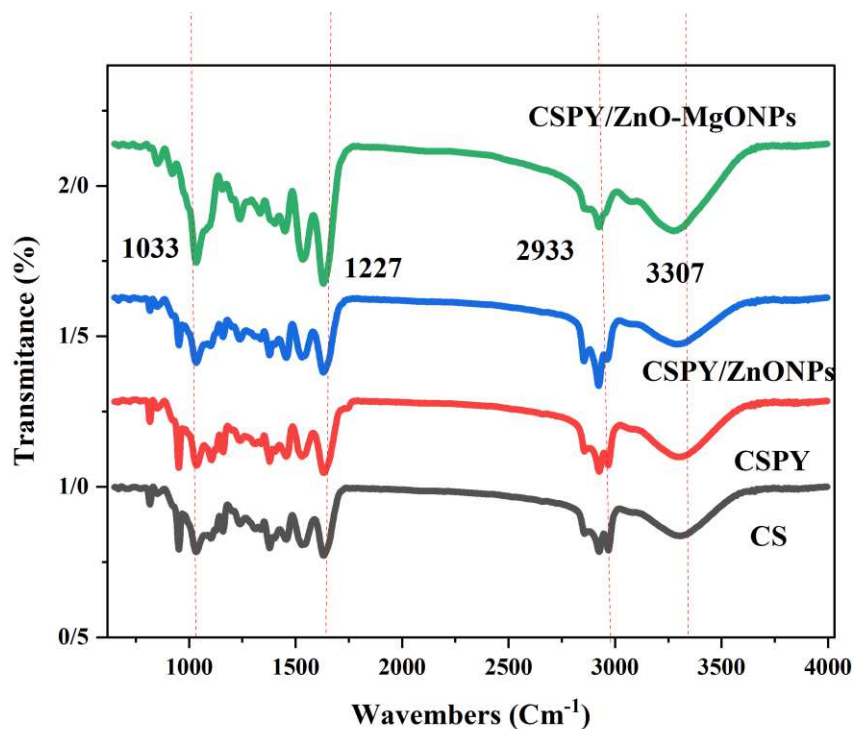


Fig. 6. FTIR spectra of composite films of CS, CSPY, CSPY/ZnONPs and CSPY/ZnO-MgONPs

3.6 XRD of Films

(Fig. 7) presents the XRD patterns of pure CS, pyruvic acid-modified chitosan (CS/PY), and their ZnO and ZnO–MgO nanoparticle-containing film nanocomposite films. Pure chitosan showed a broad diffraction peak at approximately 20° (2θ), which is typical of its semi-crystalline structure and is associated with the ordered arrangement of polymer chains through intermolecular hydrogen bonding [38]. After modification with pyruvic acid, this peak became weaker and broader, suggesting a reduction in crystallinity. This change may be attributed to the disruption of the original chain packing and hydrogen-bonding network following reaction between the primary amine groups ($-\text{NH}_2$) of chitosan and the carbonyl group of pyruvic acid, which disrupts the original chain packing and hydrogen-bonding network. As reported in [10], such covalent functionalization introduces steric hindrance and conformational disorder into the chitosan backbone, leading to a more amorphous structure with enhanced chain flexibility and processability. Furthermore, the characteristic chitosan peak at 20° (2θ) is further diminished in the nanocomposite films, particularly in the CSPY/ZnO–MgONPs sample. This reduction suggests strong interfacial interactions between the polymer matrix and the embedded nanoparticles. These interactions are likely governed by electrostatic attraction and coordination bonding between oxygen atoms on the nanoparticle surfaces (Zn–O, Mg–O) and the protonated amine ($-\text{NH}_2$) or hydroxyl ($-\text{OH}$) groups of chitosan. Such interactions restrict polymer chain mobility while enhancing the structural homogeneity of the composite [32]. The incorporation of dual metal oxide nanoparticles further amplifies the amorphous nature of the system through a synergistic effect.

