Ahmed *et al.*
Food Production, Processing and Nutrition (2024) 6:65
https://doi.org/10.1186/s43014-024-00241-9

Food Production, Processing and Nutrition

RESEARCH

Open Access

Edible microbial cellulose-based antimicrobial coatings and films containing clove extract

Mazia Ahmed[1], Pinki Saini[1*], Unaiza Iqbal[1] and Khushbu Sahu[1]

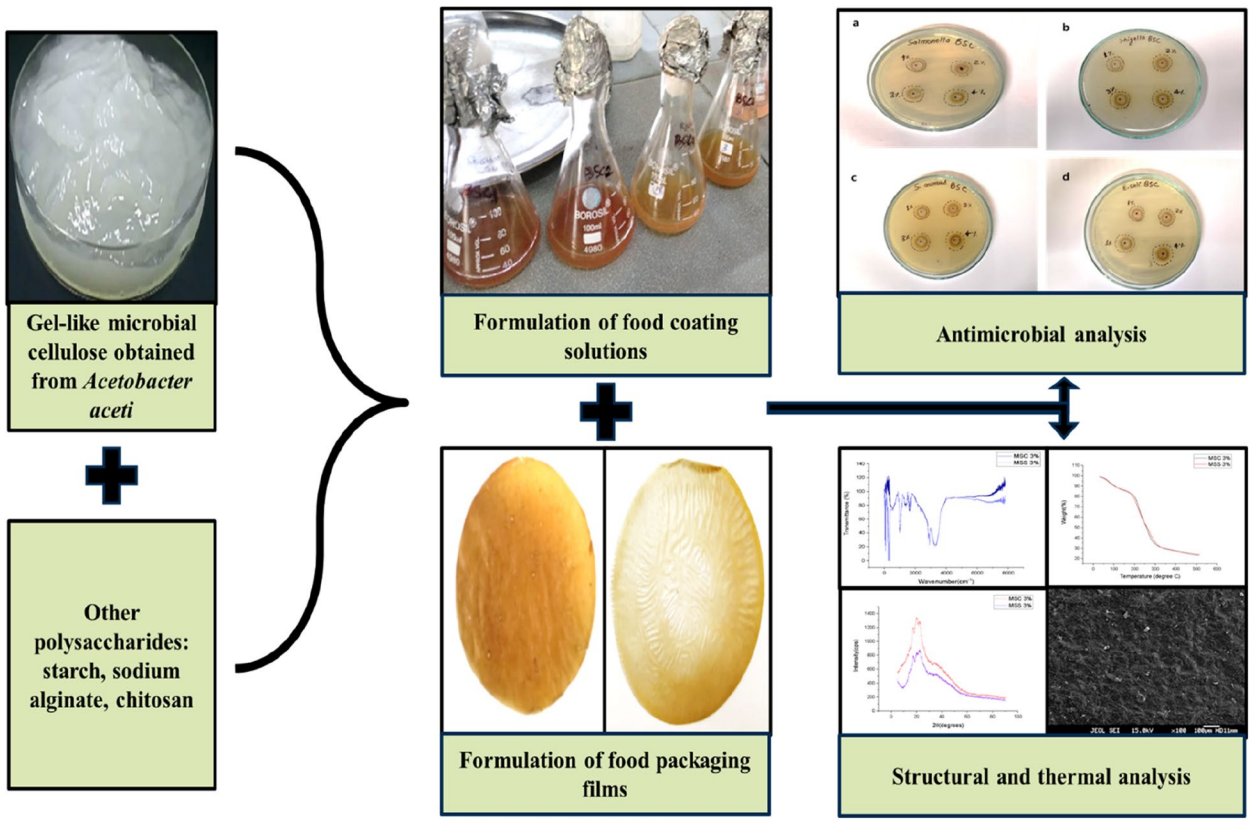
Abstract

Recently, microbial cellulose-based coatings and films have attracted substantial attention because of their promising uses in numerous fields, such as food packaging. The present work was designed to synthesize active microbial cellulose-based coatings and films with a comprehensive investigation of their antimicrobial and structural properties. Microbial cellulose was synthesized by using a gram-negative bacterium called *Acetobacter aceti*. The produced microbial cellulose was mixed with sodium alginate, chitosan and starch to obtain two different composite solutions, i.e., microbial cellulose + starch + chitosan (MSC) and microbial cellulose + starch + sodium alginate (MSS). The antimicrobial properties were achieved by incorporating four different concentrations of clove extract into the composite solutions. The resulting composite solutions were tested against *S. aureus, Shigella, Salmonella,* and *E. coli* through the agar diffusion assay method. The clove extract was found to be effective in inhibiting the growth of these pathogens, as a clear zone of inhibition was observed at all clove extract concentrations, with a maximum zone of inhibition of 4.0 ± 0.05 cm on *E. coli* for the MSC solution incorporated with 4% clove extract. The best antimicrobial solutions found were then casted into films by pouring the solutions into petri dishes and drying at 50 °C in a tray drier. The antimicrobial activity was again evaluated for the films. The results indicated that MSC 3% showed a greater zone of inhibition against all pathogens (1.7 ± 0.18 cm). Furthermore, structural and thermal analyses of the formed films were conducted. The results indicated distinctive antimicrobial and structural characteristics of the films.

Keywords Polysaccharide, Clove extract, Antimicrobial, Microbial cellulose, Coating, Edible films, *Acetobacter aceti*

*Correspondence:
Pinki Saini
pspinki55@gmail.com
Full list of author information is available at the end of the article

Graphical Abstract



Introduction

Extensive research has been conducted on edible coatings and films with the aim of enhancing food quality, elevating the value of natural polymers, and minimizing the reliance on synthetic polymers in food packaging applications (Pascall et al., 2013; Atta et al. 2022). The distinction between food materials and edible films arises from their manufacturing process and application as end products in the food industry. Specifically, when edible polymers are applied to the outer surface of food products, whether in liquid or dried form, it is commonly termed as food coating (Ahmed et al. 2023; Han 2014; Maisanaba et al. 2017). Indeed, these coatings and films play a crucial role in safeguarding edibles, slowing down the deterioration process, and providing selective barriers to water vapor and gases such as carbon dioxide and oxygen. Additionally, they contribute to enhancing texture, preserving volatile organic compounds, and reducing the growth of microorganisms on the surface of the food. In essence, these protective measures extend the shelf life of food products and maintain their quality (Atieno et al. 2018). Biodegradable films enhance the mechanical properties, moisture and gas barriers, microbial protection, sensory perception, and shelf-life of food products. Specifically, films derived from polysaccharides exhibit superior gas barrier properties, while proteins are renowned for their exceptional mechanical characteristics (Shahidi & Hossain 2022).

Microbial cellulose stands out as a remarkably pure substance, offering numerous advantages over other cellulose types, including plant cellulose (Ahmed et al. 2021). Its distinctive features encompass biodegradability, biocompatibility, high crystallinity, non-toxicity, hydrophilicity, elevated tensile strength, extensive polymerization, in-situ moldability, and porosity. It's worth noting that these specific characteristics can vary based on factors such as the microorganism type, culture conditions, composition of the culture media, and post-synthesis processing/purification (Padrao et al. 2016). The versatility of microbial cellulose is evident in its wide-ranging applications across various fields, including food (Shi et al. 2014), biomedical (Di et al. 2017; Fatima et al. 2022), biosensing (Farooq et al. 2020), and environmental

applications (Ul-Islam et al. 2016). The adaptability of microbial cellulose makes it a valuable material with diverse and promising uses.

In food processing industries, microbial cellulose is not utilized as much as intelligent or constructive packaging material. Some researchers have used microbial cellulose composites as an efficient material for food packaging that demonstrates antimicrobial properties (Cazón & Vázquez 2021; Haghighi et al. 2021). The exploration of microbial cellulose, an exceptional polysaccharide, as a potential food coating material is yet to be fully investigated. Recently, Ahmed et al. (2023) synthesized composite coating solutions by using microbial cellulose as a main element. The synthesized composite solutions were utilized as effective coating materials for extending the nutritional value and shelf life of tomatoes. Many studies have been performed to infuse antimicrobial characteristics into microbial cellulose-based packaging or coating materials. This can be achieved by combining microbial cellulose with safe-to-eat substances, such as chitosan or plant extracts.

