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ORIGINAL ARTICLE

Enhancing chitosan-zein films with *Bergenia ciliata* extracts toward smart food packaging

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Abstract

This research aims to develop chitosan-zein protein films supplemented with *Bergenia ciliata* (Bc) extract, a traditionally important medical herb of Himalayan origin. The film's physicochemical, mechanical, antioxidant, and antimicrobial properties were systematically explored. The opacity of chitosan film increased from 2.42 ± 0.97 to 10.32 ± 1.44 upon introducing zein (Z) protein in chitosan (Cs) in a 1:2 ratio (w/w); conferring enhanced UV-blocking attributes. Incorporating *B. ciliata* extracts in the chitosan-zein film (Cs-Z-Bc) under optimized conditions further increased the opacity to 16.27 ± 1.03 without compromising the tensile strength. The α-diphenyl-β-picrylhydrazyl scavenging activity of the Cs-Z-Bc film was found to be 97.07 ± 1.09%. Additionally, these optimized films displayed significant antimicrobial efficacy, with zones of inhibition of 11.4 mm measured for gram-positive strains like *C. subtilis* and *S. aureus* and 11.2 mm and 11.1 mm for *E. coli* and *K. pneumoniae* (gram-negative) bacterial strains. The film also showed excellent biodegradable properties. The shelf life study of Himalayan cheese was significantly increased when wrapped with the film. These findings suggested that *B. ciliata* extract-fortified chitosan-zein films can be an excellent food packaging material.

Highlights

- Developed chitosan-zein protein films fortified with *Bergenia ciliata* extract, a medicinal herb from the Himalayas.
- Enhanced UV-blocking properties, with film opacity increasing from 2.42 to 16.27 upon adding zein and *Bergenia ciliata* extract.
- The film maintained the tensile strength, excellent biodegradability, and high antioxidant and antimicrobial activity.

Keywords Bioactive film · Food packaging · Phytochemical screening · Chitosan · Zein protein

Introduction

Food packaging protects food from adverse environmental conditions and extends its shelf life. Traditional petroleum-based packaging materials, predominantly plastics, pose serious environmental concerns due to their non-biodegradable nature (Wang et al. 2023) and produce toxic gases when burned. They persist in the environment for hundreds or thousands of years due to their stable polymer chains. Even when they degrade, they break down into microplastics that contaminate soil, water, and air, harming ecosystems and human health (Bhardwaj et al. 2024). Therefore, the increasing environmental concerns associated with synthetic food packaging materials have driven the search for biodegradable alternatives such as chitosan, cellulose, starch, and zein (Cheng et al. 2024). Chitosan, a polysaccharide derived

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from chitin, is in high demand among these polymers because of its safety, biodegradability, non-toxicity, biocompatibility, cheapness, and readily availability (Jiménez-Gómez and Cecilia 2020). It is inherently biodegradable due to the presence of glycosidic linkages that can be broken down by microbial enzymes such as chitinases and glucosaminidases. It decomposes into non-toxic oligosaccharides and monomers, which can be further assimilated by microorganisms, making it an environmentally safe alternative to conventional plastics (Bagheri-Khoulenjani et al. 2009). In addition to this, chitosan has potent antimicrobial and metal-chelating properties (Flórez et al. 2022). Due to its rich chemistry and exceptional properties for forming thin films, chitosan has been extensively researched for applications, primarily in the food industry. However, its poor water barrier properties, low radical scavenging, and low mechanical strength limit its application to a certain extent.

Zein, a hydrophobic corn protein, is a promising material for food packaging due to its properties, such as biodegradability and renewability, and has been approved by the Food and Drug Administration. Zein degrades through enzymatic hydrolysis and microbial action (Helgeson et al. 2009). Studies have shown that zein-based films break down in soil and aqueous environments, gradually losing their structural integrity over time without leaving harmful residues. However, its inherent hydrophobicity and limited mechanical properties limit its application as a sole component in packaging material. Chitosan has demonstrated excellent synergy with zein for developing flexible and functional films with tunable properties for various applications (Zhang et al. 2019). Biodegradation studies have shown that zein-chitosan films degrade significantly faster in soil and composting conditions compared to synthetic polymers, making them an environmentally sustainable alternative for reducing plastic waste in food packaging (Chawla et al. 2021). Glycerol as a plasticizer has been used to decrease the brittleness and oxygen barrier of chitosan and zein film (Li et al. 2022). Nevertheless, researchers are focusing on enhancing the properties and functionality of chitosan and zein films by incorporating additives derived from polyphenol-rich plants. Common additives used in films include essential oils such as summer savory, cinnamon, rose, thyme, and black pepper. Plant extracts with potent antibacterial and antioxidant properties have also been increasingly utilized to enhance the functionality of films and have garnered considerable attention in recent years (Cui et al. 2020). Therefore, the extract of medicinal plants or essential oils can be incorporated into zein-chitosan film to effectively combat the spoilage and extend the shelf life of food (Anumudu et al. 2021).

Bergenia ciliata (Bc), a medicinal plant belonging to the family Saxifragaceae, is an evergreen perennial herb found in South Asian countries like Nepal, Bhutan, Pakistan, Afghanistan, and India (Kunwar et al. 2023). It is traditionally used to cure various ailments, such as the dissolution of kidney and gallbladder stones (Koul et al. 2020). In the Dolpa region of Nepal, *Bergenia rhizomes* are commonly used to treat fractured bones, fever, wounds, vomiting, fresh cuts, diarrhea, pulmonary infections, and cough (Kumar et al. 2002). The extract of *B. ciliata* is enriched with various polyphenols, including paashaanolactone, catechin, berginin, gallic acid, pyrogallol, rutin, etc. (Dhalwal et al. 2008), making it a good antioxidant and UV protector (Singh et al. 2017). These polyphenolic compounds also act as natural cross-linking agents, improving the tensile strength and flexibility of the films (Rani et al. 2021). Therefore, incorporating *B. ciliata* extract into the chitosan-zein polymer matrix would be a promising strategy for developing eco-friendly, active food packaging with excellent antimicrobial activity, antioxidant properties, and UV barrier activity. Furthermore, during the film-forming process, the active hydroxyl group of polyphenols present in the extract of the *B. ciliata* could interact with protein matrices through non-covalent interactions like hydrogen bonding and electrostatic interactions, or possible covalent linkages like enzymatic or alkaline methods, producing a protein-polyphenol conjugate (Quan et al. 2019). The protein-polyphenol conjugate can enhance the antioxidant properties, thermal stability, and mechanical strength of the film, while its bioactive components contribute to antimicrobial efficacy (Baba et al. 2021). This synergistic interaction results in a biodegradable, functional film with enhanced mechanical and barrier properties, making it suitable for applications in food packaging and biomedical fields.