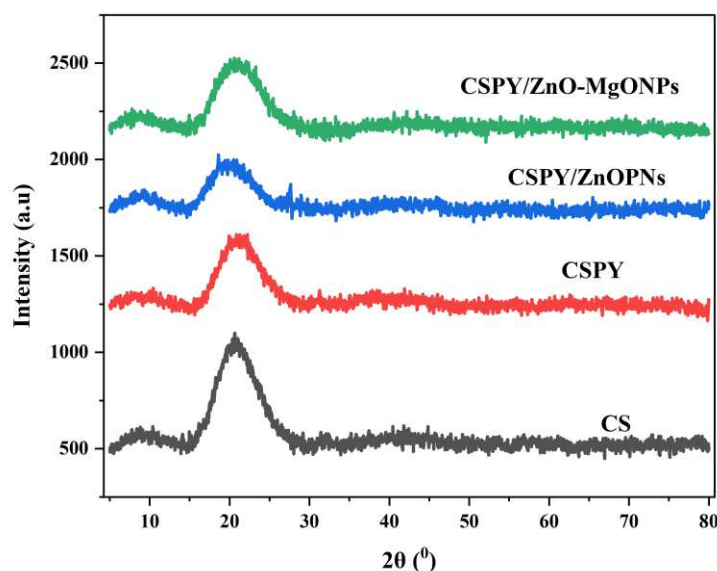


Fig. 7 XRD patterns of composite films of CS, CSPY, CSPY/ZnONPs and CSPY/ZnO–MgONPs

3.7 Thermogravimetric Analysis

The thermal stability of the chitosan-based films was evaluated by TGA and DTG, as presented in (Fig. 8). Pure CS exhibited a typical two-step degradation profile. The first stage, occurring below 120°C , was associated with the loss of physically adsorbed water. The second and most significant stage occurred between 260 and 350°C and corresponded to the

depolymerization and decomposition of the chitosan backbone, including the cleavage of glycosidic bonds and the degradation of deacetylated residues. This general degradation behavior is consistent with prior reports on chitosan. Pyruvic acid-modified chitosan (CS/PY) showed a noticeable shift in the degradation peak toward higher temperatures, indicating improved thermal stability. This indicates improved thermal stability due to stronger interfacial interactions and crosslinking [11]. Similar stabilization of chitosan after covalent modification has also been reported in previous studies. The incorporation of ZnO and ZnO–MgO nanoparticles into the CS/PY matrix further improved the thermal resistance of the nanocomposite films. In particular, the CSPY/ZnO–MgONPs formulation exhibited the highest decomposition onset temperature and the lowest mass loss rate in the main degradation region. This behavior suggests that metal oxide nanoparticles act as thermal barriers, limiting heat transfer and mass diffusion within the polymer matrix and thereby delaying the release of volatile decomposition products. Fig. 8b shows that the maximum degradation temperature $T_{\max}$ in the DTG curves shifted to higher values in the order of CSPY/ZnO and CSPY/ZnO–MgONPs, confirming a synergistic effect of covalent crosslinking by pyruvic acid and nanofiller reinforcement by bimetallic oxides. In addition, the residual char content at 650 °C was markedly higher for the nanocomposite films, especially CSPY/ZnO–MgONPs. This increased residue is attributed to the inorganic nature of ZnO and MgO nanoparticles, which do not decompose at high temperature and promote char formation through barrier and catalytic effects.

