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MULTIFUNCTIONAL SERICIN-CHITOSAN-*Aloe vera* COMPOSITE FILM FOR FOOD PACKAGING

Abstract: In recent years, the demand for innovative, sustainable, and efficient food packaging solutions has surged in response to growing concerns about environmental impact, food safety, and quality preservation. A sericin-based polymer composite film with multifunctional properties shows promise as an alternative for enhancing food packaging. In this study, sericin-based composite films were prepared by incorporating *Aloe vera* gel, chitosan, and glycerol into a sericin solution (1.5 % w/v) through facile homogenisation at 70 °C, followed by casting and subsequent drying on a glass platform. The resulting dried film exhibited uniformity, a smooth texture, and successful integration of the composite components. The film demonstrated a moisture content of 21.02 % and a porosity of 3.56 %, with a thickness of (62.1 ± 2.3) µm. It exhibited moderate transparency with reasonable water vapour permeability. Notably, the DPPH scavenging results indicated that the film has a potent antioxidant capacity with an efficacy rate of 99.1 %, supported further by a phenolic content of 11.5 mg GAE per gram of film. Controlled solute migration of components from the composite films was observed, particularly under acidic conditions. Importantly, toxicity evaluation on A549 cells revealed no adverse effects, even at higher concentrations. Due to its consistent film-forming ability, antioxidant potency, controlled migration, and safe nature, the developed sericin polymer-based film could be an effective alternative for food packaging sensitive foods, maintaining oxidative stability, reducing moisture loss, improving quality, and extending shelf life.

Keywords: sericin composite, edible, antioxidant, non-toxic, chitosan, *Aloe vera*

Introduction

In an era increasingly focused on sustainability and environmental responsibility, innovations in food packaging have gained paramount importance [1]. Conventional packaging materials and films frequently contribute to plastic waste and raise ecological

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concerns [2]. Mainly derived from petrochemicals from natural gas or crude oil, traditional food packaging materials pose significant environmental challenges due to their non-biodegradable nature and potential for pollution [3]. The production and disposal of these materials deplete resources, emit greenhouse gases, and contribute to waste accumulation [2]. Often, they are disposed of in landfills or oceans, posing threats to wildlife and ecosystems. It is imperative to make efforts to decrease their usage and enhance recyclability to reduce their environmental footprint [1, 4].

Consequently, novel strategies, such as the development of composite edible films and packaging materials using natural elements, are explored as alternative solutions [1]. In this context, the remarkable potential of silk sericin has emerged [3]. There is a need to investigate environmentally friendly and functional food packaging materials using sericin. By incorporating sericin, this study focuses on developing safe and effective packaging solutions that maintain food quality, extend shelf life, and reduce environmental impact.

Derived from *Bombyx mori* silkworm cocoons, sericin presents an exciting opportunity for repurposing [3]. A significant portion of sericin, ~12 % of the annual production of around 50,000 tonnes (tonne = 10^6 g = Mg), goes to waste after the degumming process [5]. This not only leads to environmental implications due to disposal but also overlooks sericin's valuable properties, including its antioxidative, antimicrobial, and UV-resistant attributes [3, 5]. Sericin possesses remarkable attributes such as biocompatibility, biodegradability, and the ability to form films [6]. Its incorporation into composite materials, like edible films, offers the potential to create substances that not only maintain the freshness and quality of packaged foods but also align with environment-conscious principles [3, 7, 8].

Composite films refer to films created by combining two or more distinct biopolymers to achieve synergistic effects and enhance specific properties [9]. Chitosan and *A. vera* are natural biopolymers for their roles in crafting composite and edible films, with applications spanning from food packaging to biomedicine [10-14]. Chitosan, sourced from crustacean shells, brings film-forming capabilities, biodegradability, and exceptional barrier properties against moisture and gases [11]. Its inherent antimicrobial properties elevate product safety and eco-friendliness, rendering it a sustainable packaging solution [15]. *A. vera*, renowned for its healing properties, enriches films with moisture retention and antioxidants like vitamins C and E, alongside compounds like polyphenols and flavonoids [16]. These antioxidants shield packaged foods from oxidative deterioration, sustaining their quality for a long term [17]. Both chitosan and *A. vera* can seamlessly combine with other biopolymers such as starch, protein, or cellulose to produce composite films tailored to diverse applications [12, 16].

This study undertakes an exploration of sericin's capacity to form films, synergistically combined with complementary additives like chitosan and *A. vera*. It encompasses various aspects, from the extraction and modification of sericin to the integration of chitosan's antimicrobial and film-forming qualities, as well as the infusion of *A. vera*'s bioactive advantages. Additionally, the study comprehensively investigates film characteristics and performance attributes, including moisture content, water vapour permeability, colour, antioxidant capacity, phenolic content, controlled migration behaviour, and cell line toxicity. Harnessing the multifaceted properties of sericin polymer composite film with additives like chitosan and *A. vera*, this work presents a fresh avenue for the development of edible coatings and film-forming materials. The stated key ingredients and the sericin composite film material as a whole hold the potential to safeguard and elevate the quality of

agricultural products, while simultaneously championing sustainability and economic feasibility.

Materials and methods

Materials procurement

Sericin was sourced from the Central Silk Technological Research Institute (CSTRI) in Bangalore, India. Chitosan (Low MW, extrapure, 10 m·Pa·s - 150 m·Pa·s, 90 % DA), sodium hydroxide (NaOH), DPPH (2,2'-diphenyl-1-picrylhydrazyl), Foline-Ciocalteu reagent, sodium carbonate, and glacial acetic acid were purchased from the SRL company in India. *A. vera* was obtained from a local nursery in Avadi, Chennai, while food-grade glycerine was acquired from Fisher Scientific, India. All other chemicals used were of analytical grade and were obtained from SRL in India.

Preparation of individual ingredients

Chitosan aqueous solution

The Chitosan aqueous solution was prepared following the method outlined by Escamilla-García et al. [18]. It involved dissolving 1 g of low molecular weight chitosan in 0.6 % (v/v) glacial acetic acid of 100 mL. This mixture was then stirred for an hour at 750 rpm using a REMI magnetic stirrer.

Sericin solution

The preparation of the Sericin solution was based on the procedure detailed by Ghosh et al. [19]. In this process, Sericin powder was treated with alkaline protease at a pH of 9 using 1 M NaOH. The mixture was incubated at 60 °C for 90 min and then allowed to cool to room temperature. Following this, the solution was heated at 100 °C for 10 min to inactivate the enzyme. Subsequently, the solution was subjected to centrifugation at 10,000 rpm for 10 min. The resulting supernatant was collected and used for the film preparation.

A. vera extract

The extraction of *A. vera* gel was performed using the methodology described by Qamar et al. [20]. Fresh *A. vera* leaves were washed with distilled water, and the colourless hydroparenchyma gel from the outer cortex of the leaves was extracted by carefully removing the spiky margins. The collected gel was then ground using a high-speed blender and filtered through muslin cloth to achieve a homogeneous solution. This solution was pasteurised at 70 °C for 45 min, and the gel was stabilised by cooling it to room temperature.

Preparation of sericin-based composite film

To prepare the sericin-based composite film, an enzyme-treated sericin solution (40 mL, 1.5 % w/v) was combined with *A. vera* gel (40 mL, 1 % w/v) and a chitosan solution (1 % w/v), as described in our previous study [3]. The mixture was thoroughly stirred for 20 min at 700 rpm to ensure uniform mixing. Glycerol, at a concentration of 1.5 % w/v, was then added as a plasticiser to improve the flexibility of the composite solution. The mixture was heated to 70 °C while continuously stirring at 1000 rpm until

a clear and homogeneous film-forming solution was obtained, maintaining a final volume of 40 mL. The resulting solution was casted onto levelled Petri plates and left to dry at room temperature. Once dried, the films were carefully peeled off from the Petri plates and subjected to various analyses to evaluate their properties.

