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Original Article

Production of Antimicrobial Chitosan–Pistachio Gum Composite Film for Bio-Bag Fabrication

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ABSTRACT

Functional edible films are thin polymeric layers used in food packaging to enhance product quality and shelf life. In the present study, pistachio gum and chitosan were combined to create a functional edible film. Chitosan was locally produced from fungal biomass of *Aspergillus niger* ATCC 16404 and characterized using Fourier-transform infrared spectroscopy (4000− 400 cm^{-1}). The optimal concentrations of pistachio gum, glycerol (as plasticizer), and emulsifier were found by preparing 18 distinct film formulations. The prepared films were tested for their thickness, tensile strength, elongation at break, tear strength, air permeability, water vapor permeability (WVP), solubility, field emission scanning electron microscopy (FE−SEM), and thermogravimetric analysis (TGA). The incorporation of pistachio gum substantially improved film characteristics, resulting in increased thickness from 0.13 to 0.22 mm, tensile strength up to 1.40 MPa, elongation at break of 88.07%, and tear strength of 1.73 N, suggesting more flexibility and mechanical stability. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of film solutions were evaluated against clinical multidrug-resistant (MDR) *Escherichia coli* and *Staphylococcus aureus* using the broth microdilution method. The chitosan-gum combination exhibited better antibacterial activity than chitosan alone. The antifungal activity was studied against *A. niger*; The chitosan-gum combination resulted in complete inhibition of *A. niger* growth. Additionally, the prepared chitosan-gum films exhibited lower solubility in water, acidic and alkaline solutions. Bags were successfully fabricated from chitosan- gum films. The incorporation of gum led to a more compact morphology, enhanced sealing performance, and improved functional performance.

Keywords: chitosan, pistachio gum, antimicrobial film, antifungal activity, preservation.

1 Introduction

Environmentally friendly films are important types of food packaging. This type of film uses natural, inexpensive, and biodegradable materials to protect the environment from pollution, which poses a threat to the lives of living organisms [1]. Natural films can act as a good carrier of antimicrobial and antioxidant compounds, which are important for preserving the quality and the shelf-life of food [2]. Chitosan is produced by partial deacetylation of chitin. The cationic nature of chitosan facilitates interactions with negatively charged molecules [3]. Chitosan has three effective functional groups: an amine group at the second carbon atom and two hydroxyl groups at the third and sixth carbon atoms, which enable it to form ionic bonds [4]. Chitosan is a non-toxic and biodegradable compound used to preserve food through the formation of coverings around it to maintain its quality and extend the shelf life [5]. The *Atlantic pistachio* is a medicinal plant of the Anacardiaceae family, with a wide geographical range. The subspecies *P. atlantica subsp. kurdica* is indigenous to the Kurdistan Region of Iraq, particularly thriving in the Zagros Mountains [6]. Pistachio gum is obtained through incisions made in the trunks of pistachio trees. The gum has anti-inflammatory, anti-arthritic, and analgesic properties. Traditionally, gum has been used in many countries to treat inflammatory diseases such as Crohn's disease and ulcerative colitis, diarrhea, and dysentery. Gum and its preparations have a wide range of applications, mainly in the perfume, cosmetics, and food industries. Gum consists of volatile oil, water-soluble polysaccharide, lipophilic terpenes, and insoluble substances. Polysaccharides consist of arabinose, galactose, xylose, and glucuronic acid [7]. The availability, safety for human consumption, therapeutic values, and low cost of pistachio gum have caught our interest in the incorporation of this gum with chitosan to produce antimicrobial and eco-friendly bio-bags to improve food preservation and safety.