Chitosan is extensively known for its anticoagulative and immune-stimulating nature, and it also demonstrates strength-providing and film-forming properties along with antibacterial and antifungal characteristics (Hubbe et al. 2017). Similarly, sodium alginate has excellent colloidal properties and can transform into a gel-like substance. It can increase the shelf life of food products by improving the liquid barrier characteristics, reducing the degree of shrinkage, diminishing microbial contamination, and maintaining the flavor or taste of the food products (Xu et al. 2020). Starch is another polysaccharide that is biodegradable, renewable, and easily available. It is a perfect natural substance for a wide range of industrial applications, including the synthesis of edible packaging films or coating solutions for food products (Jimenez et al. 2012).

For preparing new food materials and developing ready-to-eat or healthy foods, the most important challenge lies in maintaining the quality of packaging used. Due to food-related illness outbreaks, the current research interest revolves around designing new approaches through which microbial growth in eatables can be prevented while maintaining their nutritional status, safety, freshness, and bioactivity. Antibiotics cannot be used for the preservation of food because of the elevated antibiotic resistance developed by many foodborne pathogenic microorganisms. Therefore, it is necessary to develop new nontoxic and antimicrobial substances for food packaging applications that can provide protection for both consumer health and food (Atieno et al. 2018). An important feature that is needed to manufacture biocomposites is the incorporation or natural/synthetic antimicrobial agent or antioxidants such as enzymes, bacteriocins, vegetable oils, organic acids, and plant-based extracts for the prevention or minimization of food degradation (Huang et al. 2019). Most plant-derived oils or extracts are classified under GRAS (generally recognized as safe) by the FDA (Food and Drug Administration) and used as flavor-imparting substances in various food products. For hundreds of years, cloves have been utilized as spices. The major constituent of clove is eugenol, a well-known agent due to its antimicrobial activity. The potential of clove oil/extract for the prevention of spoilage of food has already been assessed previously (Dashipour et al. 2014).

The main objective of this research work is to synthesize microbial cellulose-based active coating solutions and food packaging films. Two types of composite solutions and films were made using microbial cellulose, chitosan, starch and sodium alginate. Furthermore, antimicrobial activity was imparted to both composites by adding clove extract. The evaluation of the biological performance of the newly synthesized composite solutions and films was performed by analyzing their antimicrobial action. The structural properties of the developed edible food packaging films were also characterized.

Material and methods

The analytical grade chemicals such as starch, sodium alginate, chitosan, acetic acid, and sodium hydroxide were obtained from Sigma–Aldrich, whereas Mannitol broth (selective media for *Acetobacter aceti*) was purchased from Himedia, India and used for the preparation of starter culture by employing methodology described by Ahmed et al. (2023). The bacterial strain MTCC 3347 (*Acetobacter aceti*) and the pathogenic strains (*Salmonella, Shigella, S. aureus,* and *E. coli*) were procured from Institute of Microbial Technology, India.

Production and purification of microbial cellulose

The production and purification of microbial cellulose was performed by slightly modifying the method of Jozala et al. (2015). The starter culture was prepared in its selective media (mannitol broth). The suspended cells were stored at − 45 °C in 20% (v/v) glycerol solution. The culture was revived by adding 100 µL of stored cell suspension into 50 mL of mannitol broth and grown at 30 °C for 48 h (two days) and used as inoculum.

The bacterial culture (2% inoculum) was inoculated in the pH range of 5.8 in fruit waste media. The fruit waste media consisted of orange, kiwi, and guava fruit peel (33.3 g each), blended in 200 mL distilled water. The mixture was then strained and centrifuged at 2000 g for 10 min to yield a clear solution. Following inoculation, the concoction was placed in an incubator at 30 °C for

seven days to facilitate bio-cellulose production. Subsequently, the synthesized cellulose pellicle underwent centrifugation at 9000 g for approximately 10 min at 4 °C to separate the cellulose from bacterial cells. The resulting supernatant was carefully transferred into chilled isopropyl alcohol, inducing the precipitation of bio-cellulose. The bio-cellulose was once again subjected to centrifugation at 9000 g for about 20 min, yielding bio-cellulose pellets. Further, these pellets were boiled in NaOH solution to eliminate any attached cells. The remaining material was filtered and rinsed with distilled water multiple times to achieve a pH-neutral bio-cellulose product (Ahmed et al. 2023).

Preparation of composite solutions

Microbial cellulose, sodium alginate, starch, and chitosan were combined in different ratios to develop two distinctive composite solutions. The rationale for employing these chemicals lies in their shared characteristic of being polysaccharides, allowing for a homogeneous mixture.

The microbial cellulose, starch, and sodium alginate composite (MSS) coating composite solution was made by mixing microbial cellulose (5 g/100 mL distilled water), starch (3 g/100 mL distilled water), and sodium alginate (2 g/100 mL distilled water) together. Thereafter, the mixture was homogenized for ten minutes at 3000 rpm by using an IKA T25 digital ultra-Turrax homogenizer.

Similarly, the microbial cellulose, starch, and chitosan composite (MSC) coating composite solution was made by mixing microbial cellulose (5 g/100 mL distilled water), starch (3 g/100 mL distilled water), and chitosan (2 g/100 mL distilled water) together. To achieve pH neutrality, a small amount of NaOH was added to the solution followed by homogenization.

Preparation of clove extract

The clove extract was prepared following the methodology outlined by Shah et al. (2014). Initially, 100 g of dried clove buds were finely powdered. The powdered clove was subjected to maceration using methanol as the solvent. After 24 h, the macerated mixture was filtered through Whatman filter paper I, resulting in a clear filtrate. Subsequently, the filtrate underwent evaporation at temperatures below 60 °C to yield a solid clove extract residue, ensuring the complete removal of residual methanol.

Development of composite solution

MSS and MSC solutions were meticulously blended with varying concentrations of clove extracts, resulting in the formulation of eight distinct combinations. The clove extracts, prepared at concentrations of 1%, 2%, 3%, and 4% in dimethylformamide, were incorporated into the solutions. The compositions of the prepared solutions included MSS control (microbial cellulose, starch, and sodium alginate without clove extract). MSS 1%, 2%, 3%, and 4% containing microbial cellulose, starch, and sodium alginate with 1%, 2%, 3%, and 4% clove extract, respectively. Similarly, different MSC solutions and control were prepared with and without clove extract, respectively. Subsequently, these solutions were tested against pathogenic microorganisms, viz. *Salmonella, Shigella, S. aureus,* and *E. coli*, through the measurement of the zone of inhibition (Casalini and Baschetti 2023).

Antimicrobial test of composite solutions

The agar well diffusion technique was used to assess the antimicrobial activity of the samples. Twenty-four-hour-old inoculum of four food pathogens (*Salmonella, Shigella, S. aureus,* and *E. coli*) was made by inoculating the stock cultures in nutrient broth (NB) and keeping them at 37 °C in an incubator. The nutrient agar plates were smeared with one hundred microliters of broth cultures that contained approximately 10^8 CFU/mL of pathogenic microorganisms. Four wells of 6 mm diameter were aseptically made on each nutrient agar plate. The composite solutions (500 µL) containing 1%, 2% 3%, and 4% clove extract were poured into each equal-sized well created on petri plates containing pathogen-smeared nutrient agar. For the control solutions (without clove extract), another petri plate was prepared in the similar manner. The petri plates were then kept at 37 °C in an incubator for 24 h. The diameter of the inhibitory zone was measured to the closest 0.02 mm with a caliper. Because the diameter of the inhibition zone was not the same across the circle, an average of three diameters for every circle was used to calculate the diameter of the inhibition zone (Casalini and Baschetti 2023).

Development of composite films

The results of the antimicrobial composite solutions were used to obtain the best solution for the formation of antimicrobial films. The optimized solution from both the composition, i.e., MSC and MSS, was used to form antimicrobial thin films. Control films of both composite solutions (without clove extract) were also prepared. Glycerol (0.5 mL/50 mL) was mixed in the composite solution as a plasticizer. About 50 mL composite solution was introduced into a petri plate and subjected to drying for twenty-four hours at 50 °C in a tray drier. Thin films obtained after drying were stripped off from the petri plates and used for further studies (Reller et al. 2009).