Physico-chemical analysis of the composite film

Film thickness measurement

Film thickness measurements were conducted using a micrometer. Prior to these measurements, the micrometer was calibrated according to the standard procedure outlined by Basiak et al. [21]. The film's thickness was measured at five different points, and the average value was calculated in mm.

Porosity assessment

The porosity, *Pr* of the films was determined using the dry-wet weight method [22, 23], employing the following equation:

$$Pr = (M1 - M2) / (\rho_w \text{ x } A \text{ x } L)$$

where $M1$ represents the weight of the wet film, $M2$ is the weight of the dry film, ρ_w denotes the water density, A stands for the film's area, and L is the film's thickness [mm] (in a wet state).

Moisture content analysis

Moisture content analysis was conducted on the films following the method outlined by Kalaycioglu et al. [24]. Small film specimens collected after conditioning were placed on a plate within a WENSAR PGB-1MB Touch screen moisture analyser (Kolkata, India). These samples were then heated at a consistent rate until they reached a stable weight at 105 °C. The percentage of weight loss relative to the original weight was used to quantify the total moisture present in the samples. This procedure was performed in triplicate for each film.

Water vapour permeability test

The water vapour permeability of the films was assessed using a gravimetric approach based on ASTM method E 96-80 with modifications [25]. Film samples were sealed over a circular opening (0.016 m^2) within a permeation cell. This cell was filled with distilled water up to a depth of 1 cm beneath the film. A humidity chamber was maintained at 50 % relative humidity (*RH*), and the permeation cells were secured to prevent water vapour leakage. Placed in a controlled environment at 25 °C and 50 % *RH*, the permeation cells' weight was measured hourly for up to 8 h, with the weight loss recorded. The water vapour transmission rate (*WVTR*) was determined from the slope of the steady-state portion of the weight loss graph [g·H_2O/s] divided by the permeation cell area [m^2]. The water vapour permeability of the film was then calculated using the formula [g·m/m^2·Pa·s]:

$$\text{WVP} = (\text{WVTP x } L) / \Delta P$$

where L represents the film's thickness [mm], and ΔP is the vapour pressure of water at room temperature (25 °C), equating to 0.0313 atm or 23.8 mm of Hg, which is equivalent to 3137.073 Pa.

Colour assessment

The absorbance was analysed using a UV-visible spectrophotometer (Cyberlab UV-100 USA) following the reported standard approach [26]. It involves determining the ratio of absorbance in nm to the film's thickness in μm. The films were fashioned into rectangular strips and placed directly into the spectrophotometer cell, using an empty cell as a reference. Absorbance was measured at 600 nm, and opacity was computed using the formula:

$$T = A_{600\text{nm}} / x$$

where T is the transparency, $A_{600\text{nm}}$ represents the absorbance of the sample at 600 nm, and x is the film's thickness in mm. This process was repeated at least three times, and the mean value was reported.

Colour assessment of the films was accomplished using a Spectra suit UV-Vis spectrophotometer. The colour parameters range from L: 0 (black) to 100 (white); a: –60 (green) to + 60 (red); b: –60 (blue) to + 60 (yellow). The total colour difference (ΔE) of the films was expressed using the equation [27]:

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

where $\Delta L^*(L - L0)$, Δa^* $(a - a0)$, and $\Delta b^*(b - b0)$ represent the differences between the sample's colour parameters and the colour parameters of a standard (L^* = 101.6, a^* = –2.4, b^* = 1.86) used as the film's background.

Water contact angle analysis

The water contact angle of the film samples was assessed using a contact angle analyser (Model DMe-211Plus, Kyowa Interface Science Co Ltd., Japan). Film pieces (3 cm × 3 cm) were affixed on a movable stage, and a water drop (~2 μL) was dispensed onto the surface. Contact angles were promptly measured, with the average of five values computed for each sample.

DPPH radical scavenging assay

The DPPH radical scavenging activity was conducted using a spectrophotometer following the methodology proposed by Siripatrawan and Harte in 2010 [28]. A film sample weighing 25 mg was dissolved in 3 mL of methanol and thoroughly mixed. Subsequently, 2.8 mL of the methanolic film extract was combined with 0.2 mL of a 1 mM methanolic DPPH solution. The prepared solution was vigorously vortexed for 1 minute at 2500 rpm and then incubated for 1 hour in darkness. After the incubation period, the absorbance of the solution was measured at 517 nm using a spectrophotometer. The percentage of radical scavenging activity was calculated using the following formula:

$$\%\text{DPPH radical scavenging activity} = \frac{\text{Abs}_{\text{DPPH}} - \text{Abs}_{\text{extract}}}{\text{Abs}_{\text{DPPH}}} \cdot 100$$

Total phenolic content

The total phenolic content of the film was determined according to the procedure outlined by Ruiz-Navajas et al. [29], utilising the Foline Ciocalteu reagent. To prepare the film extract, 25 mg of the film was dissolved in 3 mL of methanol and thoroughly mixed. Following this, 0.3 mL of the film extract was combined with 2.5 mL of the Foline

Ciocalteu reagent and 2 mL of a 7.5 % sodium carbonate solution. This mixture was then incubated at 50 °C for 5 min. After the incubation, the absorbance of the film solution was measured at 760 nm using a spectrophotometer and compared to a gallic acid calibration curve.

Solvent migration test

The migration test for films was conducted following the method described by Ni et al. [30]. In this test, three food stimulants (distilled water, 10 % alcohol, and 3 % acetic acid) were selected in accordance with Commission Regulation (EU) No 10/2011 standards. A film sample weighing 0.1 g was dissolved in 50 mL of each of the three food stimulants and placed at a minimum temperature of 25 °C for 7 days. The migration levels of the film in the food stimulants were assessed by measuring the initial and final weights of the film.

Cell line toxicity analysis

The cytotoxic effects of sericin-based films with varying weights (ranging from 3 mg to 15 mg) were assessed against human lung adenocarcinoma (A549) cell lines over 24, 48, and 72 h. This analysis was carried out with the collaboration of the Geobiotechnology Laboratory at National College, Thiruchirapalli, Tamil Nadu, and followed the methodology outlined by Grenha et al. [31]. The positive control utilised in the study was sodium dodecyl sulphate (2 % SDS). Preliminary experiments ensured the optimal cell seeding density and confirmed the absence of thiazolyl blue tetrazolium bromide (MTT) conversion in the assay medium.

All test and control samples were prepared as film/solution in pre-warmed cell culture medium (CCM) supplemented with 2 % fetal bovine serum (FBS) immediately prior to their application on the cells. After 24 h, 48 h, and 72 h of incubation with the different formulations, the culture medium of A549 cell lines at 24 h was replaced with 100 µL of fresh medium containing varying concentrations of the test substance and control solutions.

Subsequently, 50 µL of the MTT solution (0.5 mg/mL in PBS, pH = 7.3) was added to each well. After 4 h incubation, the medium was removed, and any formazan pellet formed was dissolved using a 100 µL surfactant solution containing 10 % SDS in DMF: water (1:1). Once the pellet was completely solubilised, the absorbance of each well was measured at 570 nm using a spectrophotometer; with background absorbance corrected using a wavelength of 650 nm.