In this study, utilizing the properties of *B.ciliata*, Cs, and Z, we developed and optimized Cs-Z-Bc film in a laboratory setting. The physicochemical properties, antioxidant and antimicrobial efficacies, and biodegradability of the film were systematically explored using different tools and techniques. Integrating *B. ciliata* extracts into the chitosan-zein film under optimized conditions increases opacity without compromising tensile strength. The notable α-diphenyl-β-picrylhydrazyl (DPPH) scavenging activity underscores the superior antioxidant properties of the film, which are imperative for extending the shelf life of food products. A shelf life study conducted on Himalayan cheese suggests that chitosan-zein films fortified with *B. ciliata* extract have the potential to serve as an excellent material for food packaging, aligning with the contemporary emphasis on sustainability in packaging solutions.

Materials and methods

Materials

Zein protein (grade Z3625, 22–24 kDa), Chitosan-448,877 medium molecular weight, α-diphenyl-β-picrylhydrazyl (DPPH), Quercetin, and anhydrous sodium carbonate were purchased from Sigma-Aldrich (USA). Ethanol, gallic acid, and other chemicals were purchased from Qualigens. The Mueller-Hinton agar and the liquid broth (LB) were purchased from Merck (Germany). In addition, *Escherichia coli* (ATCC 8739), *Bacillus subtilis* (ATCC 6051), *Staphylococcus aureus* (ATCC 6538P), and *Klebsiella pneumoniae* (ATCC 700603) were purchased from the American Type Culture Collection (ATCC) for the antimicrobial assay. *Candida albicans* (ATCC 2091) was used for antifungal testing. *B. ciliata* was collected from the Syangta region of the Dolpa district (altitude ~ 2500 m) of Nepal. The plant was shade-dried, and the extraction was collected in ethanol through the Soxhlet extraction process at 40 °C. To obtain the plant extracts, the filtrates were first concentrated using a rotary evaporator at 40 °C, then freeze-dried, and stored at 4 °C for further use.

Preparation of films

The 1% (w/v, g) zein solution was prepared in 70% (v/v) ethanol with constant stirring. A 2% (w/v) chitosan solution was prepared in 1% (v/v) acetic acid and mixed with 0.6 g of glycerol, with constant stirring for 4 h at 60 °C. The crude extract was dissolved in 99.9% ethanol to ensure uniform dispersion, and then added to the zein-chitosan (Cs-Z) solution, which was stirred continuously for 2 h to ensure homogeneity. To optimize the concentration, different amounts of *B. ciliata* extract (50 mg, 100 mg, 200 mg, and 400 mg) were incorporated into the film-forming solution. Subsequently, the solutions were centrifuged at 8000 rpm for 10 min to remove impurities. 35 mL of each supernatant solution was poured onto Petri dishes to ensure uniform film thickness. The Petri dishes were then transferred to a ventilated oven set at 40 °C for 12 h, after which the films were carefully peeled off. The films were prepared and labeled as Cs, Cs-Z, Cs-Z-Bc50, Cs-Z-Bc100, Cs-Z-Bc200, and Cs-Z-Bc400. The number represents *B. ciliata* concentrations of 50, 100, 200, and 400 mg per 200 mL of the chitosan and zein solution, respectively.

Material characterization

Physical, chemical, and mechanical properties

The film thickness was measured using a digital micrometer (SH1907A2522). Ten data points were collected in different regions of the film, and the mean value, along with its standard deviation, was reported. A SPECORD 200 plus UV-Vis spectrophotometer (Analytik Jena) was used to measure the opacity. The size of the films was fixed at 10 mm × 40 mm. The opacity was calculated as follows:

$$Opacity = Abs_{600}/d \quad (1)$$

where Abs_{600} is the value of the absorbance at 600 nm, and d is the film thickness in mm.

IRTracer-100 Fourier transforms infrared (FTIR) spectrometer in attenuated total reflectance mode was used to analyze the chemical composition of the films. XRD data of the film were measured in the 2θ range of 20° to 80° using an X-ray diffractometer (Rigaku, Miniflex 600) with Cu Kα radiation. The scanning rate and step size in the measurement were 2°/min and 0.02 °, respectively. The morphology of the film samples was investigated using field-emission electron microscopy (FESEM, JEOL, JSM-IT800). The samples were sputtered with gold/palladium in an argon atmosphere for 100 s. The accelerating voltage was set at 5 kV. The thermal behavior of nanofibers was measured using a thermogravimetric analyzer (TGA 5500). A known amount of sample was sealed in a platinum pan and heated from 25 to 550 °C at a ramping rate of 10 °C/min. We measured the tensile properties of the thin film based on the ASTM D882 standard test method.

Antioxidant activity

The DPPH radical scavenging assay was performed to determine the antioxidant potential, following the previous study with slight modification (Altan et al. 2018). Briefly, 50 mg of each sample was dissolved in 2 mL of ethanol. 100µL of film solution was dispensed in 96 well plates, and 100 µL of 0.1 mM DPPH solution in methanol was added in triplicate. The mixture was kept for 30 min in the dark at room temperature, and then the absorbance of the solution at 517 nm was determined spectrophotometrically.

$$\text{DPPH scavenging activity } (\%) = (\mathrm{A_{DPPH}} - A_S)/A_{DPPH} \times 100 \quad (2)$$

where $\mathrm{A_{DPPH}}$ is the absorbance of the DPPH ethanol solution, and $\mathrm{A_S}$ is the absorbance of the sample solution at 517 nm.

Antibacterial and antifungal activities

13 g of liquid broth (LB) powder (Sisco Research Laboratories Pvt. Ltd., India) was dissolved in 1 L of water. The solution mixture was autoclaved for 25 min at 15 psi pressure at 121 °C and then allowed to cool to a temperature range of 40–50 °C before being transferred into sterilized 15 mL Falcon tubes. The bacterial seeds were co-cultured with prepared media and incubated for 24 h. 39 g of Mueller-Hinton Agar (MHA) agar powder (Sisco Research Laboratories Pvt. Ltd., India) was dissolved in 1 L of water to make MHA plates. The mixture was autoclaved at 121 °C and cooled to 40–50 °C. They were stored in a refrigerator until used. 150 µL liquid bacterial seed was spread on media plates with the help of a sterile cotton swab on the surface of the media plates. The wells were made on the surface of the agar, and the samples (3 discs of each sample) and standard kanamycin 5 mg/mL (10 µL) were loaded in the prepared wells. Thus, prepared media plates were incubated at 37 °C for 24 h, and the antimicrobial properties were observed considering Cs-Z as a control sample.