Antimicrobial test of composite films

The disc diffusion method was used for carrying out the antimicrobial test of the films. In place of forming ditches and pouring down the solution, films of 6 mm diameter were kept directly on the previously smeared agar surface to calculate the zone of inhibition.

Structural analysis

X-ray Diffraction (XRD)

The X-ray patterns of the samples were studied using a X-ray diffractometer (Shimadzu XRD 7000) machine. The curve fitting method was used to determine the degree of crystallinity by employing the following formula (Güzel & Akpınar 2019):

$$\mathrm{CrI}(\%)\left(CurveFittingMethod\right) = A_{cryt}/A_{cryt} \times 100$$

where A_{cryst} = area of the crystalline peaks,
A_{total} = total area (Crystalline +Amorphous).

Scanning electron microscopy (SEM)

The morphological characteristics were evaluated using scanning electron microscope (HITACHI Model S-3000H) with a magnification × 500 to × 1500. Small strips (5 mm × 5 mm) of both MSS 3% and MSC 3% films were mounted on aluminum stubs, coated with a thin layer of graphite and observed on a Scanning Electron Microscope at an accelerated voltage of 5 kV.

Fourier transform infrared spectroscopy (FTIR)

FTIR analysis of films were performed on Jasco FT/IR-4X1typeA spectrophotometer, using samples prepared as KBr pellets. The spectra were recorded in transmittance mode in the 4,000–400 cm^{-1} region with an accumulation of 16 scans, and resolution of 4 cm^{-1}. The FTIR analysis was performed at Central Instrumentation Laboratory, Central University of Punjab, Bathinda (Punjab).

Thermogravimetric Analysis (TGA)

The test was conducted with the help of a PerkinElmer thermal analyzer at SICART (Sophisticated Instrumentation Centre for Applied Research and Testing), Anand, Gujarat. The films equivalent to 10 mg were heated in platinum crucibles for TGA analysis. The thermograms were taken at temperatures in the range of 30 to 520 °C, with a temperature increase of 10 °C/min under a nitrogen environment (Ul-Islam et al. 2013).

Statistical analysis

The triplicate readings were recorded for all the parameters by conducting the experiments thrice. The obtained data were then statistically analyzed with IBM-SPSS software. Analysis of variance (ANOVA) and Duncan's multiple range test for significant differences at $p \leq 0.05$ were used for the evaluation.

Results and discussion

Antimicrobial test of composite solutions

Microbial cellulose, starch, and sodium alginate composite (MSS)

The antimicrobial activities of the MSS control and their composites prepared with 1%, 2%, 3%, and 4% clove extracts were investigated against *Salmonella, Shigella, S. aureus,* and *E. coli*. The activity was evaluated using the agar well diffusion method. The MSS composite without clove extract, which was used as a control, did not produce any zone of inhibition against any pathogenic microbe, thus showing no antimicrobial potential. These results for the MSS composites are in agreement with those of previous investigations (Ciechanska 2004; Maneerung et al. 2008). MSS 1% showed very little inhibitory zones against all the pathogenic microbes with the maximum zone of inhibition (1.5 ± 0.07) for *E. coli*. A slight increase in the zone of inhibition was observed for the MSS 2% composite. However, the MSS 3% and MSS 4% composites displayed excellent antibacterial activities against all the tested pathogens (Table 1). MSS 3% produced clear zones of inhibition against *Salmonella, Shigella, S. aureus,* and *E. coli* with diameters of 2.8, 2.8, 2.9, and 3.1 cm, respectively. The MSS 4% composite produced even more prominent antimicrobial effects with 3.1, 3.2, 3.2, and 3.6 cm zones of inhibition against *Salmonella, Shigella, S. aureus,* and *E. coli,* respectively. These results indicated excellent antimicrobial

Table 1 Zone of inhibition of the MSS and MSC composite solution

Composite solution*	ZONE OF INHIBITION (cm)			
	Salmonella	*Shigella*	*S. aureus*	*E. coli*
MSS 1%	1.2 ± 0.01^{a}	1.2 ± 0.10^{a}	1.2 ± 0.12^{a}	1.5 ± 0.07^{a}
MSS 2%	2.3 ± 0.12^{b}	2.3 ± 0.14^{b}	2.3 ± 0.20^{b}	2.4 ± 0.06^{b}
MSS 3%	2.8 ± 0.01^{c}	2.8 ± 0.09^{c}	2.9 ± 0.05^{c}	3.1 ± 0.07^{c}
MSS 4%	3.1 ± 0.06^{d}	3.2 ± 0.09^{d}	3.2 ± 0.21^{c}	3.6 ± 0.03^{d}
MSC control	0.8 ± 0.01^{a}	0.6 ± 0.01^{c}	0.7 ± 0.00^{a}	0.8 ± 0.03^{b}
MSC 1%	1.4 ± 0.01^{a}	1.6 ± 0.01^{a}	1.6 ± 0.00^{a}	1.8 ± 0.03^{a}
MSC 2%	2.7 ± 0.00^{b}	2.8 ± 0.01^{b}	2.7 ± 0.00^{b}	2.7 ± 0.03^{b}
MSC 3%	3.8 ± 0.01^{c}	3.9 ± 0.01^{c}	3.8 ± 0.00^{c}	3.9 ± 0.02^{c}
MSC 4%	3.9 ± 0.01^{c}	3.9 ± 0.00^{d}	3.9 ± 0.01^{d}	4.0 ± 0.05^{d}

* MSS control did not show zone of inhibition against tested pathogenic strains

Mean ± SD ($n = 3$) ; Different upper case superscripts in the same column indicate the significant difference ($p < 0.05$)

properties of MSS 3% and MSS 4% against all the tested pathogenic strains.

Microbial cellulose, starch, and chitosan composite (MSC)

The antimicrobial activities of the MSC control and their composites prepared with 1%, 2%, 3%, and 4% clove extracts were investigated against *Salmonella, Shigella, S. aureus,* and *E. coli*. The activity was evaluated using the agar well diffusion method.

The MSC composite without clove extract (control) produced very little inhibition against all the pathogenic microbes, thus showing antimicrobial potential. This may be attributed to the presence of chitosan, which is antimicrobial in nature. Chitosan contains a number of active compounds and has the potential to absorb the electronegative material of living bacterial cells, disrupting their physiological functions and ultimately killing them (Zheng & Zhu 2003). The presence of chitosan enhanced the antimicrobial activity of the clove extract-incorporated MSC solution and made the results better than those of the MSS solution (Table 1). MSC 1% showed inhibitory zones against all the pathogenic microbes with the maximum zone of inhibition (1.8 ± 0.03) for *E. coli*. A slight increase in the zone of inhibition was observed for the MSC 2%. However, the MSC 3% and MSC 4% composites displayed excellent antibacterial activities against all the tested pathogens. MSC 3% produced clear zones of inhibition against *Salmonella, Shigella, S. aureus,* and *E. coli* with diameters of 3.8, 3.9, 3.8, and 3.9 cm, respectively. The 4% MSC composite produced even more prominent antimicrobial effects with 3.9, 3.9, 3.9, and 4.0 cm zones of inhibition against *Salmonella, Shigella, S. aureus,* and *E. coli,* respectively. These results indicated excellent antimicrobial properties of MSC 3% and MSC 4% against all the tested pathogenic strains.

Microbial cellulose-containing clove has previously been reported to possess antimicrobial activity (Albuquerque et al. 2021). The powerful antibacterial action of clove can be attributed mostly to eugenol. Eugenol is a phenolic component of clove that makes it efficient against microorganisms. As expected, the higher the concentration of clove extract was, the better the inhibition was observed. Even at the lowest concentration used in the formulation, clove extract inhibited all microorganisms, which was a significant finding because higher concentrations could affect the sensory attributes, changing the normal flavor of the packed food by surpassing the appropriate taste thresholds. The results are in close proximity with Fu et al. (2007). Farag et al. (1989) observed that eugenol, a main component of clove, inhibited the development of *B. cereus* by preventing the synthesis of particular enzymes needed for its growth. Zones of inhibition of approximately 25.6 mm for two and three percent clove concentrations were observed. Similarly, Bhak et al. (1990) discovered that clove had potent antibacterial properties when tested on different microorganisms.