The relative cell viability [%] was calculated as follows:

$$\text{Viability} = \frac{A - S}{CM - S} \cdot 100$$

where *A* represents the absorbance obtained for each concentration of the test substance, *S* is the absorbance obtained for the 2 % SDS, and *CM* is the absorbance obtained for untreated cells (incubated with CCM). The latter reading was taken as indicative of 100 % cell viability. The assay was conducted on three separate occasions, with six replicates at each concentration of the test substance for each instance.

SEM and FTIR analysis

The morphological assessment of the sericin-based edible films was conducted using scanning electron microscopy (SEM), TESCAN VEGA3 model, Czech Republic. Additionally, the film surfaces were examined at various magnifications.

For the film samples, the Fourier-transform infrared spectroscopy (FTIR) spectra were obtained using the Cary 600 FT-IR Spectrometer, Agilent, India which was equipped with an attenuated total reflectance (ATR) accessory. This analysis aimed to identify the functional groups present in the samples within the wave number range of 4500 cm^{-1} to 650 cm^{-1}. The acquired spectra of the samples were subjected to analysis with air serving as the background reference.

Absorbance spectra of the film

The absorbance spectra of the film was evaluated between 200 and 800 nm using a UV Spectrophotometer (Cyberlab UV-100 USA). The bio nanocomposite films were cut (60 mm long and 40 mm wide) and directly placed in a spectrophotometer test cell. An empty glass plate was used as the reference.

Results and discussion

Microscopic and FTIR analysis

Combining enzyme-treated 1.5 % sericin solution and 1 % *A. vera* gel with 1 % chitosan solution resulted in a well-mixed composite solution. The addition of 1.5 % glycerol enhanced the flexibility of sericin-based composite film. Further, mixing of ingredient mixture at 70 °C with stirring resulted in a clear, homogeneous film-forming solution forms (Fig. 1a). Casting onto Petri plates and air-drying yielded consistent and flexible films (Fig. 1b). Figure 1c,d show both low and high magnification SEM images of the prepared sericin composite film. These pictures suggest that the composite film is relatively homogenous, continuous and intact without any deformation.

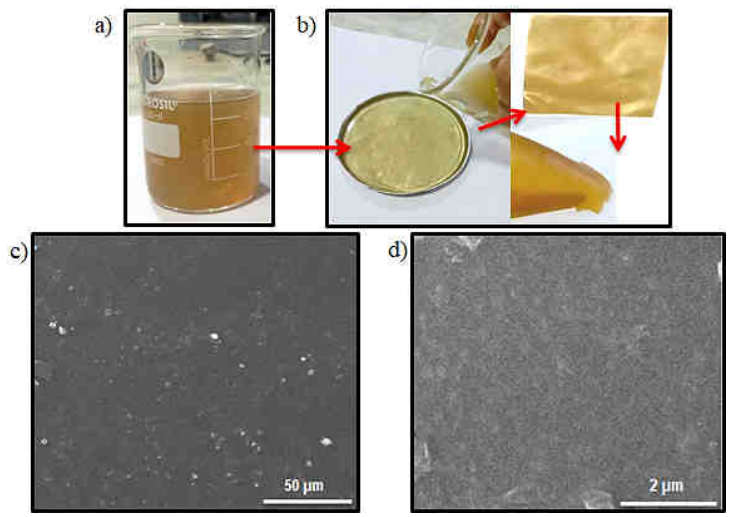

Fig. 1. a) Sericin-based composite formulation, b) the casting process and the resulting flexible dried film, and c), d) low and high magnification SEM images of the dried film

Our results are in good agreement with other reported studies where their films were obtained by individual sericin, chitosan and *A. vera* based coatings [7, 16, 20].

This microscopic analysis may be useful in optimising film production and enhancing performance for applications like food packaging, where surface characteristics influence barrier properties, stability, and aesthetics.

FTIR analysis of sericin composite film is crucial for identifying functional groups present in the film's composition [26]. It enables the characterisation of chemical bonds, polymer interactions, and additive incorporation. Sericin composite formulation used in this study was applied as a thin surface coating for tomatoes in our earlier study [3], and its lone film-forming ability through the casting solution method was explored in this study. The ATR-FTIR spectra in Figure 2 reveal the structural and compositional characteristics of chitosan, sericin, *A. vera* gel, and the sericin composite film, emphasising the functional groups present in each material and their interactions within the composite.

For chitosan (Fig. 2, curve a), the spectrum shows a broad absorption band at approximately 3430 cm^{-1} due to O-H stretching which is characteristic of polysaccharides [3, 23]. Peaks at 2925 cm^{-1} and 2875 cm^{-1} correspond to C-H stretching vibrations, while the band at ~1647 cm^{-1} is attributed to amide I (C=O stretching) [3]. The peak at 1587 cm^{-1} represents amide II (N-H bending), and a band at ~1375 cm^{-1} is assigned to C-H bending vibrations, indicating the presence of CH_2 groups. Additionally, a peak at 1013 cm^{-1} was due to C-O stretching in chitosan [3, 23].

The spectrum of sericin (Fig. 2, curve b) displays prominent peaks at 3265 cm^{-1} due to N-H and O-H stretching vibrations, indicating the protein-based nature of sericin [5, 7]. Amide I and II bands, appearing around 1640 cm^{-1} and 1540 cm^{-1}, respectively, correspond to C=O stretching and N-H bending, typical of protein structures [3, 5, 7]. A peak at 1235 cm^{-1} is associated with C-N stretching vibrations, further confirming the presence of proteins in sericin [3, 5].

The *A. vera* gel spectrum (Fig. 2, curve c) shows a broad peak at 3300 cm^{-1} due to O-H stretching vibrations, indicating the presence of polysaccharides and water content [10]. The peak at 2922 cm^{-1} is attributed to CH_2 stretching vibrations [3]. A characteristic peak at ~1700 cm^{-1} is due to C=O stretching, indicative of carbonyl groups in carboxylic acids and esters, while a peak at ~1000 cm^{-1} corresponds to C-O stretching from alcohols and ethers in *A. vera* gel [3, 10, 32].

Figure 2 also shows the FTIR spectra of the casted lone sericin composite film. The observations from sericin composite film (curve d) are as follows: the absorption band from 3500 cm^{-1} - 3200 cm^{-1} was assigned to -OH and -NH stretching vibrations. The band at 2925 cm^{-1} is attributed to the -CH stretching. The ascribed peak bands may arise due to their evidence in sericin, chitosan, *A. vera* and glycerol as reported in our earlier study [3]. Furthermore, the peak band at 1645 cm^{-1}, 1520 cm^{-1} and 1238 cm^{-1} corresponds to amide I, II and III of sericin in the composite film [33]. Characteristic peaks of chitosan are usually present at ~1640 cm^{-1} (for C=O stretching band of the acetyl group (amide I)) and at ~1550 cm^{-1} (for -NH bending and stretching (amide II)) [34]. While the peak band at ~1640 cm^{-1} merged with the sericin band, the other peak at 1559 cm^{-1} was evident in the sericin composite film. A peak at 1399 cm^{-1} is associated with O-H bending vibration, and the peak at 1028 cm^{-1} is assigned to C-O stretching from the groups of chitosan and *A. vera* [32, 35]. The absorption band at 1149 cm^{-1} and 924 cm^{-1} are ascribed to the saccharide structure of chitosan [35]. The majority of the peaks are features from the film matched with our earlier report [3] and the disappearance of the key peak bands of individual ingredients in the composite film is the indication of their participation in bonding to form

a composite matrix. Therefore, FTIR analysis supports validating composite film formulation and confirms the presence of desired components.