Biodegradation test

The biodegradability of films was assessed using a composting test, as described in the literature (Medina-Jaramillo et al. 2017). Briefly, pure chitosan-zein film and the films with varying amounts of plant extract were cut into 2×5 cm pieces and buried 6 inches deep in soil sourced from a local nursery. To simulate a consistent composting environment, the soil was moistened by spraying water in the morning and kept at room temperature. After each week, for four weeks, the film samples were extracted and weighed to determine weight loss, indicative of their biodegradability. The pure chitosan-zein sample served as a control for comparison. While this controlled test provides a baseline measure of biodegradability, it's essential to consider that real-world conditions, influenced by variables like soil pH, microbial activity, and temperature, can yield different results.

Food shelf life study

Cheese samples (200 g) were wrapped in chitosan, chitosan-zein, and chitosan-zein-*B. ciliata* films and stored for seven days. Every day, unwrapped cheese samples and film-covered samples were observed, and digital photographs were taken to monitor the changes over time.

Results and discussion

Film thickness, opacity, and transparency

Figure 1 depicts the schematic representation of the preparation process of chitosan-zein film enriched with *B. ciliata* extract. The diagram outlines the key steps involved in film fabrication, including the formulation, mixing, casting, and drying process. Additionally, it highlights the potential applications of these bioactive films in cheese preservation. The thickness and appearance of the packaging film affect the consumer's acceptance of the film. Fig. S1 shows the thickness and opacity of the different films. As seen in Fig. S1(a), the film thickness increased significantly with higher concentration of *B. ciliata* extract. This result revealed that the concentration of *B. ciliata* extract in the film-forming solution played a critical role during the drying process and significantly influenced the final thickness of the film. Opacity plays a crucial role in the effectiveness of active films used for food packaging, particularly in how these films protect food from spoilage by reducing light transmission and blocking UV rays. The opacity of films composed of chitosan, zein, and *B. ciliata* extract varies significantly depending on the concentration of the *B. ciliata* extract. Chitosan films exhibit the least opacity and are highly transparent due to their lack of unsaturation (Fig. S1(b). However, adding zein, a polypeptide, increases the film's opacity. This increase is further amplified by incorporating *B. ciliata* plant extract, raising opacity levels from 2.42 ± 0.97 to 16.27 ± 1.03. The significant opacity in films containing *B. ciliata* is attributed to the aromatic rings in flavonoids, which absorb the UV rays within the 200–400 nm wavelength range, making the film UV-blocking through optical shielding (Ghazi 2022). Additionally, there is a notable interaction between chitosan and zein when *B. ciliata* extract is added, leading to the formation of a denser and more evenly distributed structure within the film. The surface structure in the nanoscale is observed under scanning electron microscopy (SEM) imaging (*see* later section). The visual transparency of these films varies: the chitosan (Cs) film is highly transparent, and the chitosan-zein (Cs-Z) film shows a yellowish tint, indicating reduced transparency and transparency further decreases with an increased concentration of *B. ciliata* in Cs-Z film (Fig. 2).

FTIR study

The Fourier-transform infrared (FTIR) spectra were studied to evaluate the interaction between chitosan, chitosan combined with zein, and varying concentrations of *B. ciliata* samples (Fig. 3). Consistent with prior studies, the primary characteristic IR bands of the chitosan composite films were

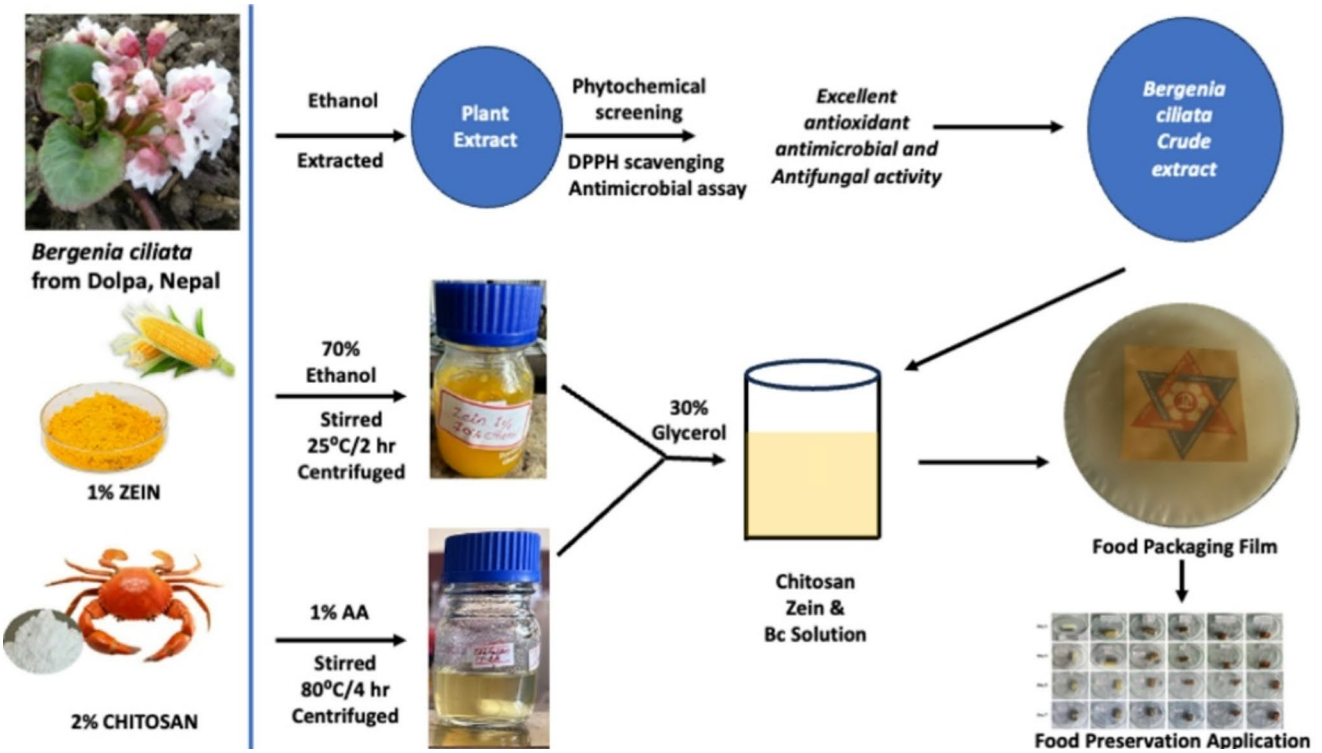


Fig. 1 Schematic representation of the preparation process for chitosan-zein film enriched with *B. ciliata* extract