Jalali et al. (2016) studied the impact of an alginate/carboxyl methylcellulose composite coating combined with clove essential oil on the quality of chilled silver carp fillet stored at 4 ± 1 °C was investigated. Total viable counts (TVC) and total psychrotrophic counts (TPC) were used in the bacteriological analysis of the control samples (c), alginate/carboxyl methylcellulose coating (C-A), and alginate/carboxyl methylcellulose composite coating incorporated with clove essential oil. The effectiveness of these treatments was also examined in relation to the management of the *Eschershia coli* O157:H7 population that had been introduced into silver carp fillets. The treated sample remained acceptable until the conclusion of the 16-day storage period, and from day 4 to the end of the storage period, it was able to lower the population of *E. Coli* O157:H7 below the acceptable threshold (< 2). The findings suggest that using clove essential oil in an alginate/carboxyl methylcellulose composite coating might be advised as a meat product preservative.

Development of composite films

As the results of MSS 3% and MSC 3% composite solutions are the best and almost similar to those of the 4% MSS and MSC composite solutions, the MSS 3% and MSC 3% composite solution was used to form composite films (Fig. 1). From 100 mL of composite solution, when casted on a petri plate, a thin sheet of packaging material of diameter 16 cm can be produced. Control films of both composite solutions (without clove extract) were also prepared. Glycerol (0.5 mL/50 mL) was also added as a plasticizer for the formation of films.

Numerous studies have been performed on the development of edible bio-composite films made from chitosan, cellulose, sodium alginate, and starch (Mahardika et al. 2019; Marvizadeh et al. 2017). These bio-composite films can be used to pack bakery products, confectionery, fresh fruits and vegetables, etc. Of course, as a food packaging material, the polysaccharide-based edible film should protect foods against deterioration due to microorganisms, moisture, dust, odors, and mechanical forces. There have been many previous reports on the synthesis and utilization of bio-composite films as a food packaging material. In a study, active edible films of potato starch, inverted sugar and sucrose was developed to coat mini panettones. All three variables significantly affected ($p < 0.05$) moisture, a_w, hardness and elasticity of panettones. From 16 to 24 days (35 °C/60% RH), panettones without coating and without additives (controls) showed

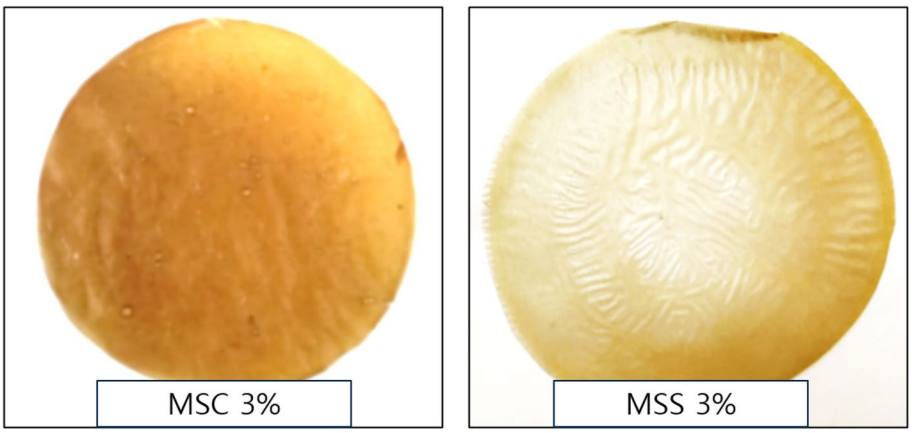


Fig. 1 Clove extract-incorporated composite films

growth of mold/yeast; while with both additives coating, fungal growth was observed from 40 days. When using potassium sorbate, mold/yeast was not detected until 48 days. During storage, there was reduced water activity, moisture, elasticity and cohesiveness of panettones with additives, while the reverse occurred in controls. The incorporation of food-graded antimicrobial compounds in the packaging films of potato starch coatings in concentrations lower than those normally used for mini panettones increased its shelf-life up to 130% and may contribute to product loss reduction during storage (Saraiva et al. 2016).

In another study, bacterial cellulose (BC) based on sago liquid waste was employed for the development of food packaging material. BC film was applied as the packaging of meat sausage, and the quality of meat sausage was measured based on weight loss, moisture content, pH, protein content, and total microbial count. The utilization of modified BC-based sago liquid waste film as the packaging of meat sausage could maintain sausage quality during 6 days of storage at room temperature. Therefore, the researchers concluded that edible BC film has the potential to be used as food packaging material (Yanti et al. 2021).

Similarly, Amorim et al. (2022) developed composite materials based on oxidized-bacterial cellulose (BC) and poly (vinyl alcohol)-chitosan (PVA-CH) nanofibers s were produced by needleless electrospinning and functionalized with the bacterial pigment prodigiosin (PG). The antimicrobial activity was evaluated and, PVA-CH_PG, and BC_PG layers exhibited the highest antimicrobial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Thus, the fabricated composites can be considered for application in active food packaging.

Antimicrobial test of composite films

The disc diffusion method was used for carrying out the antimicrobial test of the films. In place of forming ditches and pouring down the solution, films of 6 mm diameter were kept directly on the previously smeared nutrient agar surface to calculate the zone of inhibition. As predicted, no inhibitory zone was observed against all the pathogens for MSS control films. However, for MSC control films, a very minute zone of inhibition was observed. Comparing all the films, it can be concluded that MSC 3% showed a greater zone of inhibition against all the pathogens because chitosan enhanced its antimicrobial properties. Nearly the same results were found by Abral et al. (2021). They synthesized and tested a biocomposite film made up of tapioca starch/chitosan and bacterial cellulose nanofibers, and all the chitosan-based films displayed antibacterial activity.

The effect of MSC 3%, MSS 3% Film, MSC control and MSS control films on *Salmonella, Shigella, S. aureus*, and *E. coli* is depicted in Table 2. All the clove extract-incorporated films showed zones of inhibition. In the MSC 3% film, the maximum zone of inhibition (1.8 ± 0.03) was observed in *S. aureus,* and the minimum inhibitory zone of 0.3 cm was found in the MSC control film for *Shigella*

Table 2 Zone of inhibition of clove extract-incorporated and control films

Films	ZONE OF INHIBITION (cm)			
	Salmonella	*Shigella*	*S. aureus*	*E. coli*
MSC 3%	1.7 ± 0.18^{c}	1.7 ± 0.07^{c}	1.8 ± 0.03^{c}	1.7 ± 0.04^{c}
MSS 3%	1.5 ± 0.10^{b}	1.4 ± 0.07^{b}	1.4 ± 0.03^{b}	1.6 ± 0.07^{b}
MSC control	0.4 ± 0.01^{a}	0.3 ± 0.01^{c}	0.3 ± 0.00^{a}	0.5 ± 0.03^{b}
MSS control	Nil	Nil	Nil	Nil

Mean ± SD ($n = 3$) ; Different upper case superscripts in the same column indicate the significant difference ($p < 0.05$)

and *S. aureus*. Similar results were obtained by Albuquerque et al. 2021.

When employing natural extracts as antimicrobial agents, the mechanism of action occurs at the membrane level, with both the genetic material and enzyme being inactivated (Nazzaro et al. 2013). The results indicated that the composite films containing clove extract were a viable antibacterial addition for food packaging materials. Eugenol is the main constituent of clove, which inhibits the development of several harmful microbes (Mulla et al. 2017). Another critical component of an active packaging system is the facilitation of the movement of the active substance so that it may interact with the food product, hence decreasing or hindering microbial reproduction and proliferation. Thus, the microbial cellulose mixed with clove extract is sufficient for this purpose since the extract migrates within the first six hours of coming in the proximity of the microorganisms. The inclusion of several chemicals in natural polymers for the production of microbe-resistant food packaging systems has already been described. Dobre et al. (2012) showed nearly similar findings for a PVA/BC mix with sorbic acid as the antibacterial substance. In composite materials including PVA and bacterial cellulose powder, potassium (2E,4E)-hexa-2,4-dienoate was utilized as an antibacterial material (Jipa et al. 2012). Silver particles have been characterized as antibacterial substances in biocellulose membrane-based composite materials (Dobre et al. 2010), and the antimicrobial activity along with the releasing property of potassium sorbate was also examined in glycerol-tapioca starch-based films (Flores et al. 2007).