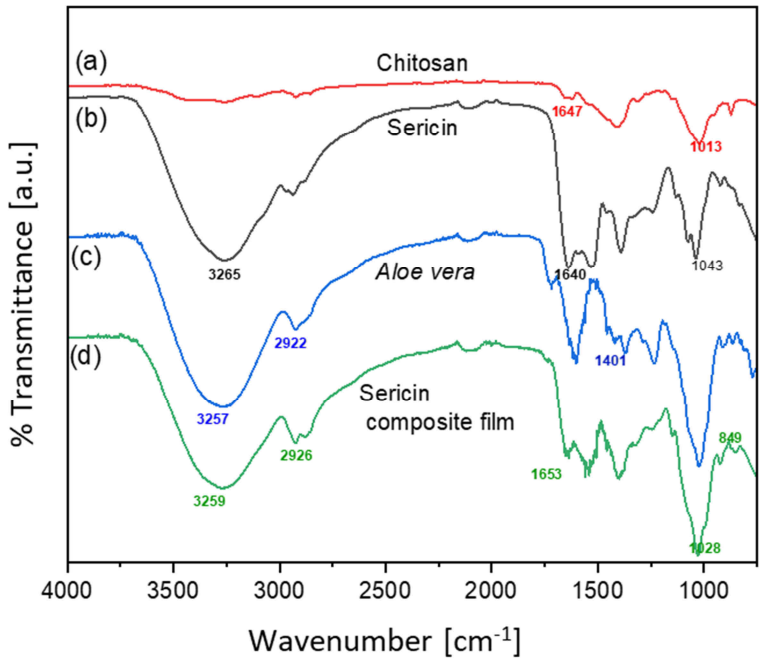

Fig. 2. ATR-FTIR spectra of chitosan, sericin, *A. vera* gel and sericin composite film

Further, the sericin composite films were subjected to various analyses to evaluate thickness, porosity, moisture content, water vapour permeability, opacity, colour, DPPH scavenging, phenolic content, solvent migration, and cell line toxicity. Table 1 shows the average thickness of the film determined by measuring at multiple points. Moreover, its moisture content relative to its initial weight was observed to be 21.02 %. The observed value is acceptable as chitosan addition to the film formulations usually decreases the moisture content [36, 37] and the same was observed with our obtained sericin composite film. Porosity assessment reveals how permeable the films are, and the percentage of porosity exhibited by the sericin-based film was 3.56 %. This value is low when compared with the reported studies with biodegradable films formed through the casting approach [38]. Porosity affects film properties like gas, moisture permeability, strength, texture, and compound release. High porosity reduces strength, affects texture, and shelf life. Edible film porosity varies by application and a 10 % - 30 % was considered as a typical value, balancing barriers and strength [39]. Therefore, the obtained film has a promising scope where enhanced barrier properties are expected.

The moisture permeability assessment

The sericin-based film was evaluated using a water vapour permeability test and weight loss measurements over time (Fig. 3a). Values for WVTR, WV permeance, and WVP were computed and presented in Table 1.

Table 1

Material properties and permeability parameters of the produced sericin composite film

Parameters	Values
Thickness [µm]	62.1 ± 2.3
Moisture content [%]	21.02
WVTR [g·H_2O/s/m^2]	2.326 · 10^{-3}
WVP [g·m/m^2·Pa·s]	1.368 · 10^{-9}
Porosity of the film [%]	3.56
Water contact angle (*WCA*) [°]	58.4 ± 3.6
Opacity, *T* [%]	4.11 ± 0.09

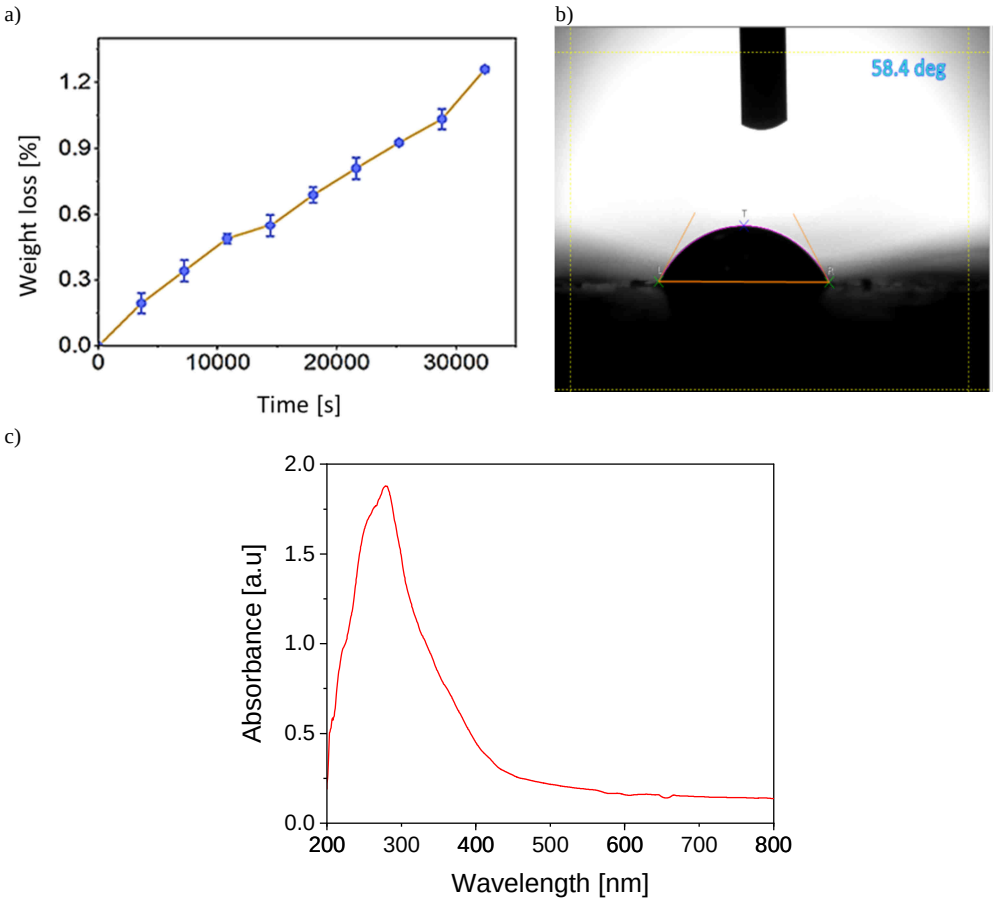

Fig. 3. a) Time-based weight loss measured for water vapour permeability using a sericin composite film placed over a circular opening within a permeation cell, b) water contact angle and c) UV-Visible absorbance spectrum of film made from sericin based composite formulation

The WVP values were analysed using analysis of variance (ANOVA), and significant differences were determined at $p < 0.05$ level. The developed film showed a momentous ($P < 0.05$) effect on the weight loss percentage (Fig. 3) and the WVP value observed was $1.368 \cdot 10^{-9}$ g·m/m^2·Pa·s, which is lower than that of the chitosan film (with a reported value of $(1.12 \pm 0.18) \cdot 10^{-9}$ g·m/m^2·Pa·s) [40]. This suggests that the formed film allowed greater water vapour transmission and prompted further analysis of its wettability. Surface wettability was determined by measuring the contact angle of a water droplet on the film's surface, as detailed in Table 1 and Figure 3b. The film's water contact angle was (58.4 ± 3.6)°, lower than the lone chitosan film's reported value (~64.5°) [40]. This confirms that the sericin film exhibits relatively lower hydrophobicity than the pure chitosan film. The presence of hydrophilic groups, such as hydroxyl, carboxylic, and amine groups in sericin, is expected to regulate water absorption in the sericin-based composite film.