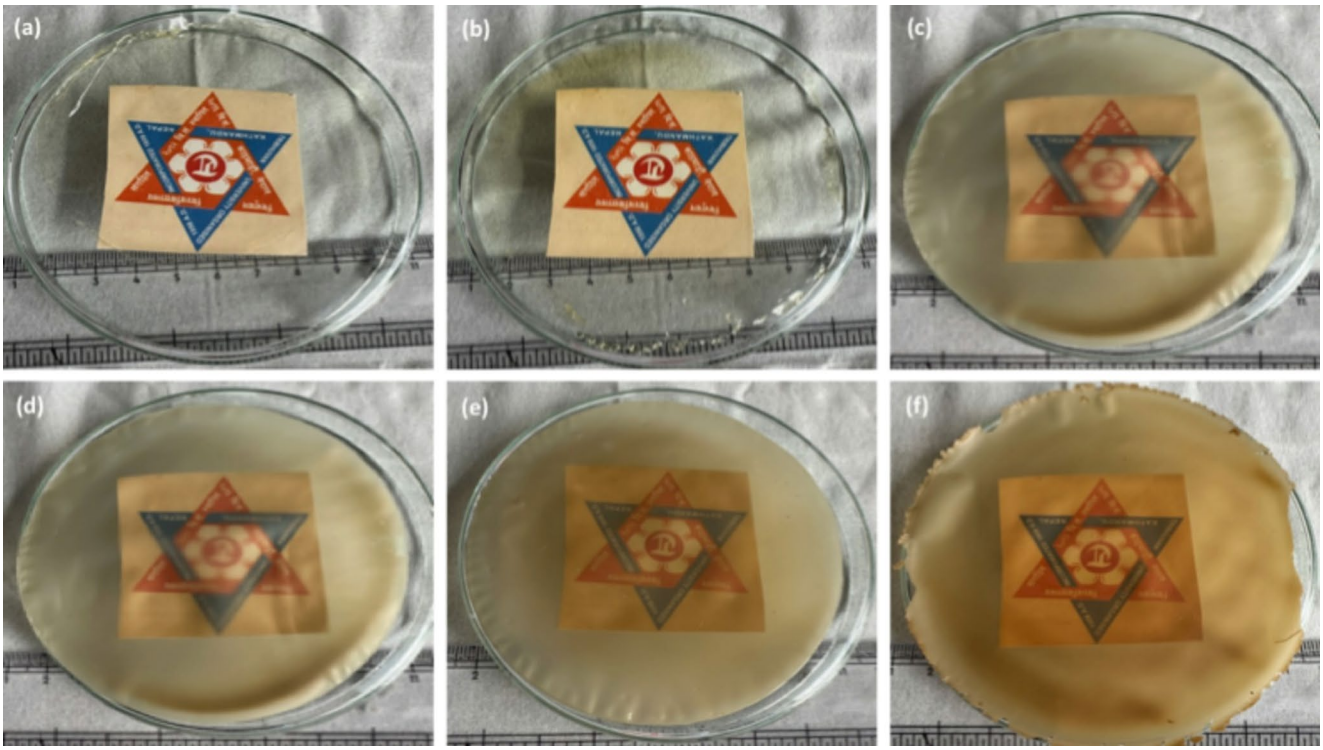


Fig. 2 Digital images showing the transparency of the different films. (**a**) Cs, (**b**) Cs-Z, (**c**) Cs-Z-Bc50, (**d**) Cs-Z-Bc100, (**e**) Cs-Z-Bc200, and (**f**) Cs-Z-Bc400