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2 Materials and Methods

2. 1 Production and Characterization of Chitosan

The chitosan was produced using *Aspergillus niger* ATCC 16404 obtained from Sigma-Aldrich, USA. The following steps were taken: A spore suspension was prepared using the method described by [8], and spores number was adjusted to 10^7 spore/mL. 1 mL of spore suspension was added to 100 mL of yeast peptone glucose medium then incubated at 25°C under submerged conditions at 100 rpm for 3 days. The mycelium was then filtered to obtain the fungal mycelium, which was washed twice with distilled water and dried at 65°C until a constant weight was reached. The dried mycelium was then thoroughly ground and suspended in a 1 M NaOH solution and autoclaved at 121°C for 15 minutes to partially deacetylate chitin groups and hydrolyze proteins and nucleic acids. The insoluble alkaline precipitate was collected after centrifugation at 12,000 × *g* for 15 minutes, then washed with distilled water and centrifuged again. The process was repeated until a neutral pH was reached. Then treated with acetic acid solution at a concentration of 20 mL/L at 95°C for 8 hours, then centrifuged at 12,000 × *g* for 15 minutes. The filtrate was then adjusted to a pH of 10 using 2 M NaOH solution and centrifuged at 12,000 × *g* for 15 minutes. The chitosan precipitate was washed with distilled water and 950 mL/L ethanol at a ratio of 1:20 (w/v), and acetone at a ratio of 1:20 (w/v). Finally, the extracted chitosan was dried at 60°C until a constant weight was reached [9]. The degree of deacetylation was estimated using the method described by [10]. The chitosan was analyzed by FTIR spectrometer and compared with the spectrum of standard chitosan according to [11].

2. 2 Preparation of Films

Chitosan solution was prepared according to the method described by [12] (with some modifications). This involved dissolving 2 g of chitosan in 100 mL of citric acid at concentration of 20 mL/L with continuous stirring at 45°C until chitosan was dissolved. The pH was adjusted to 5.7 using 1 N sodium hydroxide, then glycerol was added in varying proportions (2.5, 5, and 10 mL) to estimate the optimal percentage as a plasticizer for the treatments (T1, T2, T3, and T4). Then, different concentrations of pistachio gum were emulsified in Tween 80 and added at the following proportions (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.5, and 2 g) to each of the following formulations (T5, T6, T7, T8, T9, T10, T11, T12, T13, T14, T15, T16), respectively, as shown in Table 2. Then the effect of the emulsifier on the properties of the film was studied. Therefore, lecithin and lecithin with Tween 80 (T18, T17) were used. The resulting solution was filtered using a polyester cloth to remove undissolved particles. The mixture was poured into a 22 cm diameter Teflon tray and left to dry in an electric oven at 45°C for 24 hours.

2. 3 Viscosity Measurement of Film Solutions

The viscosity of the selected treatment film solutions (T18, T17, T9, T1) was determined using a Brookfield viscometer with spindle No. 2 at 100 rpm at room temperature.

2. 4 Films´ Resistance to Solubility in Water, Acidic, and Alkaline Solutions

The solubility of the films in water, acidic, and alkaline solutions was measured according to the method of [13]. The films were cut into small pieces and dried at 100°C for 24 hours until a constant weight was reached. The dried slices were then immersed in 100 mL of distilled water, 100 mL of hydrochloric acid (HCl, pH 4.0), or 100 mL of sodium hydroxide (NaOH, pH 10.0) for 24 hours. Then they were removed from the solutions and re-dried at 100°C for 24 hours. Final weights were recorded, and the percentage of resistance was calculated as follows:

$$\% \text{ Resistance} = [(\text{initial weight} - \text{final weight}) / \text{initial weight}] \times 100 \quad (1)$$

2. 5 Characterization of films

Physical properties including color and transparency were determined visually. Whereas, odor was assessed by direct smelling at room temperature. Moisture content was estimated using the method described by [13]. While film thickness was measured using a digital micrometer (Mitutoyo, model: PK-1012E, Japan). The measurement was performed at 7 different locations chosen randomly from the periphery to the center. Tensile strength and elongation, air and water vapor permeability, tear strength were conducted at the Ministry of Industry and Minerals/National Packaging Center.

2. 6 Morphological Characterization of Films

The surface morphology of the chitosan and chitosan with gum films was examined first with 40 × magnification using a light microscope. The dimensions of the film samples used for testing were 2 × 2 cm., followed by Field Emission Scanning Electron Microscopy (FE-SEM).

2. 7 Thermogravimetric (TGA) Analysis

Thermogravimetric analysis (TGA) was carried out using Shimadzu Simultaneous TGA/DTA Analyzer (DTG-60H). An 11 mg sample was weighed and heated from 10 °C to 600 °C with a temperature ramp rate of 10 °C/min.