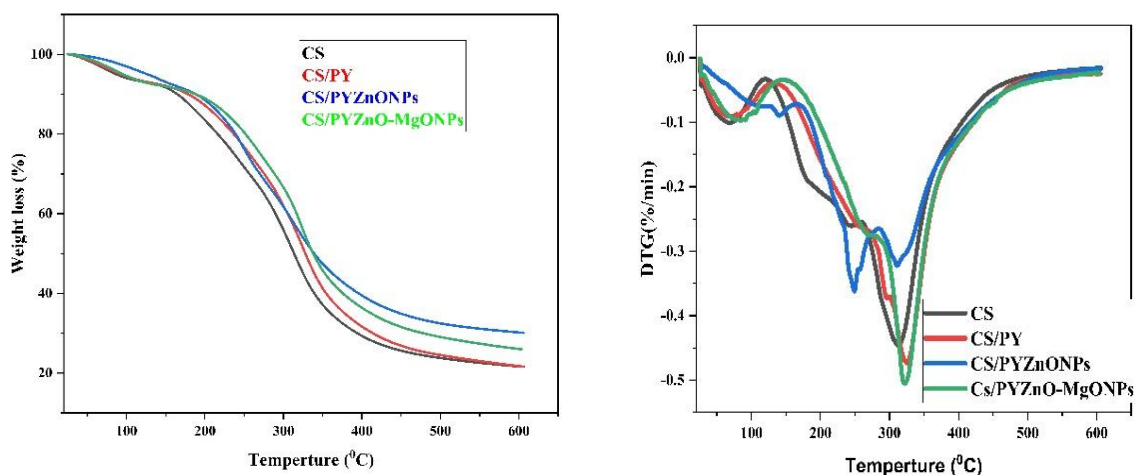


Fig. 8. TGA curves of composite films; and DTG curves of composite films.

3.8 UV-Vis spectra

The UV–Vis absorption spectra of the fabricated chitosan-based films, as shown in (Fig. 9), demonstrate significant compositional and functional changes resulting from pyruvic acid conjugation and nanoparticle incorporation. Pure CS exhibited strong absorption at wavelengths below 300 nm, mainly due to the $n \rightarrow \pi^*$ transitions of the amino ($-\text{NH}_2$) and residual acetyl ($-\text{COCH}_3$) groups [42]. After chemical modification with pyruvic acid, the CS/PY film showed a moderate increase in UV absorption in the 200–350 nm range. These imine bonds create

chromophoric structures with extended π - conjugation, leading to increased UV absorbance. A further increase in UV absorption was observed after incorporation of metal oxide nanoparticles. The films containing ZnO nanoparticles (CSPY/ZnONPs), and especially the ZnO–MgO nanocomposite films (CSPY/ZnO–MgONPs), exhibited much stronger absorption in the 200–350 nm region. This behavior is consistent with the wide bandgap semiconducting nature of ZnO and MgO, which absorb UV radiation by promoting electrons from the valence band to the conduction band. The stronger absorption of the bimetallic system compared with the ZnO-only composite may be attributed to improved nanoparticle dispersion, larger surface area, or altered electronic interactions within the chitosan matrix, all of which enhance UV-blocking capacity. Therefore, these nanocomposite films may help reduce photo-oxidative spoilage and extend shelf life by effectively blocking harmful UV photons. Recent studies also show that chitosan coatings containing ZnO and MgO can significantly reduce UV penetration without compromising fruit quality [10, 12].