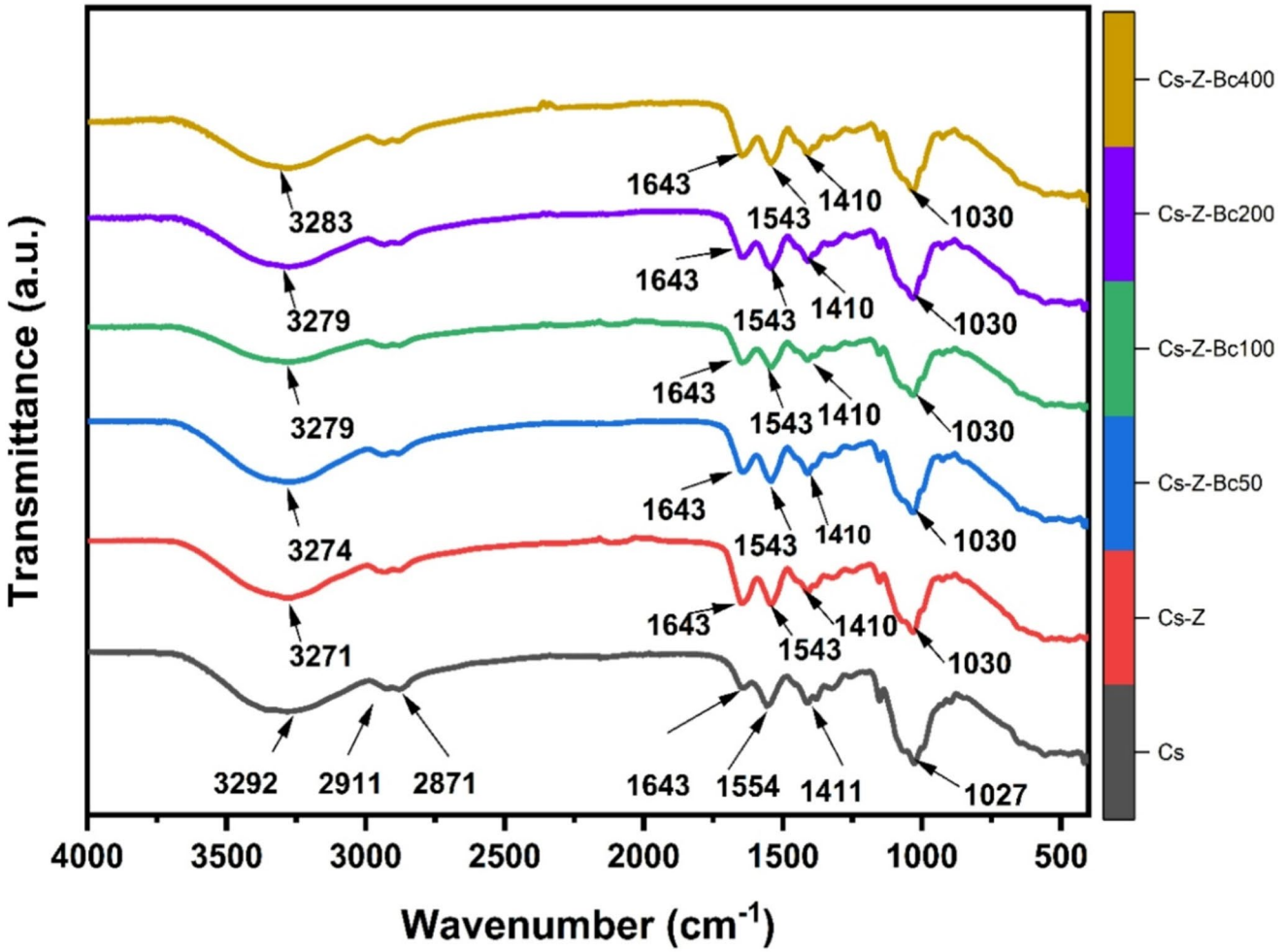


Fig. 3 FTIR spectra of different samples

identified and matched (Martínez-Camacho et al. 2010). The expansive absorption peak at 3292 cm^{-1} for Cs was associated with the stretching vibrations of N-H and O-H functional groups, revealing intermolecular hydrogen bonding. Notably, this peak shifted from the reference range of 3700–3584 cm^{-1} to a lower frequency of 3292 cm^{-1}. The presence of two distinct bands between 2911 and 2871 cm^{-1} signified the asymmetric and symmetric CH stretching, characteristic of the CH_2 group, respectively. The band was approximately 1643 cm^{-1} and was identified as the amide I band, primarily influenced by a carbonyl (C = O) stretching. Concurrently, the bands at 1554 cm^{-1} and 1411 cm^{-1} were associated with amide II and amide III stretching vibrations, respectively (Kong and Yu 2007). Another subtle band, around 1250 cm^{-1}, emerged due to the interaction of N-H bending with C-N stretching. Additionally, the peaks observed around 1027 cm^{-1} in Cs and 1030 cm^{-1} in the combined Cs-Z and *B. ciliata* compositions were attributed to C–O, C–N stretching, and C–C–N bending vibrations. Notably, the introduction of zein induced a shift in the N–H/O–H bond peak from 3292 cm^{-1} (in Cs) to 3271 cm^{-1}, suggesting interactions between chitosan and zein through hydrogen bonding and electrostatic forces (Athokpam et al. 2016). Furthermore, the spectral bands related to Cs-Z-Bc50 and Cs-Z-Bc100 films at 3279 cm^{-1} were broader.

XRD study

The XRD data of all samples are provided in Fig. S2. The broad diffraction peaks at 20° symbolize semi-crystalline chitosan. The incorporation of zein into chitosan film didn't show the diffraction peaks, indicating the amorphous nature of the zein protein. The reduction in crystallinity might be attributed to the strong interactions among chitosan, zein, and glycerol. After adding *B. ciliata*, the XRD pattern of films showed similar attenuation of peaks, which proved that *B. ciliata* is well dispersed in zein and chitosan matrix and remains amorphous. Previous studies reported that the incorporation of Chinese chive root, apple polyphenols, banana peel, and pomegranate peel extract into chitosan

composite films led to a weakening of the broad diffraction peaks (Riaz et al. 2020). Typically, materials with reduced crystallinity demonstrate enhanced elongation capabilities. The lower crystallinity observed in the Cs-Z-Bc film implies improved mechanical properties, aligning with the results from mechanical property assessments.

SEM imaging

The surface morphology of chitosan-zein films, influenced by adding *B. ciliata*. To explore this, SEM images of all the samples were observed (Figs. 4**(a-f)**. The surface morphology evolves as we move from pure chitosan to the Cs-Z blend and then through increasing concentrations of *B. ciliata*. Chitosan films exhibited a smooth and uniform surface morphology as shown in Fig. 4**(a)**. The Cs-Z film's surface revealed round, scale-like structures or globules, contributing to increased surface roughness as shown in Fig. 4**(b)**. This is likely due to phase separation between the hydrophilic chitosan and hydrophobic zein, where zein dehydrates more rapidly than chitosan during the drying process (Yu et al. 2017). This behavior is due to the protein's hydrophobic nature and its tendency to minimize its exposure to the surrounding environment by agglomerating. After blending with the *B. ciliata*, the film becomes more uniform with greater density. The increasing density and uniformity of the film may be due to the hydrogen bonding between the polyphenols of *B. ciliata* and the chitosan-zein molecule. This proves that *B. ciliata* is distributed in the chitosan zein matrix homogenously. Similar results were obtained when nano TiO_2 is dispersed in Cs-Z film (Qu et al. 2019) and Cs-Z and red radish anthocyanin (Yi et al. 2023).

Thermogravimetric analysis

Figure S3 shows the thermogravimetric analysis (TGA) and differential thermogravimetric (DTG) profile of the composite film comprising chitosan (Cs), zein (Z), and *B. ciliata*. The weight loss at 55 °C to 110 °C can be attributed to the evaporation of adsorbed water molecules. Subsequently, the onset of degradation observed at around 160 °C could be the thermal decomposition of *B. ciliata*, given that plant extracts often have volatile constituents prone to degradation at lower temperatures. In the second weight loss stage, each film showed a decrease in weight ranging from 22 to 26%, occurring between 166 °C and 286 °C. This loss is likely due to the elimination of low molecular weight proteins and glycerol, coupled with the breakdown of chitosan and *B. ciliata* (Sun et al. 2016). The peak at 281–288 °C in DTG was due to thermal degradation of the polymer structure of chitosan's polysaccharide chains, which typically exhibit higher thermal stability than other natural polymers. The film Cs-Z-Bc400 has the lowest weight loss in all the temperatures, and residual mass at 550 °C is the highest at