2. 8 Determination of the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Film Solutions

MIC and MBC were determined according to the standards provided by the CLSI [14]. The procedure involves preparing two-fold dilutions of the chitosan, chitosan-gum using 96-well microtiter plate. Samples were prepared at a final concentration of 25, 12.5, 6.25, 3.125, 1.5625, 0.78125 mg/mL. Then, each well was inoculated with a microbial suspension (0.5 McFarland turbidity standard). Clinical multi-drug resistant *Escherichia coli and S. aureus* were used as the test bacteria. After well-mixing, the inoculated 96−well microtiter plates were incubated at 35°C for 24 hours, by measuring the optical density (OD) at 630 nm using a spectrophotometer, the MIC was determined. The growth inhibition was determined by the formula,

Percentage of Inhibition = (OD of Control – OD of Test)/ (OD of Control) × 100% (2)

MBC was determined by sub-culturing a sample from wells on blood agar, the absence of microbial growth after 24 hours of aerobic incubation was recorded as MBC.

2. 9 Evaluation of the Antifungal Activity of Film Solutions

The antifungal activity was determined using plates containing PDA medium supplemented with different concentrations of chitosan solution (250, 500, 750, and 1000 µg/mL), in parallel, chitosan-gum solution at the same concentrations. A 5 mm diameter mycelial disc of *A. niger* was aseptically placed at the center of each Petri dish. Control plates consisted of PDA medium without any additives. The growth was measured after incubation at 25°C for 5 days, when mycelial growth in the control plate reached the edge of the Petri dish, following the method described by [15]. The percentage of fungal growth inhibition was calculated using the following equation:

$(\%I) = (C-T)/C \times 100$ (3)

where C is the fungal growth in the control treatment, T is the fungal growth in the different treatments, and I is the amount of inhibition [16].

2. 10 Preparation of Bio-bags

An attempt was conducted to fabricate bio-bags from chitosan film (T1) and chitosan with gum film (T9) by cutting the films into uniform dimensions and sealing the edges using a heat sealing device.

2. 11 Statistical Analysis

Data were analyzed using one-way analysis of variance (ANOVA) using **completely randomized design (CRD).** Each treatment was performed in **triplicate** and Duncan's test was applied to compare the means and statistical significance was accepted at a probability level of $P \leq 0.05$.

3 Results and Discussion

3. 1 Chitosan Production and Characterization

Fungal biomass was harvested after 72 h of cultivation, prior to the onset of sporulation, as maximum chitosan yield is achieved during the late logarithmic growth phase [17]. In the present study, *A. niger* ATCC 16404 yielded 20 g of chitosan per 100 g of dry mycelial biomass, which is considered a moderate yield compared to reported range for fungal chitosan production [9]. As reported by [18], the chitosan production and content depend on the fungal strain, age, culture medium, and cultivation conditions.

The degree of deacetylation (DD) of the produced chitosan was 86%. This value falls within the reported range of 56–99% for chitosan [10]. Chitosan obtained from the fungal cell wall exhibits high DD values. Increasing the degree of deacetylation is associated with enhanced antimicrobial activity, primarily due to a higher electrostatic interaction with microbial surfaces [18].

The spectra of FTIR spectroscopy at 400–4000 cm^{-1} showed characteristic absorption bands of functional groups, as detailed in Table 1. The FTIR analysis confirmed successful production of high-quality chitosan.

Table 1: FTIR analysis of chitosan.

	Wave number (cm^{-1})	
Functional groups	**produced chitosan**	**Pavia *et al.*, [19]**
-OH – N-H	3408.35	3500-3100
-C-H alkanes	2922.5	3000-2850
C = O amide	1647.38	1680-1630
-C-O-C stretch	1078.81	1300-1000

3. 2 Viscosity of Films Solutions

The addition of pistachio gum and emulsifiers (Tween 80 or lecithin) decreased the viscosity of solutions significantly (T9, T17, and T18) compared with the chitosan solution (T1) as presented in Figure 1. The reduction in viscosity may be due to the role of emulsifiers to decrease interfacial tension, decreasing the resistance to flow, resulting in a solution more suitable for film casting and packaging applications [20], indicating the beneficial effect of Pistachio gum and emulsifier incorporation. Reduced viscosity is advantageous in edible film casting, as it enhances moldability and uniform film formation [21]. In contrast, the absence of emulsifiers in T1 led to higher viscosity due to the capability of chitosan to form a strong network and very viscous solution [22].