Another recent research aimed to develop 'green' bacterial cellulose (BC)-based bioactive and biocompatible food packaging material by using plant oils (olive oil and ginger oil) as antimicrobial agents. A composite film containing 2 wt.% BC slurry, 30% wt.% carboxymethylcellulose (CMC), and 30% wt.% glycerol (Gly) was ex-situ developed and separately impregnated with 1–2 wt.% olive oil and ginger oil. The BC/CMC/Gly/Olive oil edible film showed good antimicrobial activity against three bacterial strains (*Staphylococcus aureus, Pseudomonas aeruginosa*, and *Escherichia coli*) and two fungal strains (*Candida albicans* and *Trichosporon* sp.) by producing clear inhibition zones of 0.1 cm, 0.1 cm, 0.22 cm, 0.08 cm, and 0.15 cm, respectively, after 24 h, while the BC/CMC/Gly/Ginger oil film respectively produced inhibition zones of 0.1 cm, 0.11 cm, 0.1 cm, 0.04 cm, and 0.05 cm after 24 h. The findings of this study indicate that the developed BC/CMC/Gly/Oil composite films could be potentially used in developing edible packaging materials (Atta et al. 2022).

Structural analysis

X-ray Diffraction (XRD)

In materials research, XRD analysis is employed to analyze the structure of the crystals, the ratio of the crystalline and amorphous areas, crystal organization forms,

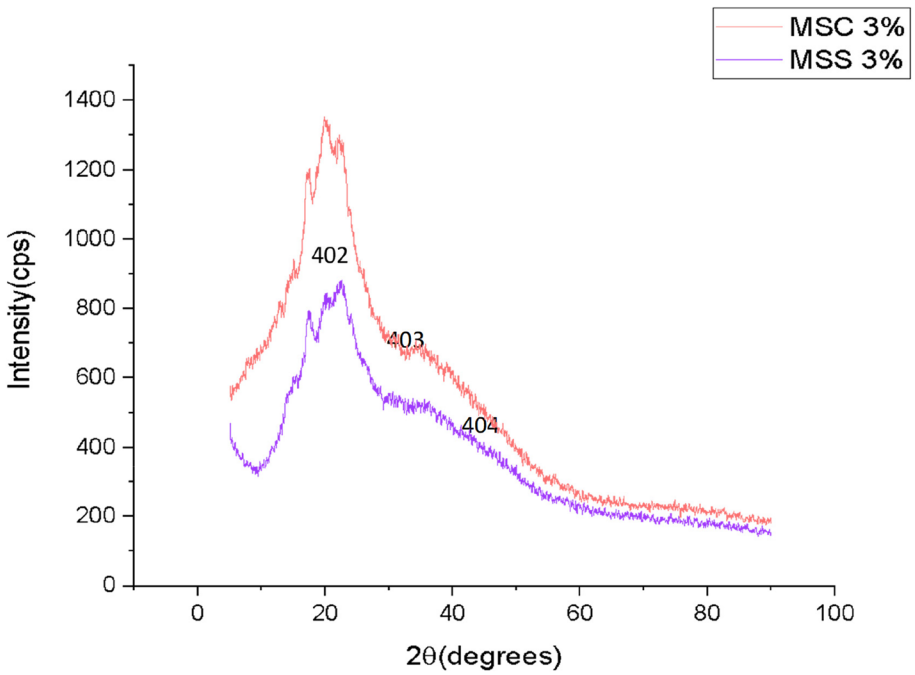


Fig. 2 XRD patterns of the MSC 3% and MSS 3% films

and size of the crystals (Aritonang et al. 2020). The XRD patterns for both the examined films (MSC 3% and MSS 3%) are shown in Fig. 2. Nearly the same semicrystalline phase was observed in both tested films, with distinctive peaks at 2 theta angles of 21° and 24°. The crystallinity of the MSC 3% film was found to be 28.23%. The X-ray diffraction of the MSC 3% film has shown intensity peaks at 1200 cps, 1400 cps and 1300 cps at small 2Θ angles, such as 19°, 20°, and 21°. Thus, a narrow width peak was observed, implying that these were larger crystals of crystalline nature (the peak width is inversely related to crystal size; a narrower peak correlate to a larger crystal). The intensity peak maxima were observed at 1400 cps. Additionally, the peak width was found to be wide compared to other peak widths at different intensities, showing that the crystal achieved an amorphous nature. A larger peak indicates a smaller crystal, a flaw in the crystalline structure, or that the sample is amorphous in nature, meaning it lacks complete crystallinity. Similarly, the crystallinity of the MSS 3% film was found to be 20.41%. The X-ray diffraction pattern of MSS 3% showed intensity peaks at 800 cps, 910 cps and 912 cps at 2Θ angles of 18°, 20°, and 22°, respectively. Thus, a wide width peak was observed, implying that these were larger crystals of crystalline nature. The intensity peak maximum was observed at 912 cps, while the peak width at 800 cps was wide compared to other peak intensities depicting the amorphous state of the crystal and a smaller size. Adding microbial cellulose fibers to the polysaccharide blend increased the crystallinity of the biocomposite films. This improved value signifies enhanced filler distribution in the cellulose matrix. These results are consistent with earlier research (Abral et al. 2021; Merino et al. 2018).

Scanning electron microscopy (SEM)

The detailed morphological characterization of the MSC 3% and MSS 3% film samples was carried out using SEM, which is also known as field emission scanning electron microscopy (FE-SEM). Both the MSC 3% and MSS 3% films demonstrated a flat surface and strong structural integrity, with no holes or fractures. After drying, the films remained smooth and compressed. The cellulose nanofibril network configuration was observed in both films (Fig. 3, a-b). Chitosan, alginate, and starch might be smoothly disseminated throughout the fibril network, with reasonably high interfacial bonds between the materials (Wenling et al. 2005). These findings might be attributable to the strong connections between microbial cellulose, starch, and chitosan, as well as microbial cellulose, starch, and sodium alginate, which were induced by hydrogen bonding between microbial cellulose's -OH (hydroxyl groups) and the C=O (carbonyl group) in other polymers. The nanofibers of the produced microbial cellulose exhibited void space dominated by rod-shaped, porous fibers with a diameter of 100 nm and width of 11 mm. It also showed strong interfacial adhesion between the cellulosic fibers and other composites. These ultrafine fibrils improved the polymer's tensile strength and elongation while also smoothing the surface by minimizing vapor evaporation. Furthermore, the arrangement of fibers at the nanoscale helped in increasing the surface area. Due to the presence of pores in nanofibers, it can hold ample amounts of water along with molecules of other composites, resulting in a higher water holding capacity (WHC) and water absorption rate (WAR). The lower hardness and higher tensile strength, WHC and WAR can be utilized in many fields, such as food packaging materials, wound dressings and face mask production. The result of SEM analysis is consistent with previous studies performed by MohammadKazemi et al. (2015) and Avcioglu et al. (2021).