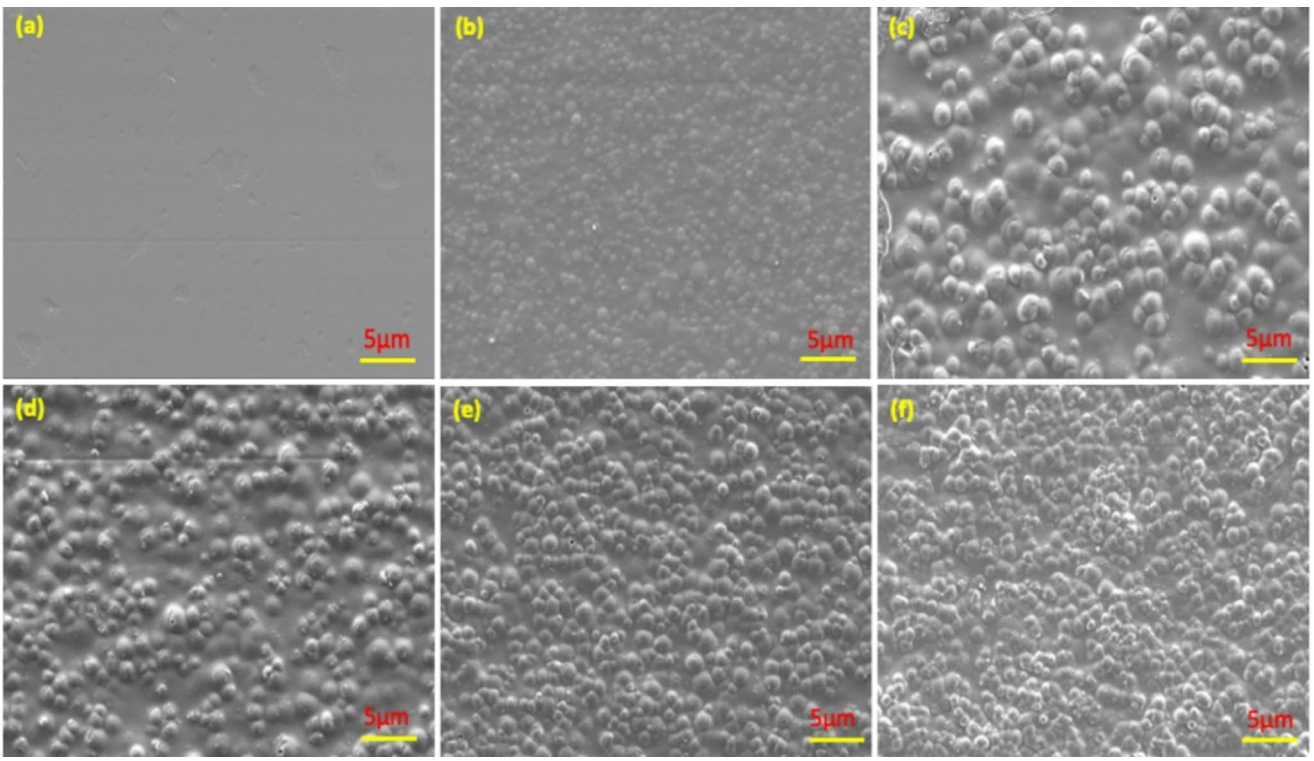


Fig. 4 SEM images of the film samples. (**a**) Cs, (**b**) Cs-Z, (**c**) Cs-Z-Bc50, (**d**) Cs-Z-Bc100, (**e**) Cs-Z-Bc200, (**f**) Cs-Z-Bc400

29.61%, which means that the higher thermal stability of the film was achieved. Similar behavior was observed when zein film was reinforced with chitosan and cinnamon essential oil (Vahedikia et al. 2019).

Mechanical properties

The mechanical properties of all blended films are shown in Table S1. Chitosan film showed the lowest peak loads and break load, indicating its relatively weaker mechanical strength. However, incorporating zein and *B. ciliata* into chitosan films significantly enhanced these properties. Specifically, the Cs-Z-Bc100 sample showed the highest peak load and break load, while Cs-Z-Bc50, Cs-Z-Bc200, and Cs-Z-Bc400 also show improved strength compared to pure Cs and Cs-Z films, but not as much as Cs-Z-Bc100. A significant increase in Cs-Z film as compared to chitosan could be due to increased bonding between chitosan and zein protein. Furthermore, the study highlights the impact of these additives on the films' elongation at break. Zein films demonstrate the least elongation, suggesting lower ductility, whereas the Cs-Z-Bc100 sample shows the highest, indicating a significant enhancement in flexibility. The other *B. ciliata* samples (Cs-Z-Bc50, Cs-Z-Bc200, Cs-Z-Bc400) exhibit similar elongation properties, reinforcing the idea that the addition of zein and *B. ciliata* consistently improves the ductility of chitosan films. It could be suggested that at lower concentrations of *B. ciliata*, there is increased bonding, but at higher concentrations, interaction decreases due to phase segregation. This enhancement in tensile strength, stiffness, and ductility makes these modified films more suitable for food packaging applications where durability and flexibility are crucial. The mechanical properties of the as-synthesized Cs-Z-Bc400 sample were compared with chitosan-zein films reported in the literature, as shown in Table S2. The results indicate that the mechanical properties of Cs-Z-Bc400 are comparable to those reported in previous studies.

Antioxidant activity

Among all six films, chitosan film exhibits poor antioxidant activity, while Cs-Z-Bc400 has the highest scavenging properties (Fig. 5). The low antioxidant activity of chitosan is due to the absence of easily donated hydrogen atoms, making them less effective as antioxidants. Additionally, the poor antioxidant activity of chitosan is due to the weak free radical scavenging ability of amino groups at the C-2 position in chitosan chains (Xie et al. 2001). The high antioxidant activity of *B. ciliata* is due to the presence of easily donated hydrogen atoms in its polyphenols (bergenin, catechin, gallic acid, pyrogallol, etc.), making it highly effective as an antioxidant (Cheng et al. 2015). Additionally, the scavenging property of *B. ciliata* is dependent on its concentration in the Cs-Z-Bc film. The antioxidant activity of the as-prepared samples was compared with that of a previous study, and the results are shown in Table S2. It