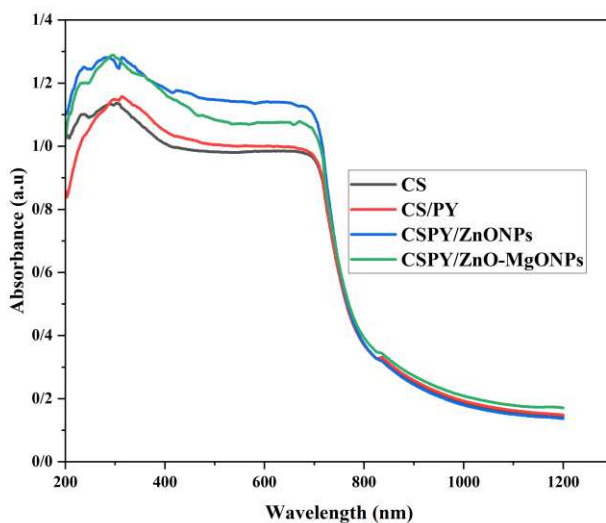


Fig. 9. UV spectra of Chitosan, CS/PY, CSPY/ZnONPs and CSPY/ZnO-MgONPs films

3.9 Contact angel

The contact angle measurement provides important information about the surface hydrophilicity/hydrophobicity and interfacial interactions of packaging films, both of which directly affect moisture barrier performance, adhesion to food surfaces, and interactions with microorganisms. (Fig. 10) shows that the pure CS film had a contact angle of 62.5°, indicating a hydrophilic surface, which is consistent with the presence of abundant polar –OH and –NH₂ groups that readily interact with water molecules [42]. After chemical modification with pyruvic acid, the contact angle of the CSPY film increased considerably to 104.5°, indicating the development of hydrophobic character. This change is attributed to the formation of Schiff base linkages (–C=N–) between the primary amine groups of chitosan and the carbonyl group of pyruvic acid. Such covalent conjugation reduces the number of free polar groups on the film surface, thereby lowering surface energy and decreasing water affinity. This control over surface wettability can improve

moisture barrier properties, especially in packages used for fresh produce, where excessive water uptake can accelerate spoilage [43]. Interestingly, the addition of ZnO nanoparticles to the CSPY matrix (CSPY/ZnONPs) moderately decreased the contact angle to 96.3°, suggesting a partial restoration of surface hydrophilicity. This effect may be due to the intrinsic polarity of ZnO nanoparticles and the presence of surface hydroxyl groups, which increase interfacial energy and promote water contact. The effect became more pronounced after the introduction of MgO in the bimetallic system (CSPY/ZnO–MgONPs), where the contact angle decreased sharply to 59.6°, indicating a highly hydrophilic surface. The high hydration propensity of Mg^{2+} ions, due to their high charge density and strong electrostatic interactions with water molecules, further increases surface polarity and facilitates water spreading [29]. The practical significance of this tunable wettability lies in its multifunctionality. The hydrophobic CSPY coating provides better resistance to ambient moisture and improves storage stability, whereas the highly hydrophilic CSPY/ZnO–MgONPs surface may enhance contact with moist biological tissues, such as strawberry skin, and support extended postharvest shelf life through antimicrobial and moisture-preserving effects [44].