Fourier transform infrared spectroscopy (FTIR)

FTIR spectroscopy has frequently been used to determine particular chemical bonds or functional groups present in a substance. Since the molecular structures

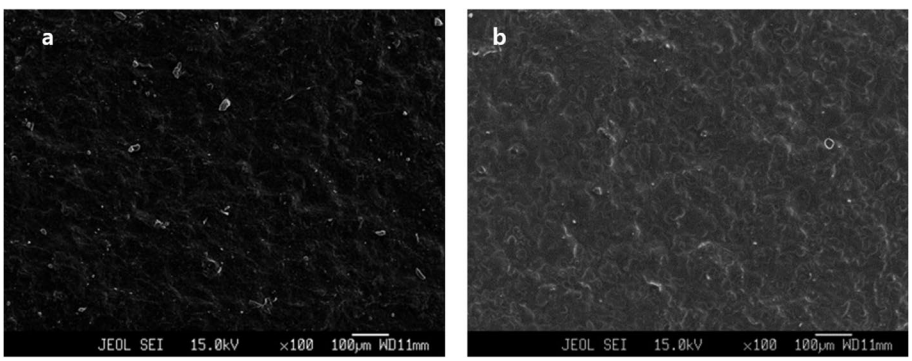


Fig. 3 SEM micrographs of (**a**) MSS 3% film and (**b**) MSC 3% films

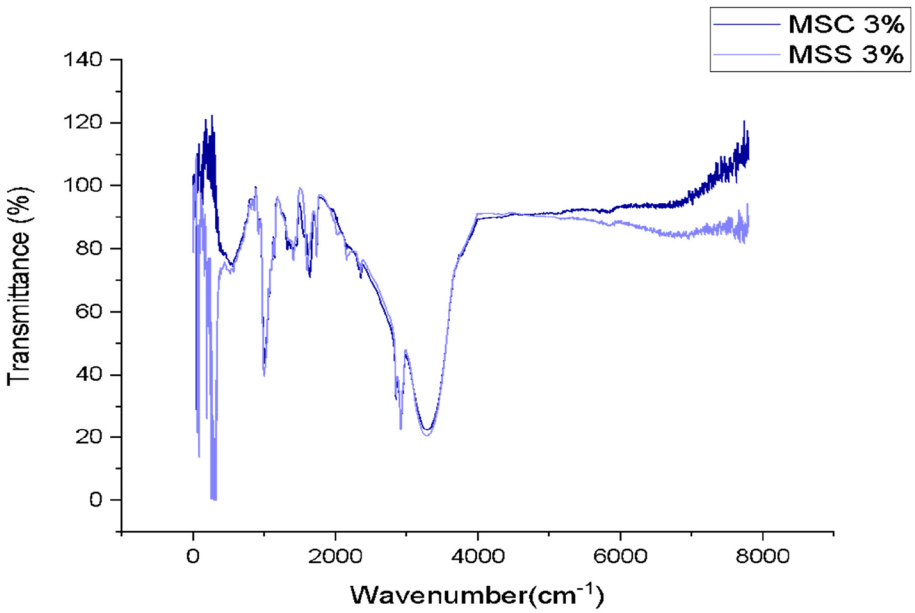


Fig. 4 FTIR spectra of MSC 3% and MSS 3% films

of microbial cellulose, sodium alginate, starch and chitosan are very similar, it is anticipated that these polysaccharides have excellent compatibility and miscibility. The spectra of MSC 3% and MSS 3% are shown in Fig. 4, depicting the peak locations of numerous functional groups. The spectra of MSC 3% depicted distinctive peaks for hydroxide (O–H) vibration at 3292.86 cm^{-1}, which has been found earlier (Ul-Islam et al. 2012). Likewise, for the C-H group, peaks were observed at 2919.7 cm^{-1}, which also coincides with previous research (Ul-Islam et al. 2013). The presence of the C-H group was further substantiated by the development of multiple peaks at 1454 to 1244 cm^{-1} in accordance with the C-H bending curve. The vibrations for the C-O or carbonyl group were observed at 1643.05 cm^{-1}, as mentioned by previous researchers (Ul-Islam et al. 2012). The C–O–C group resulted in the formation of peaks at 1020.16 cm^{-1}, which corresponded with previous work (Shah et al. 2010).

The spectrum of MSS 3% indicated the peak locations of many functional groups. The spectra of MSS 3% demonstrated distinctive peaks for hydroxide (OH) vibration at 3271.64 cm^{-1}, which has been found earlier (Ul-Islam et al. 2013). Likewise, for the C-H group, peaks were observed at 2360.7 cm^{-1}, which also coincided with previous research (Ul-Islam et al. 2013). The presence of the C-H group was further substantiated by the development of multiple peaks at 1455 to 1245 cm^{-1} in accordance with the C-H bending curve. The vibrations for the C-O or carbonyl group were observed at 1605.45 cm^{-1}, as mentioned by previous researchers (Ul-Islam et al. 2012). The C–O–C group resulted in the formation of peaks at 999.91 cm^{-1}, which corresponded with previous work (Shah et al. 2010).

Thermogravimetric Analysis (TGA)

The heat sensitivity of microbial cellulose composites, particularly at high temperature, is a very important characteristic for utilizing it commercially. Figure 5 shows TGA curves of both the MSC 3% and MSS 3% films as a function of temperature. The TGA curves of both films were similar. The graph depicted three different stages of weight reduction of the films. The first stage (100–200 °C) is associated with weight loss in the film as a result of evaporation of absorbed water. For the MSC 3% films, approximately 20% of the total weight loss occurred in this temperature range, while for the MSS 3% films, approximately 23% of the weight loss occurred. The greater loss in weight at this particular temperature could be because of the higher water holding capacity of microbial cellulose.

In the second stage (200–300 °C), sharp and sheer weight loss was observed because of the degradation of microbial cellulose and other polymers. During this period, the MSC 3% film lost up to 66% of its original weight, while the MSS 3% film lost approximately 64% of its original weight, thus depicting the highest weight loss. The third zone of weight reduction was observed

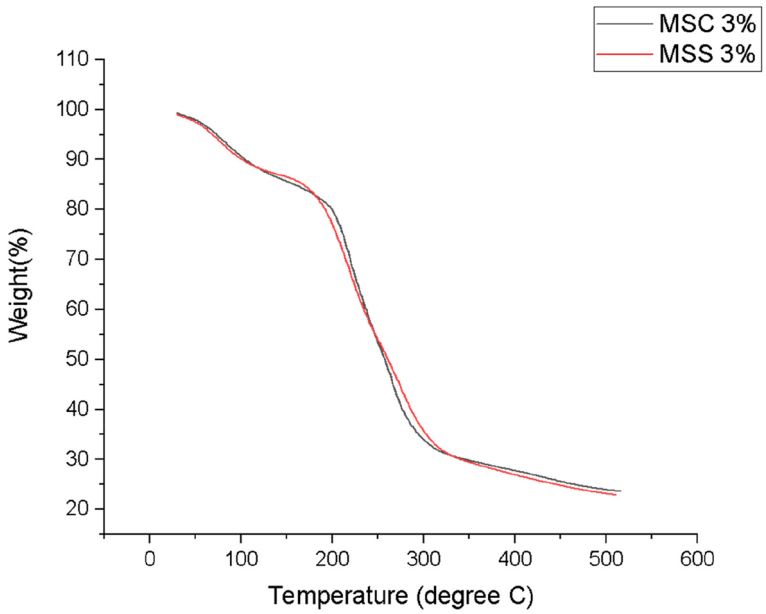


Fig. 5 TGA analysis of MSC 3% and MSS 3%

at 300–500 °C. During this phase, the final decomposition of both the MSC 3% and MSS 3% films takes place, changing them into ash. The results signified the thermal stability of the composite films. The thermal stability is mainly because of the well-arranged and compact structure of the microfibrils present in the microbial cellulose. Furthermore, the improved interfacial hydrogen bonds between polymers and homogeneously dispersed cellulosic nanofibers resulted in enhanced heat resistance (Abral et al. 2018, 2021).

Conclusion

The objective of this research work focused on the synthesis and characterization of active microbial cellulose-based films with enhanced properties and functionalities. Through systematic investigation and experimentation, several key findings and achievements were obtained, contributing to the advancement of active film technology. The synthesis of microbial cellulose was successfully achieved using a microbial fermentation process. The obtained microbial cellulose-based composites exhibited high purity and structural integrity, making them excellent candidates for coating applications in various foods and film formation. The antimicrobial activity of the clove extract-incorporated composite solution and films was evaluated against various microorganisms, indicating their potential application as both coating and food packaging materials.

Acknowledgements
The authors are thankful to Central Instrumentation Laboratory, Central University of Punjab, Bathinda (Punjab) and Sophisticated Instrumentation Centre for Applied Research and Testing, Anand, Gujarat for providing the analytical facilities.

Authors' contributions
PS designed the research and supervised the whole work. MA conducted the research, analysed the data, and wrote the manuscript. UI and KS helped in result analysis and manuscript writing.

Funding
The research work does not receive any fund from agencies in the public, commercial, or nonprofit segments.

Availability of data and materials
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have "no competing interests" in this section.

Author details
[1]Centre of Food Technology, University of Allahabad, Prayagraj 211002, India.