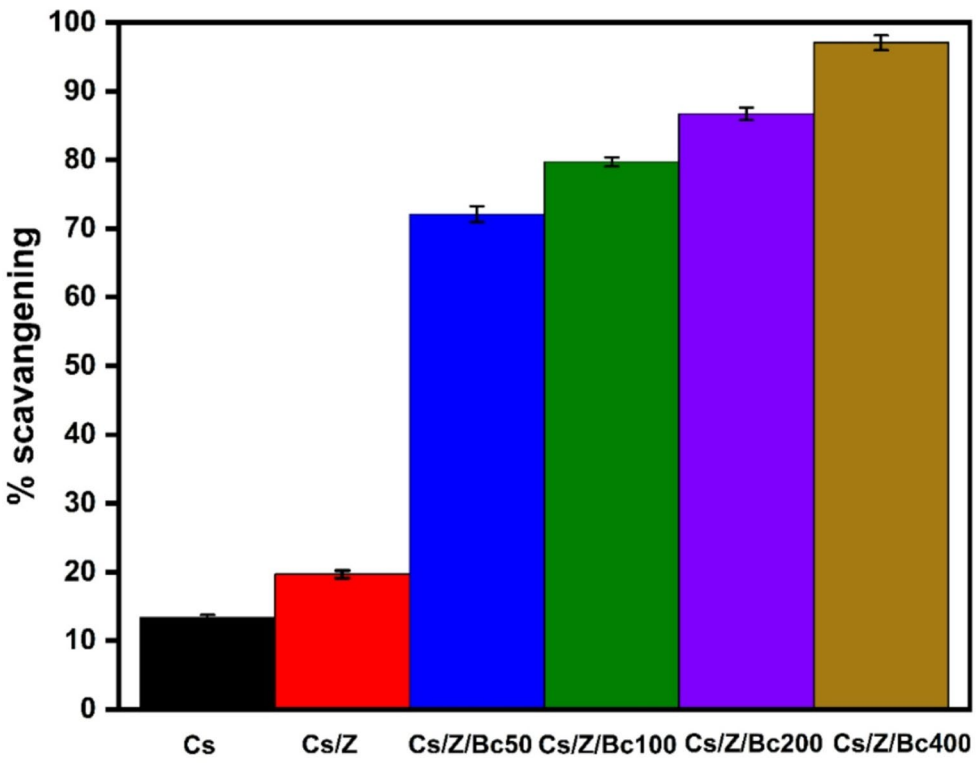


Fig. 5 DPPH radical scavenging activity of all the film samples. The histogram displays the mean value, and the error bars represent the standard deviation from three independent measurements

can be seen that the *B. ciliata* incorporated sample exhibits superior DPPH scavenging activity.

Antibacterial assay

In our study, a crude extract of *B. ciliata* obtained from the high altitude of Dolpa district, Nepal, exhibited excellent antibacterial and antifungal activity as shown in Fig. S4. The extract showed a zone of inhibition (ZOI) of 22.1 mm against *E. coli*, *S. aureus*, *and C. albicans*, and 22.2 mm against *C. subtilis* and *K. pneumoniae.* The kanamycin (5 mg/mL) was used as a control for comparison. The outstanding antibacterial behavior of *B. ciliata* can be attributed to the high phenolic and flavonoid compounds present in it, as confirmed by TPC, TFC, and DPPH assays. Previous studies have reported that the methanol extract of *B. ciliata* (tested at 200–1000 µg/disc) exhibited ZOI of 12 and 14.5 mm against *E. coli* ATCC 10,536 and *S. aureus* ATCC 29,737 (Sinha et al. 2001), respectively. In another study, ethanolic extract of *B. ciliata* showed ZOI of 06 to 10 mm against gram+ve *B. subtilis* (ATCC 6633) and *S. aureus* (ATCC 6538), and 11 to 15 mm against gram -ve *E. coli* (ATCC 10536) bacteria (Azhar et al. 2002). Based on these findings, *B. ciliata* extract was incorporated into the chitosan-zein matrix to develop an active film aimed at enhancing food safety and extending the shelf life by inhibiting microbial growth (Wu et al. 2016). Table S3 shows the antimicrobial activity of different films. It can be observed that the Cs-Z and Cs-Z-Bc50 displayed no antibacterial activity, while Cs-Z-Bc100 exhibited a ZOI of 11.1 mm against *B. subtilis* alone. Cs-Z-Bc 200 showed ZOI of 11.3 mm and 11.1 mm against *B. subtilis* and *S. aureus*, respectively. Notably, Cs-Z-Bc400 film demonstrated enhanced ZOI ranging from 0 to 11.2 mm against *E. coli* and *K. pneumoniae*. The ZOI for *B. subtilis* and *S. aureus* also saw a marginal rise with this concentration. These findings indicate that beyond a specific concentration, *B. ciliata* extract exhibits strong antibacterial properties against both gram-positive and gram-negative bacteria, making it a promising candidate for active packaging applications. Furthermore, we compared the antimicrobial activity of the as-synthesized films with chitosan-zein composite films reported in the literature, with the results summarized in Table S2. The comparison reveals that the as-synthesized films in this study exhibit superior antimicrobial performance, demonstrating enhanced efficacy against a range of microorganisms compared to previously published studies. This improved performance underscores the potential of the synthesized films for practical applications in antimicrobial packaging.

Biodegradability

Analyzing the biodegradability of the samples based on weight loss over 28 days reveals interesting insights. Fig. S3 shows the digital images of the various samples at different time intervals during biodegradation, and the corresponding % weight loss is summarized in Table S4. The blank (Cs-Z) sample and Cs-Z-Bc400 samples exhibit a lower degree of weight loss, exhibiting lower biodegradation compared to the Cs-Z-Bc50, Cs-Z-Bc100, and Cs-Z-Bc200 samples. The reduced biodegradability of Cs-Z-Bc400 samples can be attributed, in particular, to their higher resistance to microbes in the soil, which is likely linked to their increased phenolic and flavonoid content compared to other samples, as supported by TPC and antimicrobial tests. In contrast, Cs-Z-Bc100 and Cs-Z-Bc200 demonstrated a more significant weight loss, suggesting a higher biodegradation rate, likely due to their lower resistance to microbial action. This variation in biodegradability among the samples highlights the influence of chemical composition, especially phenolics and flavonoids, on microbial resistance. Notably, Bc400 has lost 44.09% of its initial weight, suggesting that it is moderately biodegradable and holds significant potential for use in packaging applications.