UV-Visible and colour analysis

The absorbance spectrum of the sericin-based composite film (Fig. 3c) shows a distinct peak around 200 nm - 300 nm, which is characteristic of protein-based materials like sericin [3, 32]. The peak observed in the UV region (around 200 nm - 280 nm) is likely attributed to the presence of aromatic amino acids (such as tyrosine and tryptophan) in sericin, which absorb strongly in the UV range [3, 32]. This suggests that the sericin protein retains its native structure within the composite film. The gradual decrease in absorbance beyond 300 nm indicates a lack of significant chromophores absorbing in the visible region, which is expected for a film primarily composed of natural biopolymers such as sericin, *A. vera* and chitosan [3, 10, 12]. The low absorbance in the visible range also suggests that the film would be relatively transparent, which could be advantageous for applications where optical clarity is required, such as in food packaging or biomedical uses [3, 10, 12].

The presence of chitosan and *A. vera*, known for their film-forming and antimicrobial properties, does not introduce additional absorbance peaks, indicating compatibility and uniform blending with sericin [10, 12]. Glycerol, as a plasticiser contributes to the flexibility of the film but does not significantly affected the UV-Vis absorbance profile in Figure 3c. Thus, the UV-Visible spectral profile suggests that the sericin-based composite formulation forms a uniform film with desirable optical properties and maintains the structural integrity of its protein components.

In addition to the above, insights into film opacity and colour attributes provide valuable information regarding their visual properties and potential applications. The colour of the sericin composite film is a significant factor influencing its appearance and consumer acceptance. This is particularly important for packaged food items, as the film's colour can impact how consumers perceive the contents. The film's colour parameters, such as L^*, a^*, and b^* values, were analysed. A higher L^* value indicates greater colourlessness and brightness, while positive a^* and b^* values indicate redness and yellowness, respectively. The total colour difference (ΔE) helps assess overall colour changes. The sericin film exhibited specific colour coordinates with values $L^* = 83$, $a^* = -6.3$, and $b^* = 34.9$. The value of ΔE was found to be 36.61. Higher ΔE values indicate films with higher colour intensity. The colour values of pure *Bombyx mori* sericin are usually near to white ($L^* = 100$), greenness ($a^* = -60$), and yellowness ($b^* = -60$) which indicate more lightness and more green and yellow colour intensities [41]. These values can be influenced by additives and plasticisers such as saccharides and lipids [42]. Therefore, in comparison to

prior studies, the inclusion of chitosan and *A. vera* in the composite film influenced the brightness (L^*) and colour intensity (ΔE) values, resulting in slightly reduced overall values. However, these alterations are considered acceptable for edible films [43].

The sericin composite film that was produced exhibited an average thickness of (62.1 ± 2.3) mm, and the calculated opacity value was ~4.11 (Table 1). Typically, sericin tends to yield films that are somewhat translucent or transparent due to its protein-based nature, and the additives utilised in this study are known to contribute to transparent film formation. Given that the sericin composite film comprises a blend of sericin, chitosan, and *A. vera* gel, it is quite likely to result in a film that possesses a reasonable level of translucency [28]. This expectation was confirmed by a slightly higher T value compared to other films, such as chitosan films with or without additives. However, this opacity value remains within an acceptable range for antioxidant composite films.

The DPPH radical scavenging assay

The sericin-based film's antioxidant properties were examined. The film exhibited a remarkable scavenging activity of 99.1 %, indicating heightened antioxidant potential attributed to the incorporation of sericin with additives, *A. vera*, and chitosan. When antioxidants interact with DPPH, the solution's absorbance diminishes, reflecting scavenging ability [44]. The sericin film's constituents, including glutathione, cysteine, phenolic compounds, along with *A. vera*'s phenolic content, and chitosan's residual amino groups, possibly contributed to the observed enhanced scavenging activity. Furthermore, this study utilised a film concentration of ~7.7 mg/mL to achieve a ~99 % free radical scavenging effect in the DPPH assay. This value is significantly lower than the doses reported in other studies, for instance maximum effect was noticed with chitosan-based films of concentration ~300 mg/mL [28]. As a result, the substantial antioxidant effect of the sericin composite film reported in this study could prove advantageous for long-term food storage applications.

The total phenolic content assay

The produced sericin film gauges the concentration of antioxidant-rich phenolic compounds [45]. This indicates the film's potential to counteract free radicals, prolong food quality, and convey potential health benefits to consumers *via* packaged foods. An estimated total phenolic content (TPC) of 11.5 mg/GAE per gram of sericin composite film suggests a positive presence of antioxidant-rich phenolic compounds [46]. This is generally considered good, as higher TPC values indicate potential health benefits and enhanced oxidative stability in the film and the foods it interacts with. The antioxidant potential of the composite film is influenced by the TPC, which varies depending on the individual antioxidant properties of its components. The composite film developed in this study comprises *A. vera*, chitosan, and sericin as key ingredients. Among these, *A. vera* and sericin are primarily responsible for the antioxidant activity in the film [16, 41]. Sericin, constituting significant portion (1.5 % w/v) of the film is rich in amino acids and could contribute to antioxidant activity by providing protection against oxidative stress [3, 41]. Moreover, *A. vera* also contribute with its known bioactive compounds such as polyphenols, flavonoids, and vitamins towards antioxidant potential [16]. The combination of these components synergy could enhance the overall antioxidant capacity of the composite film making it effective for food packaging.

Solvent migration test

This test assessed sericin-based film components migration into the simulants by comparing initial and final film weights, indicating potential substance transfer between the films and the food simulants. Migration testing was conducted using three distinct food stimulants: distilled water, 10 % alcohol, and 3 % acetic acid, representing neutral, fatty, and acidic solutions, respectively. The migration percentages of film content into these solvents are illustrated in Figure 4. Notably, the sericin-based film exhibited higher migration levels in the acidic solution compared with water and alcohol solutions. Approximately 56 % of the sericin composite film content dissolved in the acidic solution. Conversely, migration was lower in the 10 % alcohol solution compared with water solution. *A. vera* gel has partial solubility in distilled or deionised water, while chitosan readily dissolves in mild acids due to its protonated amino groups, enhancing interaction with polar compounds. Furthermore, sericin's solubility characteristics make it exhibit more significant migration in acidic solutions. Despite the desirable properties of edible film components like sericin, chitosan, and *A. vera*, controlled migration can enhance packaged food quality and shelf life [47].