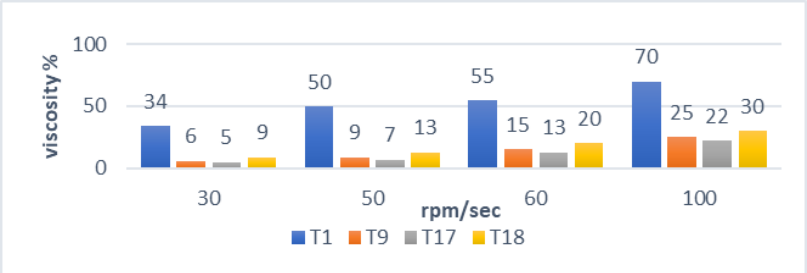

Figure 1: Effect of pistachio gum and emulsifiers on the viscosity (mPa s) of chitosan solutions.

3. 3 Film Production

The optimal concentrations of glycerol, pistachio gum, and emulsifier were determined through the preparation of 18 different formulations in three stages as summarized in Table 2. First stage demonstrated that 2.5 mL of glycerol provided the most favorable film elasticity. Based on sensory evaluation, the ratio of 0.5 g of gum (T9) was adopted as the best ratio in terms of edibility. In the third stage of screening, the importance of emulsifiers was studied. The results showed that the absence of an emulsifier meant that complete dissolution of the pistachio gum in the chitosan could not be achieved, thus limiting the functional benefits of gum incorporation. Finally, four formulations were selected for films production. Consequently, Tween 80 and lecithin were evaluated as emulsifiers, and the films were prepared using treatments T18, T17, T9, and T1.

Table 2: Formulation composition of edible film.

Treatments	Chitosan (g)	Glycerol (ml)	Gum (g)	Tween 80 (ml)	Lecithin (g)
T1	2	2.5	0	0	0
T2	2	5	0	0	0
T3	2	10	0	0	0
T4	2	15	0	0	0
T5	2	2.5	0.1	2	0
T6	2	2.5	0.2	2	0
T7	2	2.5	0.3	2	0
T8	2	2.5	0.4	2	0
T9	2	2.5	0.5	2	0
T10	2	2.5	0.6	2	0
T11	2	2.5	0.7	2	0
T12	2	2.5	0.8	2	0
T13	2	2.5	0.9	2	0
T14	2	2.5	1.0	2	0
T15	2	2.5	1.5	2	0
T16	2	2.5	2.0	2	0
T17	2	2.5	0.5	0	1.0
T18	2	2.5	0.5	2	1.0

As shown in Figure 2a-d, significant differences in film morphology and handling properties were observed among treatments. Films prepared with Tween 80 were smooth, and easy to handle, whereas films containing lecithin exhibited incomplete resin dissolution and poor structural integrity. The simultaneous use of two emulsifiers resulted in complete failure of film formation. Based on these findings, T1 was selected as the control, and T9 was identified as the optimal formulation for subsequent analyses.

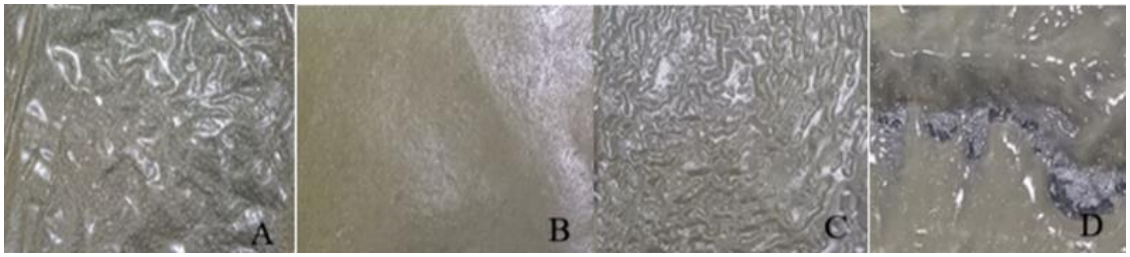

Figure 2: Appearance of chitosan –based films prepared with different formulations:(A) chitosan (T1), (B) chitosan with gum with Tween 80 (T9), (C) chitosan with gum and lecithin (T17), (D) chitosan with gum and mixed emulsifier (T18).

The incorporation of pistachio gum and emulsifiers facilitated folding, shaping, and handling of the film. In contrast, chitosan films were adhesive and difficult to handle, maybe due to extensive hydrogen bonding within the polymer network (Figure 3A-B). As found by [23], chitosan possesses a lot of reactive amino side group that allow for chemical modification and the creation of a wide range of useful derivatives. Films containing pistachio gum and Tween 80 were less sensitive to moisture exposure (Figure 3C), as the emulsifier enhanced film stability under humid conditions. These results indicate that the incorporation of pistachio gum and Tween 80 into chitosan improved the mechanical and handling properties like folding and bending without cracking or tearing. While previous studies reported increased brittleness in chitosan films prepared with other gums [24-26]. Thus, gum plays an important role in enhancing film processability and usability in packaging applications.