Food shelf life study

Figure 6 presents the digital images of the Himalayan cheese samples, both bare and wrapped in various films, over different time intervals. The images clearly show that the cheese wrapped in Cs-Z-Bc400 film exhibits minimal signs of degradation after 7 days at room temperature, in contrast to the other samples, including the unwrapped control. This result suggested that the Cs-Z-Bc400 films provide superior protection, effectively minimizing microbial growth and spoilage, thereby enhancing the shelf life of the cheese compared to other films tested. The superior performance of the Cs-Z-Bc400 is likely due to its enriched content of bioactive components, such as phenolics and flavonoids, which are known to inhibit microbial proliferation. These findings align with results from antimicrobial and biodegradation tests. The cheese wrapped with the Cs-Z-Bc400 film maintains its original appearance with negligible evidence of spoilage or desiccation, indicating that the wrap's bioactive matrix is effective in retaining the cheese's condition. Consequently, the application of the *B. ciliata* to chitosan and zein matrix made the film bioactive, which enhanced the shelf life of food by inhibiting microbial decay.

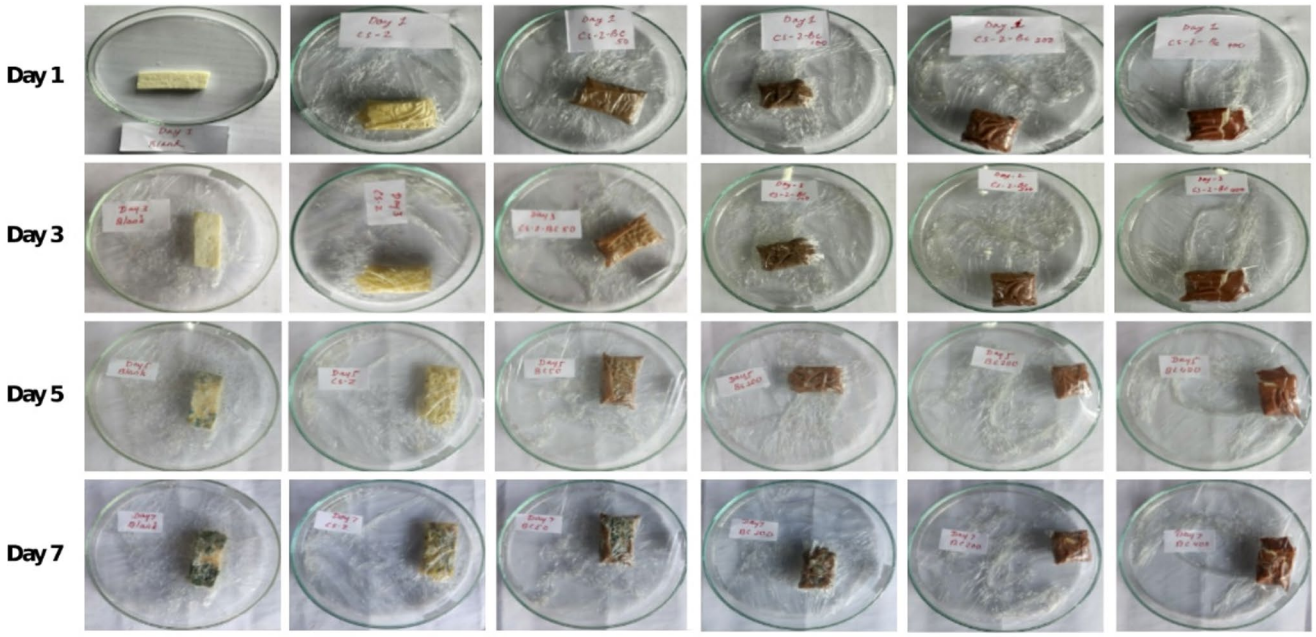


Fig. 6 Digital photographs of Himalayan cheese samples, both bare and wrapped in different films at various time intervals

Potential applications and commercialization prospects of the developed films

Plants have long been a rich source of therapeutic compounds with significant applications. *B. ciliata*, abundantly available in the Himalayan and hilly regions of Nepal, is traditionally used by Amchis and Baidya for medicinal purposes. As a non-endangered, easily cultivable plant, *B. ciliata* can be grown commercially. The extract of *B. ciliata* has been incorporated into zein-chitosan films in this study. These films exhibit excellent antimicrobial, antioxidant, and UV barrier properties, along with tunable mechanical properties, making them a promising alternative to conventional plastic packaging. Due to their biodegradability and ability to preserve food quality, these films can be used for packaging perishable items such as fruits, vegetables, ready-to-eat meals, and Himalayan cheese. Their natural properties help extend shelf life while reducing the need for synthetic preservatives. Additionally, they can serve as edible coatings for fresh produce, dairy, and meats, offering protection without generating waste.

The commercialization of these plant extract-incorporated zein-chitosan films presents opportunities in both large-scale and niche markets. Increasing regulatory pressure to reduce plastic waste, combined with the global shift toward sustainable packaging, creates a favorable market environment. The cost-effectiveness of plant extracts and biodegradable polymers, along with growing demand for eco-friendly solutions, further enhances their market potential. Collaborations with food manufacturers, environmental groups, and packaging companies will facilitate broader adoption. For widespread commercialization, further research is necessary to optimize production processes, enhance film properties, and explore applications in other industries, such as pharmaceuticals and cosmetics, while focusing on cost reduction and scaling up production.

Conclusions

In this study, the physicochemical, mechanical, and biological properties of the chitosan-zein films enhanced with *B. ciliata* extract were systematically explored. Chitosan-zein films fortified with *B. ciliata* extract mark a significant advancement in biodegradable food packaging, demonstrating a remarkable improvement in UV protection, mechanical strength, antioxidant, and antimicrobial properties. Zein provides moisture resistance, and introducing glycerol diminishes brittleness and barrier to oxygen, while other polar compounds augment the film's adaptability. The films' increased opacity effectively blocks UV rays, while their enhanced tensile strength and ductility ensure durability. The high DPPH scavenging activity highlights superior antioxidant properties, which are crucial for extending the shelf life of food products. Additionally, the films exhibit strong antimicrobial efficacy against various bacterial strains, contributing to food safety. Importantly, their excellent biodegradability aligns with environmental sustainability goals, and their application in packaging, such as Himalayan cheese, significantly increases food shelf life,

underscoring their potential as effective, eco-friendly packaging solutions.

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Data availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

Code availability NA.

Declarations

Ethics approval Ethics approval is not required for this research.

Consent to participate NA.

Consent for publication NA.

Conflict of interest The authors declare that there is no conflict of interest.

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