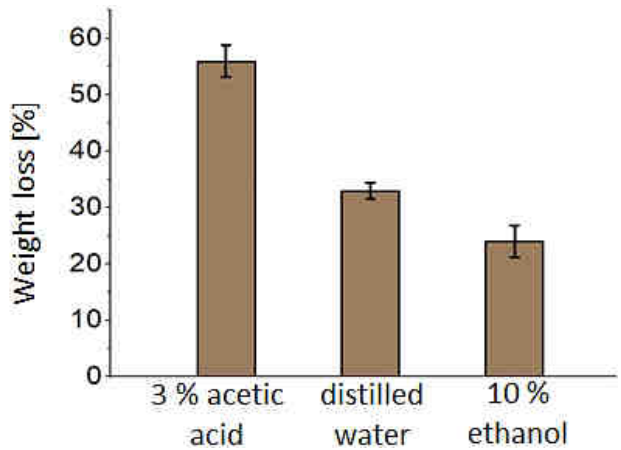

Fig. 4. Comparative analysis of weight loss from the sericin composite film in different solutions of food stimulants

Cell line toxicity analysis

This test evaluates the impact of materials/substances on cell viability. It is crucial for evaluating potential harm to living cells, aiding in determining safe concentrations for applications in diverse fields ranging from medicine to materials [48]. Cell viability assessment in toxicity analysis with various test samples, differing in their weights (Fig. 5), demonstrated consistent effects on the cell viability of A549 cell lines. The percentage of cell viability is presented in Figure 5, while the corresponding confocal microscopic images are displayed in Figure 6. As the film weight approached 15 mg or consisted of 5 film discs, the cell viability exhibited a decline, reaching ~94.9 % and 88.8 % after 48 h and 72 h of storage, respectively. No significant difference was observed in the control group over the 72 h test period. The notable ~12.2 % reduction in cell viability at 72 h was attributed

to the application of the highest film concentration (15 mg) on A549 cells. Remarkably, the sericin film (3 mg) demonstrated only a ~2.5 % reduction in cell viability after 72 h.

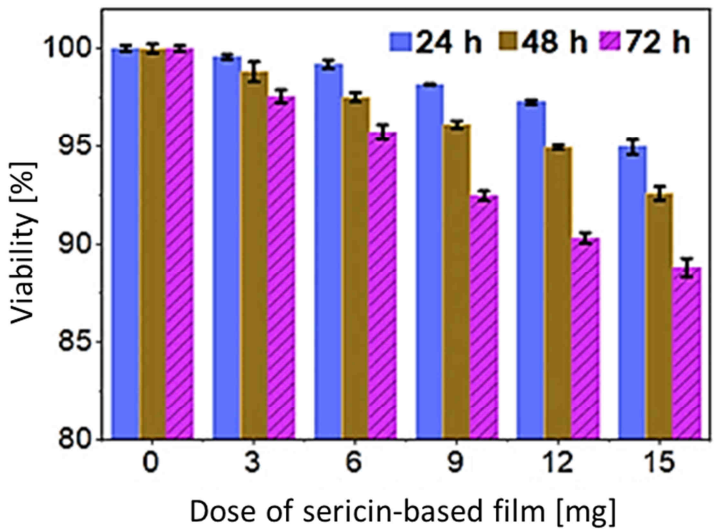

Fig. 5. Bar graph depicting the cell viability percentage in response to various doses of sericin-based film, ranging from 0 mg to 15 mg in 100 µL of medium

In the sericin composite film, chitosan's antimicrobial properties can contribute to cell death by disrupting cell membranes. However, the film's safety for edible applications depends on the concentrations of each component and adherence to regulatory guidelines to ensure non-toxicity and suitability for food contact or consumption. The concentrations tested in this study ranged from 0 mg to 15 mg in 0.1 mL of media, corresponding to strengths of 0 %, 3 %, 6 %, 9 %, 12 %, and 15 % and A549 cells were used for testing.

The U.S. Food and Drug Administration (FDA) categorises chitosan as 'Generally Recognised as Safe' (GRAS) for use in foods [49]. Human studies have shown that doses of 1 g to 6 g of chitosan per day produce no significant adverse effects [50]. The concentrations used in our study are significantly higher than the reported safety limits. Despite this, the sericin-*A. vera*-chitosan composite film did not show significant toxicity to A549 cells even at higher doses, such as 3 mg/0.1 mL (3 %). Therefore, based on these findings, the developed films are likely safe for use as edible coatings apart from packagings as well.

This study presents the development and comprehensive characterisation of a sericin-based composite film, enhanced with *A. vera* and chitosan additives. The investigation provides a detailed understanding of the film's physical, mechanical, optical, and biological properties, demonstrating its potential to be used as food packaging material and as an edible coating. Compared to conventional food packaging materials like polyethylene, polypropylene, and cellulose-based films [22, 24], sericin based film offers distinct advantages as they exhibit good barrier properties by being biodegradable and non-toxic to the environment [3, 7, 8]. The sericin based composite film developed in this study displayed moderate water vapour permeability and good transparency similar to

cellulose-based films [22, 24]. Wherein these films provide additional antioxidant protection which is not typically present in synthetic or cellulose-based packaging materials.

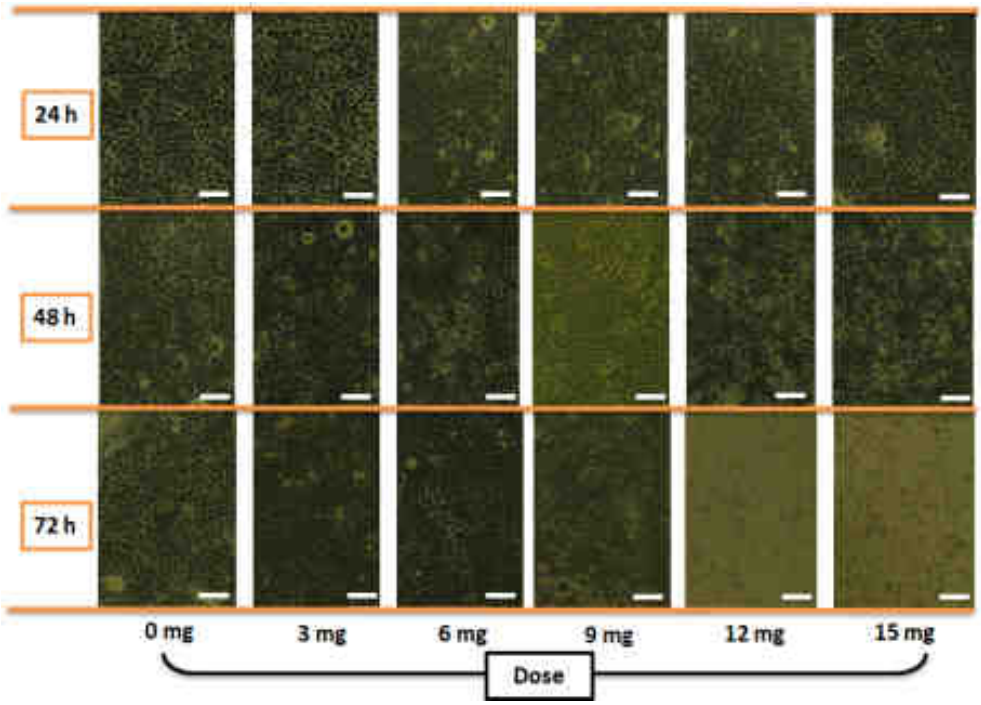

Fig. 6. Confocal microscopic images of A549 cell lines with 0 mg to 15 mg dose of sericin composite film at 24 h, 48 h, and 72 h. The scale bar in each Figure corresponds to 50 µm

Furthermore, in this study, the sericin-based composite film's gradual release of its components makes it a good candidate for food coating applications, as it could help maintain food quality and extend shelf life. In contrast to other edible coatings, such as alginate, pectin, or starch-based coatings - which despite their potential [1, 20, 21], have limitations like relatively low antioxidant activity and may alter the taste or texture of food. The sericin composite formulation with film forming ability observed in this study offers superior antioxidant activity and could be better compatible with food surfaces. The film's non-toxic nature demonstrated in this study along with the biocompatibility and biodegradability of its individual components (sericin, chitosan, *A. vera*) [8, 11, 16, 51], makes it a sustainable alternative that can effectively enhance food safety and prolong shelf life. Thus, there is considerable potential for further research on this sericin-based composite formulation, particularly for innovative edible coating applications on various foods, which our research group is actively pursuing.