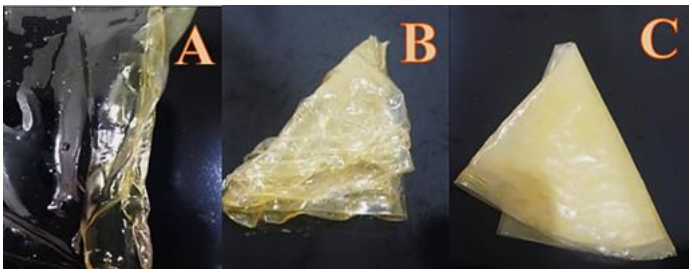

Figure 3: Effect of high relative humidity on the folding and bending of: (a) and (b) chitosan (T1), (c) chitosan with gum (T9).

3. 4 Films´ Resistance to Solubility in Water, Acidic, and Alkaline Solutions

The incorporation of pistachio gum into the chitosan solution enhanced film resistance to solubility in water, acidic, and alkaline solutions (Figure 4). This improvement may be attributed to the addition of emulsified pistachio gum with Tween 80. Low solubility of film is a critical and desirable characteristic for edible films used in food preservation as reduced solubility limits water vapor permeability and contributes to the preservation of food freshness, especially during refrigerated or frozen storage [27].

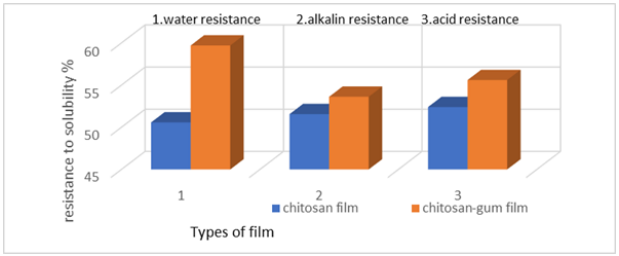

Figure 4: Films resistance to Water, Acidic, and Alkaline Solubility.

3. 5 The physical properties and moisture content of films

The physical properties of films including smell, color, and transparency are presented in Table 3, all films were odorless. The addition of gum resulted in a transition of films from transparent to opaque. Opacity is a desirable feature in food packaging, as it protects sensitive food items from photo-oxidation and damage to vitamins and fats caused by exposure to ultraviolet (UV) light. Moisture content is an important factor influencing the stability and flexibility of edible films. The moisture content significantly increased in gum-containing films (T9) compared to chitosan without gum films (T1) as shown in Table 3. As glycerol which is a highly hydrophilic plasticizer along with Tween 80 and gum, this triple assembly distributed within the chitosan network helped maintain the film moisture content and provided a flexible film, which decreased film brittleness, increased elasticity and tear resistance.

Table 3: The physical properties and moisture content of the films.

Treatments	Odor	Color	Transparency	moisture content
Chitosan film (T1)	Odorless	Amber	Transparent	11.77 ± 0.45^{b}
Chitosan-gum film (T9)	Odorless	Amber	Opaque	16.78 ± 0.62^{a}

*Different superscript letters within each column refer to a significant difference ($p \leq 0.05$).

3.6 The thickness, Barrier, and Mechanical properties of Films

A balance of thickness, barrier and mechanical properties is required for edible films. As shown in Table 4, there were significant differences in mechanical performance of the films before and after the addition of gum. The incorporation of pistachio gum increased film thickness, perhaps because gum molecules filled the cavities in the chitosan network. Increasing thickness within acceptable limits is very important, as it improves isolation against external environmental factors. Film thickness plays an important role in determining film quality, as it directly affects the mechanical and barrier properties of film [13]. According to the Japanese Industrial Standard [28], the maximum acceptable film thickness is 0.25 mm. The results showed that the tensile strength of gum-containing films increased significantly from 0.27 MPa (T1) to 1.40 MPa (T9). The measured tensile strength values complied with JIS standard. Films containing gum showed higher elongation at break (88.07%), exceeding the minimum requirement of 70% according to [29]. The improvement in elongation is due to increased plasticity, allowing the films better handling, folding, bending, and stretching [30]. Tear resistance was also increased from 1.32 N to 1.73 N, which is beneficial for preventing tearing during handling, storage, and transportation. Additionally, (T9) shows reduced water permeability, ensuring effective food protection.