Received: 31 October 2023 Accepted: 19 February 2024
Published online: 14 March 2024

References

Abral, H., Pratama, A. B., Handayani, D., Mahardika, M., Aminah, I., Sandrawati, N., & Ilyas, R. A. (2021). Antimicrobial edible film prepared from bacterial cellulose nanofibers/starch/chitosan for a food packaging alternative. *International Journal Polymer Science, 2021*, 1–11. https://doi.org/10.1155/2021/6641284

Abral, H., Anugrah, A. S., Hafizulhaq, F., Handayani, D., Sugiarti, E., & Muslimin, A. N. (2018). Effect of nanofibers fraction on properties of the starch based biocomposite prepared in various ultrasonic powers. *International Journal Biological Macromolecules, 116*, 1214–1221. https://doi.org/10.1016/j.ijbiomac.2018.05.067

Ahmed, M., Saini, P., & Iqbal, U. (2021). Microbial cellulose based films and composites for food packaging: a review. The annals of the University Dunarea de Jos of Galati. *Fascicle VI-Food Technology, 45*(1), 178–198. https://doi.org/10.35219/foodtechnology.2021.1.12

Ahmed, M., Saini, P., & Iqbal, U. (2023). Bio cellulose-based edible composite coating for shelf-life extension of tomatoes. *Food and Humanity, 1*, 973–984. https://doi.org/10.1016/j.foohum.2023.08.016

Albuquerque, R. M., Meira, H. M., Silva, I. D., Silva, C. J. G., Almeida, F. C. G., Amorim, J. D., & Sarubbo, L. A. (2021). Production of a bacterial cellulose/poly (3-hydroxybutyrate) blend activated with clove essential oil for food packaging. *Polymers Polymer Composites, 29*(4), 259–270. https://doi.org/10.1177/0967391120912098

Amorim, L. F., Mouro, C., Riool, M., & Gouveia, I. C. (2022). Antimicrobial food packaging based on prodigiosin-incorporated double-layered bacterial cellulose and chitosan composites. *Polymers, 14*(2), 315.

Aritonang, H. F., Kamea, O. E., Koleangan, H., & Wuntu, A. D. (2020). Biotemplated synthesis of Ag-ZnO nanoparticles/bacterial cellulose nanocomposites for photocatalysis application. *Polymer-Plastics Technology and Materials, 59*(12), 1292–1299.

Atieno, L., Owino, W., Ateka, E. M., & Ambuko, J. (2018). Effect of surface coatings on the shelf life and quality of cassava. *Journal of Food Research, 7*, 46–60. https://doi.org/10.5539/jfr.v7n1p46

Atta, O. M., Manan, S., Ul-Islam, M., Ahmed, A. A. Q., Ullah, M. W., & Yang, G. (2022). Development and characterization of plant oil-incorporated carboxymethyl cellulose/bacterial cellulose/glycerol-based antimicrobial edible films for food packaging applications. Advanced Composites and Hybrid Materials, 1–18. https://doi.org/10.1007/s42114-021-00408-9

Avcioglu, N. H., Birben, M., & Bilkay, I. S. (2021). Optimization and physicochemical characterization of enhanced microbial cellulose production with a new Kombucha consortium. *Process Biochemistry, 108*, 60–68. https://doi.org/10.1016/j.procbio.2021.06.005

Bhak, J., Yousef, A. E., & Martha, E. H. (1990). Behavior of *Listeria monocytogens* in the presence of clove oil. *Lebensm Wiss U Technol, 23*, 66–69.

Casalini, S., & Giacinti Baschetti, M. (2023). The use of essential oils in chitosan or cellulose-based materials for the production of active food packaging solutions: a review. *Journal of the Science of Food and Agriculture, 103*(3), 1021–1041. https://doi.org/10.1002/jsfa.11918

Cazón, P., & Vázquez, M. (2021). Bacterial cellulose as a biodegradable food packaging material: a review. *Food Hydrocolloids, 113*, 106530. https://doi.org/10.1016/j.foodhyd.2020.106530

Ciechanska, D. (2004). Multifunctional bacterial cellulose/chitosan composite materials for medical applications. *Fibers Text East Eur, 12*(4), 69–72.

Dashipour, A., Khaksar, R., Hosseini, H., Shojaee-Aliabadi, S., & Ghanati, K. (2014). Physical, antioxidant and antimicrobial characteristics of carboxymethyl cellulose edible film cooperated with clove essential oil. *Zahedan Journal of research in medical Sciences, 16*(8):34–42.

Di, Z., Shi, Z., Ullah, M. W., Li, S., & Yang, G. (2017). A transparent wound dressing based on bacterial cellulose whisker and poly (2-hydroxyethyl methacrylate). *International Journal of Biological Macromolecules, 105*, 638–644. https://doi.org/10.1016/j.ijbiomac.2017.07.075

Dobre, L. M., Stoica, A., Stroescu, M., Jinga, S., Jipa, I., & DOBRE, T. (2010). Characterization of composite materials based on biocellulose membranes impregnated with silver particles as antimicrobial agent. *UPB Sci Bull Series B, 72*, 53–58.

Dobre, L. M., Stoica-Guzun, A., Stroescu, M., Jipa, I. M., Dobre, T., Ferdeş, M., & Ciumpiliac, Ş. (2012). Modeling of sorbic acid diffusion through bacterial cellulose-based antimicrobial films. *Chemical Papers, 66*, 144–151. https://doi.org/10.2478/s11696-011-0086-2

Farag, R. S., Daw, Z. Y., & Abo-Raya, S. H. (1989). Influence of some spice essential oils on *Aspergillus parasiticus* growth and production of aflatoxins in a synthetic medium. *Journal of Food Science, 54*(1), 74–76. https://doi.org/10.1111/j.1365-2621.1989.tb08571.x

Farooq, U., Ullah, M. W., Yang, Q., Aziz, A., Xu, J., Zhou, L., & Wang, S. (2020). High-density phage particles immobilization in surface-modified bacterial cellulose for ultrasensitive and selective electrochemical detection of *Staphylococcus aureus*. *Biosensors and Bioelectronics, 157*, 112163. https://doi.org/10.1016/j.bios.2020.112163

Fatima, A., Yasir, S., Ul-Islam, M., Kamal, T., Ahmad, M. W., Abbas, Y., ... & Yang, G. (2022). Ex situ development and characterization of green antibacterial bacterial cellulose-based composites for potential biomedical applications. Advanced Composites and Hybrid Materials, 1–15. https://doi.org/10.1007/s42114-021-00369-z

Flores, S., Haedo, A. S., Campos, C., & Gerschenson, L. (2007). Antimicrobial performance of potassium sorbate supported in tapioca starch edible films. *European Food Research and Technology, 225*, 375–384. https://doi.org/10.1007/s00217-006-0427-5

Fu, Y., Zu, Y., Chen, L., Shi, X., Wang, Z., Sun, S., & Efferth, T. (2007). Antimicrobial activity of clove and rosemary essential oils alone and in combination. *Phytotherapy Research, 21*(10), 989–994. https://doi.org/10.1002/ptr.2179

Güzel, M., & Akpınar, Ö. (2019). Production and characterization of bacterial cellulose from citrus peels. *Waste and Biomass Valorization, 10*, 2165–2175. https://doi.org/10.1007/s12649-018-0241-x

Haghighi, H., Gullo, M., La China, S., Pfeifer, F., Siesler, H. W., Licciardello, F., & Pulvirenti, A. (2021). Characterization of bionanocomposite films based on gelatin/polyvinyl alcohol blend reinforced with bacterial cellulose nanowhiskers for food packaging applications. *Food Hydrocolloids, 113*, 106454. https://doi.org/10.1016/j.foodhyd.2020.106454

Han, J. H. (2014). Edible films and coatings: a review. *Innovations in food packaging*, 213–255. https://doi.org/10.1016/B978-0-12-394601-0.00009-6

Huang, T., Qian, Y., Wei, J., & Zhou, C. (2019). Polymeric antimicrobial food packaging and its applications. *Polymers, 11*(3), 560. https://doi.org/10.3390/polym11030560

Hubbe, M. A., Ferrer, A., Tyagi, P., Yin, Y., Salas, C., Pal, L., & Rojas, O. J. (2017). Nanocellulose in thin films, coatings, and plies for packaging applications: a review. *BioResources, 12*(1), 2143–2233.