Conclusion

The study focuses on a composite film composed of sericin, *A. vera* gel, and chitosan. The formulated film solution exhibited homogeneity and formed consistent films upon drying. Microscopic analysis confirmed the film's uniform structure. FTIR analysis validated ingredient incorporation into a composite matrix. The film displayed lower water vapour permeability compared with chitosan while maintaining acceptable transparency. The film demonstrated exceptional antioxidant scavenging activity (99.1 %) due to combined sericin, *A. vera*, and chitosan. Adequate total phenolic content suggested potential oxidative stability in packaged foods. Controlled migration tests indicated varying substance transfer, with higher migration in acidic solutions. Cell viability tests revealed minimal toxicity, even at elevated concentrations. This study highlights the composite film's potential for versatile applications, particularly in food packaging, aligning with contemporary standards and consumer preferences for enhanced barrier properties, stability, and visual appeal.

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References

[1] Versino F, Ortega F, Monroy Y, Rivero S, López OV, García MA. Sustainable and bio-based food packaging: a review on past and current design innovations. Foods. 2023;12:1057. DOI: 10.3390/foods12051057.

[2] Sid S, Mor RS, Kishore A, Sharanagat VS. Bio-sourced polymers as alternatives to conventional food packaging materials: A review. Trends Food Sci Technol. 2021;115:87-104. DOI: 10.1016/j.tifs.2021.06.026.

[3] Tarangini K, Kavi P, Jagajjanani Rao K. Application of sericin-based edible coating material for postharvest shelf-life extension and preservation of tomatoes. eFood. 2022;3:e36. DOI: 10.1002/efd2.36.

[4] Mollah MZI, Miah MS, Akram MW, Mahmud SH, Faruque MRI, Al-mugren KS. Thermoplastic-polymer matrix composite of banana/betel nut husk fiber reinforcement: Physico-mechanical properties evaluation. E-Polymers. 2024;24. DOI: 10.1515/epoly-2023-0158.

[5] Silva T, Silva A, Vieira M, Gimenes M, Silva M. Production and physicochemical characterization of microspheres made from sericin and alginate blend. Chem Eng Trans. 2014;39:643-8. DOI: 10.3303/CET1439108.

[6] Cheerarot O, Baimark Y. Biodegradable silk fibroin/chitosan blend microparticles prepared by emulsification-diffusion method. E-Polymers. 2015;15:67-74. DOI: 10.1515/epoly-2014-0134.

[7] He H, Cai R, Wang Y, Tao G, Guo P, Zuo H, et al. Preparation and characterization of silk sericin/PVA blend film with silver nanoparticles for potential antimicrobial application. Int J Biol Macromolecules. 2017;104:457-64. DOI: 10.1016/j.ijbiomac.2017.06.009.

[8] Yang C, Yao L, Zhang L. Silk sericin-based biomaterials shine in food and pharmaceutical industries. Smart Materials Medicine. 2023;4:447-59. DOI: 10.1016/j.smaim.2023.01.003.
[9] Martins JT, Cerqueira MA, Bourbon AI, Pinheiro AC, Souza BWS, Vicente AA. Synergistic effects between κ-carrageenan and locust bean gum on physicochemical properties of edible films made thereof. Food Hydrocolloids. 2012;29:280-9. DOI: 10.1016/j.foodhyd.2012.03.004.
[10] Ali J, Pandey S, Singh V, Joshi P. Effect of coating of Aloe vera gel on shelf life of grapes. Current Res Nutrition Food Sci J. 2016;4:58-68. DOI: 10.12944/CRNFSJ.4.1.08.
[11] Ghosh T, Katiyar V. Nanochitosan functionalized hydrophobic starch/guar gum biocomposite for edible coating application with improved optical, thermal, mechanical, and surface property. Int J Biol Macromol. 2022;211:116-27. DOI: 10.1016/j.ijbiomac.2022.05.079.
[12] Khatri D, Panigrahi J, Prajapati A, Bariya H. Attributes of Aloe vera gel and chitosan treatments on the quality and biochemical traits of post-harvest tomatoes. Scientia Horticulturae. 2020;259:108837. DOI: 10.1016/j.scienta.2019.108837.
[13] Rahman S, Konwar A, Majumdar G, Chowdhury D. Guar gum-chitosan composite film as excellent material for packaging application. Carbohydrate Polymer Technologies and Applications. 2021;2:100158. DOI: 10.1016/j.carpta.2021.100158.
[14] Jampílek J, Kráľová K. Application of nanotechnology in agriculture and food industry, its prospects and risks. Ecol Chem Eng S. 2015;22:321-61. DOI: 10.1515/eces-2015-0018.
[15] Bandara S, Du H, Carson L, Bradford D, Kommalapati R. agricultural and biomedical applications of chitosan-based nanomaterials. Nanomaterials (Basel). 2020;10:1903. DOI: 10.3390/nano10101903.
[16] Nicolau-Lapeña I, Colàs-Medà P, Alegre I, Aguiló-Aguayo I, Muranyi P, Viñas I. Aloe vera gel: An update on its use as a functional edible coating to preserve fruits and vegetables. Progress Organic Coatings. 2021;151:106007. DOI: 10.1016/j.porgcoat.2020.106007.
[17] Kapusta-Duch J, Leszczyńska T, Borczak B, Florkiewicz A, Ambroszczyk A. Impact of different packaging systems on selected antioxidant properties of frozen-stored broccoli. Ecol Chem Eng S. 2019;26:383-96. DOI: 10.1515/eces-2019-0027.
[18] Escamilla-García M, Calderón-Domínguez G, Chanona-Pérez JJ, Farrera-Rebollo RR, Andraca-Adame JA, Arzate-Vázquez I, et al. Physical and structural characterisation of zein and chitosan edible films using nanotechnology tools. Int J Biolog Macromol. 2013;61:196-203. DOI: 10.1016/j.ijbiomac.2013.06.051.
[19] Ghosh S, Rao R, Nambiar K, Haragannavar V, Augustine D, Sv S. Sericin, a dietary additive: Mini review. J Medicine, Radiology, Pathology Surgery. 2017;4:13-7. DOI: 10.15713/ins.jmrps.89.
[20] Qamar J, Ejaz S, Anjum MA, Nawaz A, Hussain S, Ali S, et al. Effect of Aloe vera gel, chitosan and sodium alginate based edible coatings on postharvest quality of refrigerated strawberry fruits of cv. Chandler. J Hortic Sci Technol. 2018:8-16. DOI: 10.46653/jhst180101008.
[21] Basiak E, Lenart A, Debeaufort F. How glycerol and water contents affect the structural and functional properties of starch-based edible films. Polymers. 2018;10:412. DOI: 10.3390/polym10040412.
[22] Vetrivel S, Saraswathi MSA, Rana D, Nagendran A. Fabrication of cellulose acetate nanocomposite membranes using 2D layered nanomaterials for macromolecular separation. Int J Biolog Macromol. 2018;107:1607-12. DOI: 10.1016/j.ijbiomac.2017.10.027.
[23] Wang W, Xue C, Mao X. Chitosan: Structural modification, biological activity and application. Int J Biolog Macromol. 2020;164:4532-46. DOI: 10.1016/j.ijbiomac.2020.09.042.
[24] Kalaycıoğlu Z, Kahya N, Adımcılar V, Kaygusuz H, Torlak E, Akın-Evingür G, et al. Antibacterial nano cerium oxide/chitosan/cellulose acetate composite films as potential wound dressing. Eur Polymer J. 2020;133:109777. DOI: 10.1016/j.eurpolymj.2020.109777.
[25] Gennadios A, Weller CL, Gooding CH. Measurement errors in water vapor permeability of highly permeable, hydrophilic edible films. J Food Eng. 1994;2:395-409. DOI: 10.1016/0260-8774(94)90062-0.
[26] Mikus M, Galus S, Ciurzyńska A, Janowicz M. Development and characterization of novel composite films based on soy protein isolate and oilseed flours. Molecules. 2021;26:3738. DOI: 10.3390/molecules26123738.
[27] Maria TMC, de Carvalho RA, Sobral PJA, Habitante AMBQ, Solorza-Feria J. The effect of the degree of hydrolysis of the PVA and the plasticizer concentration on the color, opacity, and thermal and mechanical properties of films based on PVA and gelatin blends. J Food Eng. 2008;87:191-9. DOI: 10.1016/j.jfoodeng.2007.11.026.
[28] Siripatrawan U, Harte BR. Physical properties and antioxidant activity of an active film from chitosan incorporated with green tea extract. Food Hydrocolloids. 2010;24:770-5. DOI: 10.1016/j.foodhyd.2010.04.003.
[29] Ruiz-Navajas Y, Viuda-Martos M, Sendra E, Perez-Alvarez JA, Fernández-López J. In vitro antibacterial and antioxidant properties of chitosan edible films incorporated with Thymus moroderi or Thymus piperella essential oils. Food Control. 2013;30:386-92. DOI: 10.1016/j.foodcont.2012.07.052.