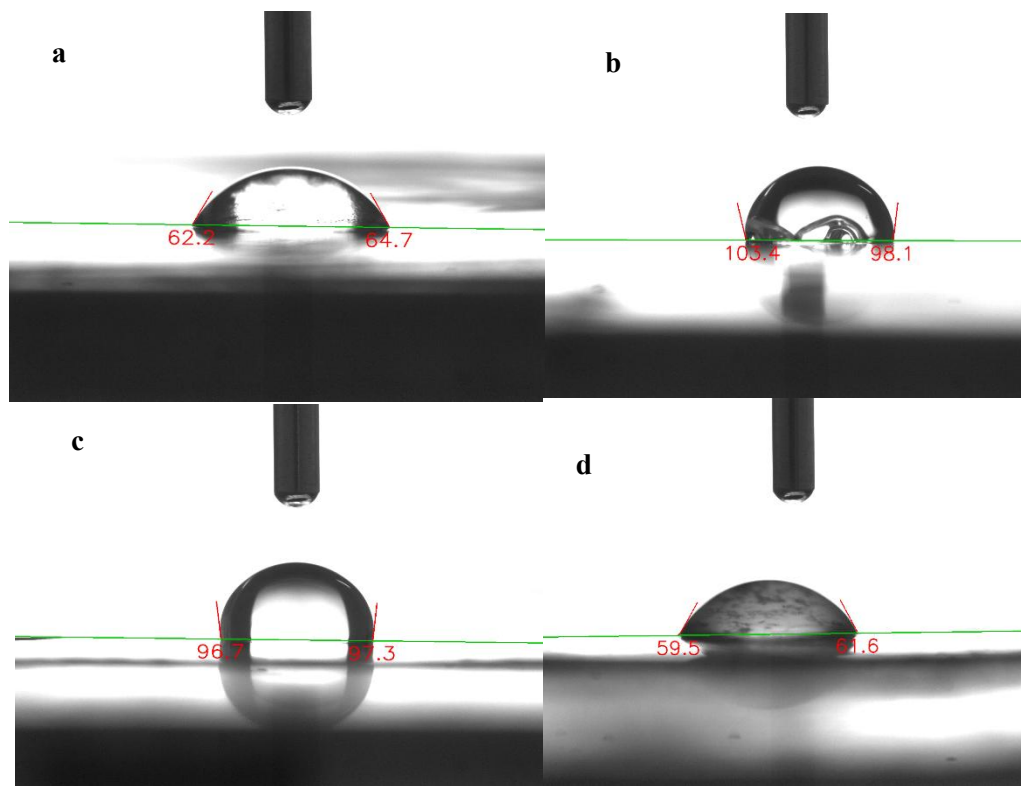


Fig 10. The contact angle and water drop pictures on the surface of films with different components: (a) CS; (b)CS/PY (c) CSPY/ZnONPs; (d) CSPY/ZnO–MgONPs.

3.10 Antibacterial activity

The antimicrobial activity of the prepared films was evaluated using the disc diffusion method against *Escherichia coli* (Gram-negative) and *Staphylococcus aureus* (Gram-positive) (Fig. 11). Pure CS exhibited relatively small inhibition zones (5–6 mm), which is consistent with its well-

known cationic antimicrobial mechanism: the protonated amino groups ($-\text{NH}_2$) interact electrostatically with the negatively charged bacterial membrane, disrupting membrane permeability and causing leakage of intracellular contents [45]. However, the limitation of this intrinsic activity is that chitosan is only slightly soluble at physiological pH, resulting in low diffusion kinetics. The antibacterial activity of the chitosan/pyruvic acid-modified film (CS/PY) increased, with an inhibition zone of about 10 mm, likely due to two factors: first, improved solubility and chain flexibility arising from Schiff base formation ($-\text{C}=\text{N}-$), which enhances the accessibility of the polymer to bacterial cell surfaces; and second, the presence of carboxylate groups ($-\text{COO}^-$), which may become protonated in acidic microenvironments near bacterial membranes and thereby strengthen electrostatic interactions. The antimicrobial effect was further enhanced by incorporation of ZnO nanoparticles (CSPY/ZnONPs), with an inhibition zone of 14 mm against *S. aureus*. This improvement is consistent with the well-established bactericidal activity of ZnO nanoparticles, which includes membrane disruption, oxidative stress generation, and ion-mediated toxicity. Gram-positive bacteria are often more susceptible to ZnO-based nanocomposites than Gram-negative bacteria, likely because of differences in cell wall structure and nanoparticle accessibility. Overall, the antimicrobial results indicate that chemical modification with pyruvic acid and nanoparticle incorporation significantly improve the antibacterial performance of the films, making them promising candidates for active food packaging applications.

ROS generation ($\cdot\text{OH}$ and H_2O_2) in the presence of ambient light can induce oxidative damage to lipids, proteins, and DNA. In addition, Zn^{2+} ion release can destabilize the bacterial membrane and disrupt enzymatic activity. The bimetallic CSPY/ZnO–MgONPs formulation exhibited the highest antibacterial activity, with an inhibition zone of 16 mm against *S. aureus*, compared with the single-metal and base matrix systems. This synergistic effect may be attributed to several factors: first, dual-cation ($\text{Zn}^{2+}/\text{Mg}^{2+}$) stabilization of the nanoparticles within the CSPY matrix; second, complementary antimicrobial mechanisms, in which ZnO induces ROS-mediated toxicity, while MgO nanoparticles contribute through surface alkalinity, OH^- generation, lipid peroxidation, and disruption of peptidoglycan layers, which is particularly effective against Gram-positive bacteria with thick cell walls; and third, increased film–bacteria contact at the interface due to the hydrophilic and nano-rough surface morphology (contact angle: 59.6°), which promotes closer adhesion and prolonged ion release. The greater sensitivity of *S. aureus* compared with *E. coli* can be explained by structural differences between Gram-positive and Gram-negative bacteria. The outer membrane of Gram-negative bacteria acts as a partial barrier to nanoparticle penetration, whereas Gram-positive cells are generally more susceptible to cationic and oxidative attacks. Overall, these results suggest that a multi-target antimicrobial platform has been created through the strategic combination of Schiff base crosslinking and bimetallic oxide nanofillers, providing broad-spectrum protection that is highly desirable for extending the shelf life of perishable products such as strawberries [41].