Table 4: The thickness, barrier, and mechanical properties of films.

Property	Chitosan film (T1)	Chitosan-gum film (T9)
Thickness (mm)	0.13 ± 0.01^{b}	0.22 ± 0.02^{a}
tensile strength (MPa)	0.27 ± 0.02^{b}	1.40 ± 0.05^{a}
elongation at break (%)	82.00 ± 2.1^{b}	88.07 ± 2.4^{a}
Tear resistance (N)	1.32 ± 0.06^{b}	1.73 ± 0.08^{a}
air permeability ($cm^3\ m^{-2}\ s^{-1}\ Pa^{-1}$)	impermeable	impermeable
WVP ($\times 10^{-9}$ $kg \cdot m^{-2} \cdot Pa^{-1} \cdot s^{-1}$)	1454 ± 20.1^{b}	280 ± 3.8^{a}

*Different superscript letters within each column refer to a significant difference ($p \leq 0.05$)

3. 7 Morphology and Microstructure of Films

As shown in Figure 5A, chitosan films when examined by light microscope with 40x magnification exhibited fine lines and cavities. In contrast, films containing gum appeared without visible cavities or lines Figure 5B.

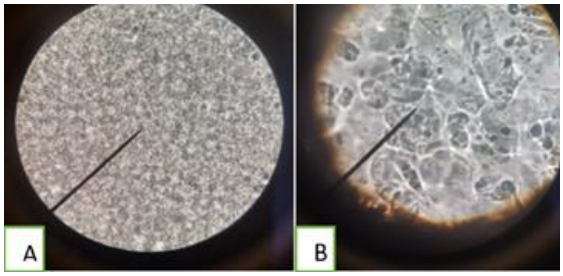

Figure 5: Surface morphology (40X) of (A) chitosan and (B) chitosan with gum films.

This morphological uniformity was confirmed by Field Emission Scanning Electron Microscopy (FE-SEM) which revealed distinct differences in the morphology of chitosan (Figure 6) and chitosan-gum (Figure 7) films. The pure chitosan film exhibited a smoother and more homogeneous surface, indicating uniform film formation. In contrast, the composite film displayed a rougher and denser morphology characterized by the presence of gum aggregates that linked at their ends to surrounding chitosan. This may suggest strong intermolecular interactions, particularly hydrogen bonding between the hydroxyl groups of the gum and the amino/hydroxyl groups of chitosan. At higher magnifications, the composite film showed compact nanoscale domains (46.85 nm, 70.80 nm, and 71.75 nm) implying that the gum was successfully dispersed acting as a nanostructured filler and polymeric bridges as observed as interconnected clusters, suggesting excellent compatibility between the two biopolymers and the absence of significant phase separation. The denser nanostructured surface observed in FE-SEM correlates with improved heat-sealing, mechanical and handling properties, water soluble resistance, transition of films from transparent to opaque, increases of thickness, and thermal stability, as nano-scale fillers restrict the mobility of the polymer chains. Better insulating film is essential for ensuring film quality during food storage, as reported by [31].

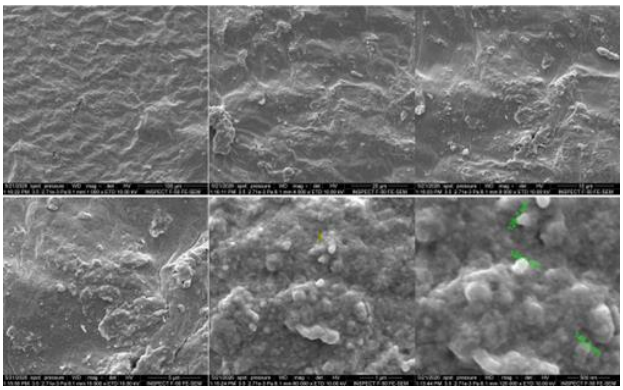

Figure 6: FE-SEM surface micrographs of the chitosan film at various magnifications.