[30] Ni S, Zhang H, Dai H, Xiao H. Starch-based flexible coating for food packaging paper with exceptional hydrophobicity and antimicrobial activity. Polymers. 2018;10:1260. DOI: 10.3390/polym10111260.
[31] Grenha A, Grainger CI, Dailey LA, Seijo B, Martin GP, Remuñán-López C, et al. Chitosan nanoparticles are compatible with respiratory epithelial cells in vitro. Eur J Pharm Sci. 2007;31:73-84. DOI: 10.1016/j.ejps.2007.02.008.
[32] Anju TR, Parvathy S, Valiya Veettil M, Rosemary J, Ansalna TH, Shahzabanu MM, et al. Green synthesis of silver nanoparticles from Aloe vera leaf extract and its antimicrobial activity. Materials Today: Proc. 2021;43:3956-60. DOI: 10.1016/j.matpr.2021.02.665.
[33] Castrillón Martínez DC, Zuluaga CL, Restrepo-Osorio A, Álvarez-López C. Characterization of sericin obtained from cocoons and silk yarns. Procedia Eng. 2017;200:377-83. DOI: 10.1016/j.proeng.2017.07.053.
[34] Bujňáková Z, Dutková E, Zorkovská A, Baláž M, Kováč J, Kello M, et al. Mechanochemical synthesis and in vitro studies of chitosan-coated InAs/ZnS mixed nanocrystals. J Mater Sci. 2017;52:721-35. DOI: 10.1007/s10853-016-0366-x.
[35] Fernandes Queiroz M, Melo KRT, Sabry DA, Sassaki GL, Rocha HAO. Does the use of chitosan contribute to oxalate kidney stone formation? Marine Drugs. 2015;13:141-58. DOI: 10.3390/md13010141.
[36] de Oliveira LIG, de Oliveira KÁR, de Medeiros ES, Batista AUD, Madruga MS, dos Santos Lima M, et al. Characterization and efficacy of a composite coating containing chitosan and lemongrass essential oil on postharvest quality of guava. Innovative Food Sci Emerging Technologies. 2020;66:102506. DOI: 10.1016/j.ifset.2020.102506.
[37] W. Apriliyani M, Purwadi P, Manab A, W. Apriliyanti M, D. Ikhwan A. Characteristics of moisture content, swelling, opacity and transparency with addition chitosan as edible films/coating base on casein. AJFST. 2020;18:9-14. DOI: 10.19026/ajfst.18.6041.
[38] Boyandin AN, Dvoinina LM, Sukovatyi AG, Sukhanova AA. Production of porous films based on biodegradable polyesters by the casting solution technique using a co-soluble porogen (Camphor). Polymers. 2020;12:1950. DOI: 10.3390/polym12091950.
[39] Malhotra B, Keshwani A, Kharkwal H. Natural polymer based cling films for food packaging. Int J Pharm Sci. 2015;7.
[40] Shankar S, Rhim JW. Preparation of sulfur nanoparticle-incorporated antimicrobial chitosan films. Food Hydrocolloids. 2018;82:116-23. DOI: 10.1016/j.foodhyd.2018.03.054.
[41] Seo SJ, Das G, Shin HS, Patra JK. Silk Sericin protein materials: characteristics and applications in food-sector industries. Int J Molecular Sci. 2023;24. DOI: 10.3390/ijms24054951.
[42] Prakoso FAH, Indiarto R, Utama GL. Edible film casting techniques and materials and their utilization for meat-based product packaging. Polymers. 2023;15:2800. DOI: 10.3390/polym15132800.
[43] Maruddin F, Malaka R, Baba S, Amqam H, Taufik M, Sabil S. Brightness, elongation and thickness of edible film with caseinate sodium using a type of plasticizer. IOP Conf Ser: Earth Environ Sci. 2020;492:012043. DOI: 10.1088/1755-1315/492/1/012043.
[44] Wu S, Li G, Li B, Duan H. Chitosan-based antioxidant films incorporated with root extract of Aralia continentalis Kitagawa for active food packaging applications. E-Polymers. 2022;22:125-35. DOI: 10.1515/epoly-2022-0017.
[45] Takechi T, Wada R, Fukuda T, Harada K, Takamura H. Antioxidant activities of two sericin proteins extracted from cocoon of silkworm (Bombyx mori) measured by DPPH, chemiluminescence, ORAC and ESR methods. Biomed Rep. 2014;2:364-9. DOI: 10.3892/br.2014.244.
[46] Bajić M, Novak U, Likozar B. Development of bio-based chitosan films with incorporated chestnut extract. 5th World Congress on Mechanical, Chem Material Eng. 2019. DOI: 10.11159/iccpe19.107.
[47] Dordevic S, Dordevic D, Sedlacek P, Kalina M, Tesikova K, Antonic B, et al. Incorporation of natural blueberry, red grapes and parsley extract by-products into the production of chitosan edible films. Polymers. 2021;13:3388. DOI: 10.3390/polym13193388.
[48] Taghizadeh M, Mohammadifar MA, Sadeghi E, Rouhi M, Mohammadi R, Askari F, et al. Photosensitizer-induced cross-linking: A novel approach for improvement of physicochemical and structural properties of gelatin edible films. Food Research Int. 2018;112:90-7. DOI: 10.1016/j.foodres.2018.06.010.
[49] US FDA. GRAS Notice No. GRN 000997. US FDA Center for Food Safety Applied Nutrition. 2021. Available from: https://www.fda.gov/media/158880/download [accessed September 4, 2024].
[50] Abourehab MAS, Pramanik S, Abdelgawad MA, Abualsoud BM, Kadi A, Ansari MJ, et al. Recent advances of chitosan formulations in biomedical applications. Int J Molecular Sci. 2022;23:10975. DOI: 10.3390/ijms231810975.
[51] Tarangini K, Huthaash K, Nandha Kumar V, Kumar ST, Jayakumar OD, Wacławek S, et al. Eco-friendly bioplastic material development via sustainable seaweed biocomposite. Ecol Chem Eng S. 2023;30:333-41. DOI: 10.2478/eces-2023-0036.