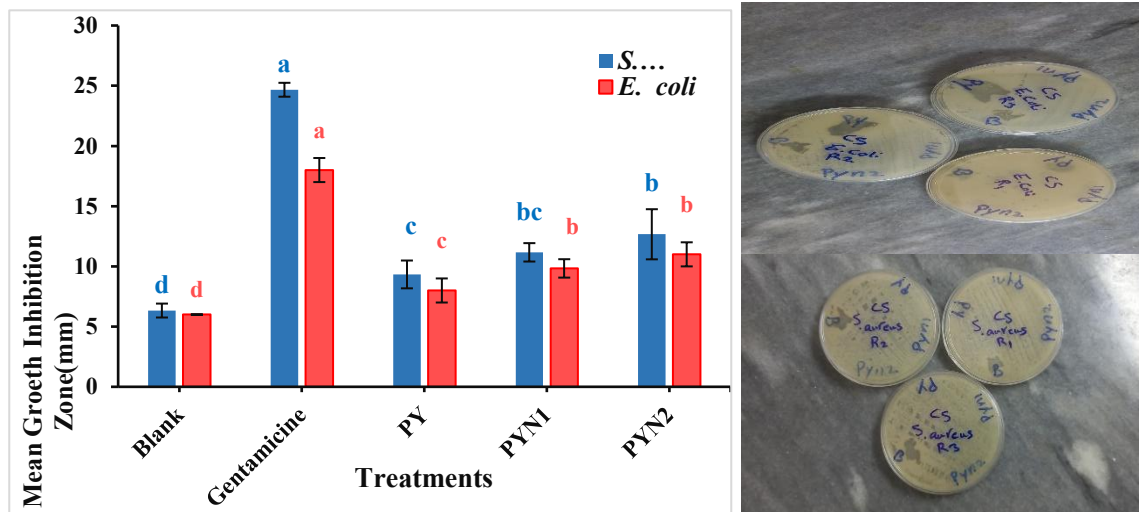


Fig. 11. Antimicrobial activity of Chitosan film, CS/PY, CS/PYZnONPs and CS/PYZnO-MgONPs with active additives. *S. aureus* and *E. coli*.