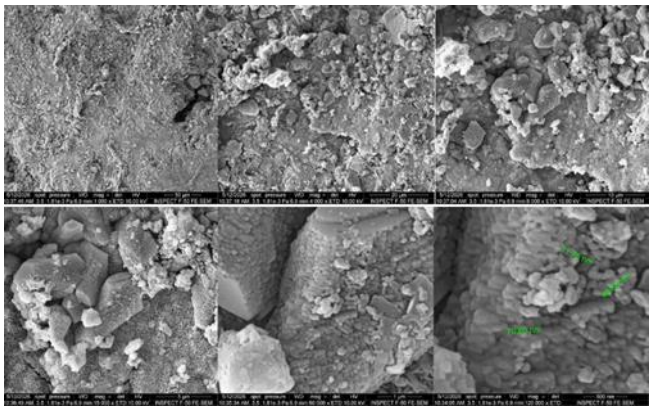

Figure 7: FE-SEM surface micrographs of the chitosan-gum composite film at various magnifications.

3.8 Thermogravimetric Analysis (TGA)

TGA plots of chitosan (Figure 8) and chitosan –gum composite film (Figure 9) show that chitosan film exhibited higher thermal stability. The thermal degradation temperature was lowered in chitosan –gum film, while the total mass loss was increased. The initial weight loss detected below 120 °C in both films was attributed to the water loss. The final degradation stage above 400 °C was associated with decomposition of residual organic constituents. The lower degradation temperature in chitosan-gum composite film could be attributed to the chemical nature of the pistachio gum and the action of Tween 80 that acted as an emulsifier, preventing phase separation and gum precipitation. These findings aligned with the heat-sealing performance, where the composite required lower temperatures for sealing.

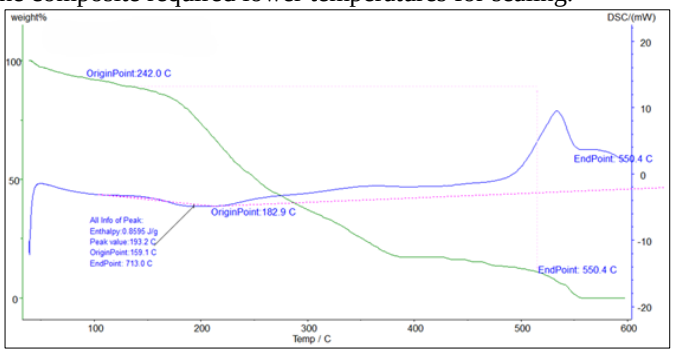

Figure 8: The thermal behavior of chitosan film.

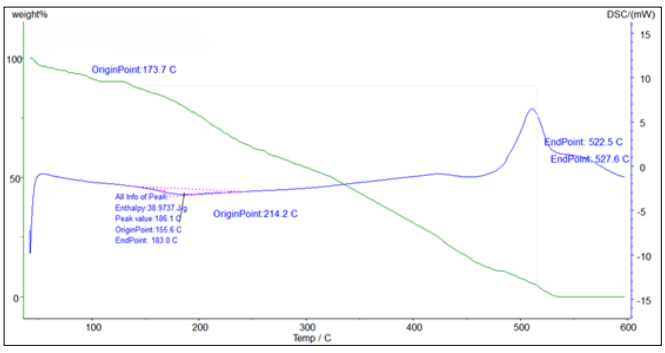

Figure 9: The thermal behavior of chitosan-gum film.

3. 9 Antibacterial Activity of Chitosan-Gum Film Solutions

The incorporation of gum into the chitosan solution significantly enhanced the inhibitory effect of the solution against bacteria Table 5. The lowest MIC was observed for chitosan-gum solution (3.125 mg/mL) and MBC was (6.25 mg/mL) against *Staphylococcus aureus*, whereas the MIC was (12.5 mg/mL) and MBC was (25 mg/mL) against *Escherichia coli*. The antibacterial effects may be attributed more to the action of pistachio gum rather than the synergistic effects of chitosan with gum. The observed antibacterial activity may be related to the presence of antimicrobial compounds in pistachio gum, particularly phenolic constituents and boswellic acids, which have been reported to disrupt bacterial cell membranes and inhibit microbial growth [32,33].

Table 5: MIC and MBC of chitosan, chitosan-gum, and Pistachio Gum solutions.