Jalali, N., Ariiai, P., & Fattahi, E. (2016). Effect of alginate/carboxyl methyl cellulose composite coating incorporated with clove essential oil on the quality of silver carp fillet and Escherichia coli O157: H7 inhibition during refrigerated storage. *Journal of Food Science and Technology, 53*, 757–765.

Jimenez, A., Fabra, M. J., Talens, P., & Chiralt, A. (2012). Edible and biodegradable starch films: A review. *Food and Bioprocess Technology, 5*, 2058–2076. https://doi.org/10.1007/s11947-012-0835-4

Jipa, I. M., Stoica, A., Stroescu, M., Dobre, L. M., Dobre, T., Jinga, S., & Tardei, C. (2012). Potassium sorbate release from poly (vinyl alcohol)-bacterial cellulose films. *Chemical Papers, 66*, 138–143. https://doi.org/10.2478/s11696-011-0068-4

Jozala, A. F., Pértile, R. A. N., dos Santos, C. A., de Carvalho Santos-Ebinuma, V., Seckler, M. M., Gama, F. M., & Pessoa, A. (2015). Bacterial cellulose production by *Gluconacetobacter xylinus* by employing alternative culture media. *Applied Microbiology and Biotechnology, 99*, 1181–1190. https://doi.org/10.1007/s00253-014-6232-3

Mahardika, M., Abral, H., Kasim, A., Arief, S., Hafizulhaq, F., & Asrofi, M. (2019). Properties of cellulose nanofiber/bengkoang starch bionanocomposites: Effect of fiber loading. *Lwt, 116*, 108554.

Maisanaba, S., Llana-Ruiz-Cabello, M., Gutiérrez-Praena, D., Pichardo, S., Puerto, M., Prieto, A. I., & Cameán, A. M. (2017). New advances in active packaging incorporated with essential oils or their main components for food preservation. *Food Reviews International, 33*(5), 447–515. https://doi.org/10.1080/87559129.2016.1175010

Maneerung, T., Tokura, S., & Rujiravanit, R. (2008). Impregnation of silver nanoparticles into bacterial cellulose for antimicrobial wound dressing. *Carbohydrate Polymers, 72*(1), 43–51. https://doi.org/10.1016/j.carbpol.2007.07.025

Marvizadeh, M. M., Oladzadabbasabadi, N., Nafchi, A. M., & Jokar, M. (2017). Preparation and characterization of bionanocomposite film based on tapioca starch/bovine gelatin/nanorod zinc oxide. *International Journal of Biological Macromolecules, 99*, 1–7.

Merino, D., Mansilla, A. Y., Gutiérrez, T. J., Casalongué, C. A., & Alvarez, V. A. (2018). Chitosan coated-phosphorylated starch films: Water interaction, transparency and antibacterial properties. *Reactive and Functional Polymers, 131*, 445–453. https://doi.org/10.1016/j.reactfunctpolym.2018.08.012

Mohammadkazemi, F., Azin, M., & Ashori, A. (2015). Production of bacterial cellulose using different carbon sources and culture media. *Carbohydrate Polymers, 117*, 518–523. https://doi.org/10.1016/j.carbpol.2014.10.008

Mulla, M., Ahmed, J., Al-Attar, H., Castro-Aguirre, E., Arfat, Y. A., & Auras, R. (2017). Antimicrobial efficacy of clove essential oil infused into chemically modified LLDPE film for chicken meat packaging. *Food Control, 73*, 663–671. https://doi.org/10.1016/j.foodcont.2016.09.018

Nazzaro, F., Fratianni, F., De Martino, L., Coppola, R., & De Feo, V. (2013). Effect of essential oils on pathogenic bacteria. *Pharmaceuticals, 6*(12), 1451–1474. https://doi.org/10.3390/ph6121451

Padrao, J., Gonçalves, S., Silva, J. P., Sencadas, V., Lanceros-Méndez, S., Pinheiro, A. C., & Dourado, F. (2016). Bacterial cellulose-lactoferrin as an antimicrobial edible packaging. *Food Hydrocolloids, 58*, 126–140. https://doi.org/10.1016/j.foodhyd.2016.02.019

Pascall, M. A., & Lin, S. J. (2013). The application of edible polymeric films and coatings in the food industry. *Food Processing & Technology, 4*, e116. https://doi.org/10.4172/2157-7110.1000e116

Reller, L. B., Weinstein, M., Jorgensen, J. H., & Ferraro, M. J. (2009). Antimicrobial susceptibility testing: a review of general principles and contemporary practices. *Clinical Infectious Diseases, 49*(11), 1749–1755. https://doi.org/10.1086/647952

Saraiva, L. E. F., Naponucena, L. D. O. M., da Silva Santos, V., Silva, R. P. D., de Souza, C. O., Souza, I. E. G. L., & Druzian, J. I. (2016). Development and application of edible film of active potato starch to extend mini panettone shelf life. *LWT, 73*, 311–319.

Shah, A., Jani, M., Shah, H., Chaudhary, N., & Shah, A. (2014). Antimicrobial effect of Clove oil (*Laung*) extract on *Enterococcus faecalis*. *Journal of Advanced Oral Research, 5*(3), 36–38.

Shah, N., Ha, J. H., & Park, J. K. (2010). Effect of reactor surface on production of bacterial cellulose and water soluble oligosaccharides by *Gluconacetobacter hansenii* PJK. *Biotechnology and Bioprocess Engineering, 15*, 110–118. https://doi.org/10.1007/s12257-009-3064-6

Shahidi, F., & Hossain, A. (2022). Preservation of aquatic food using edible films and coatings containing essential oils: A review. *Critical Reviews in Food Science and Nutrition, 62*(1), 66–105. https://doi.org/10.1080/10408398.2020.1812048

Shi, Z., Zhang, Y., Phillips, G. O., & Yang, G. (2014). Utilization of bacterial cellulose in food. *Food Hydrocolloids, 35*, 539–545. https://doi.org/10.1016/j.foodhyd.2013.07.012

Ul-Islam, M., Wajid Ullah, M., Khan, S., Kamal, T., Ul-Islam, S., Shah, N., & Kon Park, J. (2016). Recent advancement in cellulose based nanocomposite for addressing environmental challenges. *Recent Patents on Nanotechnology, 10*(3), 169–180.

Ul-Islam, M., Khan, T., Khattak, W. A., & Park, J. K. (2013). Bacterial cellulose-MMTs nanoreinforced composite films: Novel wound dressing material with antibacterial properties. *Cellulose, 20*, 589–596. https://doi.org/10.1007/s10570-012-9849-3

Ul-Islam, M., Khan, T., & Park, J. K. (2012). Nanoreinforced bacterial cellulose–montmorillonite composites for biomedical applications. *Carbohydrate Polymers, 89*(4), 1189–1197. https://doi.org/10.1016/j.carbpol.2012.03.093

Wenling, C., Duohui, J., Jiamou, L., Yandao, G., Nanming, Z., & Xiufang, Z. (2005). Effects of the degree of deacetylation on the physicochemical properties and Schwann cell affinity of chitosan films. *Journal of Biomaterials Applications, 20*(2), 157–177. https://doi.org/10.1177/0885328205049897

Xu, L., Zhang, B., Qin, Y., Li, F., Yang, S., Lu, P., & Fan, J. (2020). Preparation and characterization of antifungal coating films composed of sodium alginate and cyclolipopeptides produced by *Bacillus subtilis*. *International Journal of Biological Macromolecules, 143*, 602–609. https://doi.org/10.1016/j.ijbiomac.2019.12.051

Yanti, N. A., Ahmad, S. W., Ramadhan, L. O. A. N., Jamili, M., Walhidayah, T., & Mamangkey, J. (2021). Properties and application of edible modified bacterial cellulose film based sago liquid waste as food packaging. *Polymers, 13*(20), 3570. https://doi.org/10.3390/polym13203570

Zheng, L. Y., & Zhu, J. F. (2003). Study on antimicrobial activity of chitosan with different molecular weights. *Carbohydrate Polymers, 54*(4), 527–530. https://doi.org/10.1016/j.carbpol.2003.07.009

Publisher's Note