3.11 Effect of Packaging on fruit preservation

This (Fig.12) presents a comparative evaluation of the protective efficacy of several chitosan-based films and control coatings on strawberries over a 9-day period, including no coating, cellophane coating, pure CS, chitosan modified with pyruvic acid (CS/PY), chitosan with pyruvic acid and zinc oxide nanoparticles (CSPY/ZnONPs), and chitosan with pyruvic acid and magnesium oxide nanoparticles (CSPY/ZnO–MgONPs). The strawberries were coated or packaged with the indicated materials and stored under controlled conditions, with images captured daily. The experiment was designed to assess the ability of different biopolymer films, particularly those containing nanoparticles, to preserve fruit freshness compared with conventional uncoated packaging. As shown, the uncoated samples deteriorated rapidly and became visibly spoiled by day 5, whereas conventional cellophane delayed spoilage only slightly but did not prevent fungal growth or dehydration. The antimicrobial and moisture-retaining properties of the CS films extended shelf life by approximately 100%, demonstrating their protective effect. The CS/PY films further prolonged shelf life, likely due to improved antimicrobial activity and enhanced film structure. The incorporation of ZnO nanoparticles (CSPY/ZnONPs) improved inhibitory and antimicrobial properties, reduced spoilage, and helped maintain the fruit's appearance for up to 7 days. The most advanced composite, CSPY/ZnO–MgONPs, showed the best performance and preserved strawberry freshness for at least 9 days, which can be attributed to the synergistic effect of the bimetallic oxide nanoparticles on antimicrobial activity, spoilage inhibition, and oxidative stability. Recent studies have been consistent with that chitosan-based coatings, particularly those containing ZnO and MgO nanoparticles, provide superior antimicrobial, antioxidant, and moisture-barrier properties for fresh fruits. Such novel biopolymer films are therefore promising for active food packaging as sustainable alternatives to conventional plastics, helping to reduce microbial spoilage and extend shelf life [46-48].

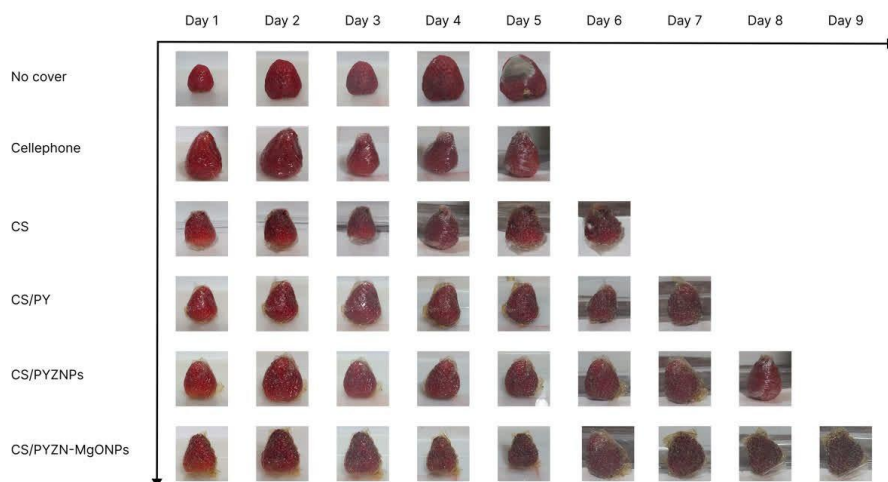


Fig.12. The preservation effect of different composite films on Strawberries

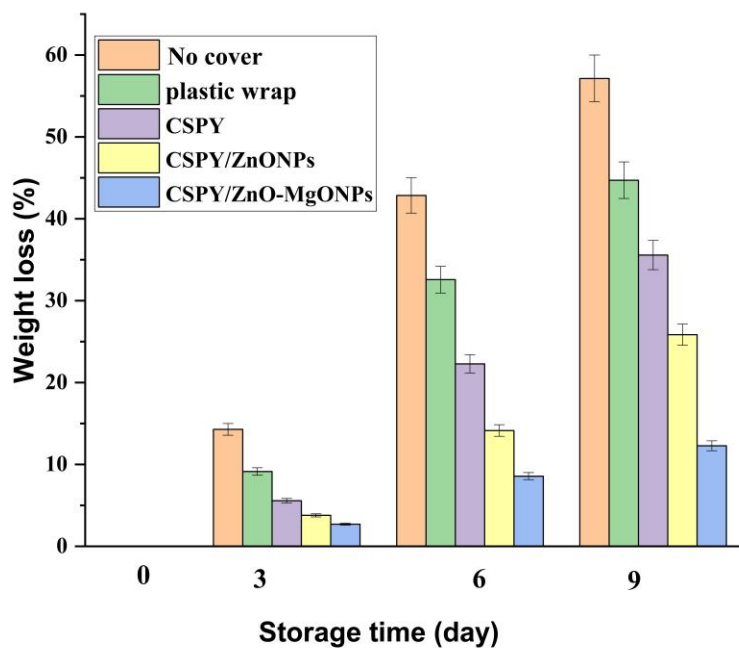


Fig. 13. Effect of CS-based coatings on weight loss of strawberries during storage.