Anti-bacterial agents	*E. coli MIC mg/mL*	*E. coli MBC mg/mL*	*S.aureus MIC mg/mL*	*S. aureus MBC mg/mL*
Chitosan	12.5	25	12.5	25
Chitosan-gum	6.25	12.5	3.125	6.25
Pistachio Gum	6.25	12.5	3.125	6.25

3.10 Antifungal activity of chitosan-gum film solutions

The antifungal activity of chitosan with gum film solutions against *A. niger* is shown in Table 6. The incorporation of pistachio gum at concentrations of 250, 500, 750, and 1000 µg/mL into PDA medium significantly enhanced fungal growth inhibition compared with both the control treatment and chitosan without gum solutions. Complete inhibition (100%) of A. niger growth was achieved at a gum concentration of 750 µg/mL. The strong antifungal effect of the chitosan-gum combination may be attributed to the presence of phenolic compounds, essential oil, and boswellic acid within the gum, which are known to interfere with fungal cell wall integrity and metabolic processes [34]. These results showed the potential of the chitosan with gum as an effective antifungal for food preservation application.

Table 6: The antifungal activity of chitosan and chitosan-gum solutions against *A. niger* growth.

chitosan and Chitosan-gum concentration (µg/mL)	250	500	750	1000
% inhibition by chitosan solution	44.0 ±2.1^{c}	76.0 ±3.0^{b}	81.0 ±2.5^{b}	87.0 ±3.2^{b}
% inhibition by chitosan-gum solution	67.0 ±2.8^{b}	87.0 ±3.1^{b}	100.0 ±0.0^{a}	100.0 ±0.0^{a}

*Different superscript letters within each column refer to a significant difference ($p \leq 0.05$)

3.11 Preparation of bio-bags from films

The sealing of film edges for fabrication of bags failed completely when chitosan films (T1) were used. Chitosan film exhibited melt resistance when exposed to heat, preventing adequate softening and adhesion during the sealing process and the same results were reported by [35]. while sealing was successfully achieved when chitosan-gum films (T9) were used as shown in Figures 10A –C. The enhancement of meltability, thermal softening, and plasticity upon heat exposure may be due to the presence of gum that ensures adequate sticking strength between film edges. Such effective sealing is important for good insulation from the external environment and contributes to improved food shelf life. To evaluate the seam strength of the fabricated bio-bags, dry food models (flour and rice) were packed in fabricated bags as shown in Figure 10. These materials were selected specifically to test the ability of bags to contain fine particulate foods and to confirm the mechanical reliability of the heat-sealed edges. The results showed that the bags were successfully sealed without any leakage. Previous studies have reported the same results in chitosan film performance when blended with certain polysaccharides. [24] reported that addition of Tara gum resulted in brittle chitosan film, while [25] and [26] observed similar brittleness with Arabic gum incorporation, limiting their applicability in edible film preparation. Many studies have reported that chitosan-based films show great potential for the preservation of foods, but their antibacterial and antioxidant effectiveness is limited [36]. So the bio-bags prepared in this study offer additional advantages, including therapeutic and antimicrobial properties besides its biodegradability, edibility, reducing environmental pollution associated with plastics, and possess antimicrobial properties [37]. The addition of gum reduced light transmission by making the film opaque, protect the packaged food from oxidative reactions that cause spoilage [38].

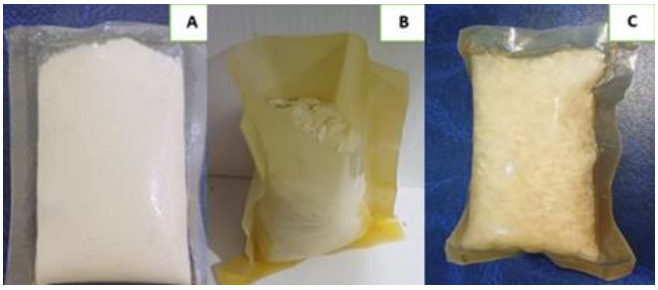

Figure 10: The fabrication of bio-bags using chitosan-gum film (T9) (A) filled with wheat flour (B & C) filled with rice.

Conclusions

Pistachio gum was incorporated into chitosan solution to produce novel antimicrobial bio-bags. The incorporation of pistachio gum significantly enhanced the mechanical, handling, and antimicrobial properties of films, making them a promising

option for food preservation. The most significant benefit of adding the gum was the improvement of heat sealing. This was attributed to the decrease in the glass transition temperature (Tg). The prepared bio-bags in this study offer a bioactive alternative to plastic packaging, thereby bridging the gap between environmental safety and food preservation.

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Conflict of interests

There are no conflicts of interest to declare.

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