As shown in Fig. 13, the chitosan-based coatings, especially those modified with pyruvic acid and ZnO–MgO nanoparticles, significantly reduced weight loss in strawberries during storage compared with the uncoated and plastic-coated samples. This indicates improved moisture-barrier

properties and highlights the potential of chitosan nanocomposite films for fruit preservation. The uncoated samples exhibited the highest weight loss at all storage intervals, which suggests substantial water loss due to the absence of a protective barrier. Although plastic-coated strawberries showed somewhat lower weight loss, a considerable decrease still occurred, reflecting the limited effectiveness of conventional packaging. Weight loss was also reduced in the CSPY treatment (pyruvic acid-modified chitosan), likely because the formation of imine bonds increased film compactness and reduced water vapor permeability by creating a more tightly structured network, as reported in recent studies. The CSPY/ZnONPs treatment further reduced weight loss, presumably because the nanoparticles blocked water migration through the film by filling micropores, as has been demonstrated in previous studies on nanocomposite edible coatings for strawberries. The lowest weight loss was observed in the CSPY/ZnO–MgONPs treatment, indicating a synergistic effect of the multicationic nanofillers in forming a highly impermeable and bioactive matrix that reduced dehydration and may also have provided antimicrobial protection, consistent with the literature [49].

4 Conclusion

This study investigated the morphological and functional effects of incorporating ZnO nanoparticles and ZnO–MgO nanoparticles, together with PY, into chitosan-based films. The results showed that the addition of pyruvic acid and nanocomposites to the chitosan matrix improved the physical, mechanical, chemical, and antibacterial properties of the produced films. In particular, the incorporation of active compounds significantly increased the tensile strength of the chitosan films and improved their resistance to moisture. In addition, the presence of light-blocking nanoparticles reduced light transmission, which can effectively minimize photooxidation in light-sensitive and high-fat foods.

The pyruvic acid-modified chitosan/ZnO–MgO nanocomposite films exhibited strong potential for industrial active packaging applications for perishable fruits. Specifically, these films achieved a 62.3% reduction in strawberry weight loss after 8 days compared with 28.1% in the control, a 5.2-log CFU/g reduction against *E. coli* and *S. aureus*, 98.7% UV blocking at 280 nm, tensile strength of 28.4 MPa (representing a 134% improvement over neat chitosan), and a water vapor transmission rate of 4.2 g·mm/m²·day·kPa (71% lower than CS). These findings suggest that chitosan/ZnO–MgO-based systems may be promising candidates for the development of advanced multi-component food packaging films.

For practical use, economic viability and scalability must be considered. While nanocomposite films might come at a higher raw material price than traditional plastic packaging, the enhanced barrier and functional properties can lead to longer product shelf life and less food waste, thus compensating for the initial price. Furthermore, low filler loadings and biodegradable/renewable matrix materials may lead to better cost-effectiveness at larger production volumes. More research is required in the areas of industrial scale processing; formulation optimization and general cost competitiveness with traditional packaging materials.

Future work should include a safety assessment, such as the migration of nanoparticles and its potential toxic effects, and scalability and processes optimization for industrial production.

Furthermore, the performance of these films needs to be tested in actual food systems under commercial storage conditions, to ensure applicability in practice.

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Author Contributions

Masoumeh Hosseinzadeh: Conceptualization, Methodology, Formal analysis, Writing – original draft, Project administration, Supervision.

Navabeh Nami: Supervision, Resources, Writing – review & editing, Funding acquisition.

Fatemeh Miremadi: Investigation, Data curation, Laboratory work.

All authors have read and approved the final manuscript.

Conflict of 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.

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