Sustainable
Food Technology

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Sustainability Spotlight

Biodegradable coatings developed from chitosan, polyvinyl alcohol, gelatin, and Rosa rubiginosa extract present an environmentally friendly technique for fruit preservation. These natural and synthetic biopolymers are combined to produce films with improved antioxidant, antibacterial and barrier properties, providing a sustainable alternative to traditional plastic packaging. By applying these coatings to strawberries, the study shows a significant increase in the shelf life while minimizing food waste and environmental impact. The addition of Rosa rubiginosa, a plant rich in bioactive compounds, enhances the value of underutilized botanical resources. This work aligns with the goals of circular bioeconomy and supports the global transition to sustainable, biodegradable materials for food packaging applications.

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Chitosan/PVA/Gelatin Derived Biodegradable Coatings Enriched with *Rosa rubiginosa* for Extended Shelf Life and Preservation of Strawberries

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Abstract

This study developed biodegradable composite coatings and films using chitosan (CS), polyvinyl alcohol (PVA), and gelatin enriched with *Rosa rubiginosa* extract (RRE) to enhance the shelf life of strawberries. The coatings were prepared through solution blending and applied via dip-coating, while the corresponding films were fabricated using solution casting. FTIR and SEM analyses confirmed the effective molecular interactions and uniform microstructure within the composite matrix. Increasing the proportion of RRE improved functional performance by reducing the moisture content, water solubility, water absorption, and oxygen ingress. The formulation with the highest RRE content (BS-03) exhibited the strongest antioxidant activity (78.8 ± 1.01% DPPH inhibition; IC_{50} = 0.3 mg/mL) and enhanced antibacterial potential against *Staphylococcus aureus*, *Bacillus subtilis*, and *Escherichia coli*. Soil burial tests revealed that the films were biodegradable, with BS-02 and BS-03 showing mass losses of up to 91.29% and 86.79% after 45 days, respectively. When applied to strawberries, the coatings significantly reduced weight loss and delayed visible spoilage, extending the shelf life by up to 10 days compared to uncoated fruit. Sensory evaluation also confirmed better color, firmness, and acceptability of the coated samples. These findings demonstrate that CS/PVA/gelatin coatings enriched with RRE offer a promising, safe, and eco-friendly approach for prolonging the freshness of perishable fruits.

Keywords: Antibacterial, Antioxidant, Dip coating technique, Solution casting, Food packaging

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1. Introduction

Approximately 1.3 billion tons of food is wasted annually, making food waste one of the major global challenges of our time, results in resource depletion and environmental damage [1]. Packaging protects food from harmful ultraviolet rays and preserves its quality, aroma, taste, and physicochemical characteristics. By preserving food, we can enhance its use and protect it from microbial decay [2]. With the rising demand for eco-friendly packaging solutions, innovative biopolymer-based coatings play a vital role in preserving food and prolonging the shelf life of perishable items, thereby positively affecting the environment [3]. Films containing plasticizers such as glycerol, honey, and propylene glycol are applied to food products to improve their nutritional, antimicrobial, and antioxidant properties [4]. The addition of natural extracts, such as essential oils or plant extracts, to chitosan-based films improves their functional properties [5]. A variety of semi-synthetic and edible biopolymers are used in food packaging applications, including chitosan, agarose, alginate, agar, starch, cellulose, guar gum, polyvinyl alcohol and carboxymethyl cellulose [6, 7]. Although plastic materials are lightweight, economical, and flexible, they contribute to waste, raising environmental concerns [3].

Recently, there has been growing interest in utilizing biodegradable materials for food packaging and coatings[8]. Bio-based plastics are favoured for film coatings because they are abundantly available, biodegradable and capable of forming films [9]. However, these materials often lack mechanical strength and offer poor resistance [10]. To address these limitations, structural modifications [11] or blending of bio-based polymers with synthetic polymers have been performed to obtain specific properties [12]. Chitosan, a naturally occurring biopolymer with inherent anti-microbial [13] and anti-oxidant properties [14-16], has been widely used in food packaging [17]. It is non-toxic [18] and has chelating ability to bind with vital trace metals selectively, bacteria and prevent toxin and bacterial growth [19, 20], thus making it perfect for food packaging applications [21, 22]. In addition, chitosan has high susceptibility to water and low

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thermal and mechanical strength, which limits it use in food packaging materials. To address these limitations, different techniques such as cross-linking [23-26], enzymatic treatment [27], graft copolymerization [28-30], complex formation [29], surface coating [31], incorporation of fillers [32], high-energy radiation [33] and blending with other biopolymers or synthetic polymers can be used [34, 35].

PVA is a synthetic and water-soluble polymer that is an ideal biomaterial for making polymer blends. It is biocompatible, flexible, and non-toxic [36] and can be used in food packaging applications because of its low cost, eco-friendly nature, durability, transparency, mechanical strength, chemical stability, and film-forming properties [37, 38]. Blending PVA with chitosan not only enhanced the mechanical strength but also improved the antibacterial activity of the fabricated films.

In contrast, *Rosa rugosa*, a common petal variety, has been traditionally used in biomedical applications [39]. *Rosa* species contain components including flavonoids, terpenes, glycosides, polyphenols, and anthocyanins. The volatile oil extracted from *Rosa* flowers possesses therapeutic properties and is primarily used for analgesic, anticonvulsant, cardiovascular, and laxative purposes [40]. Given the numerous bioactive components found in *Rosa rubiginosa* (RR), our objective was to explore the effects of its incorporation and proportion when combined with CS/PVA/gelatine solutions. These coatings and films are expected to exhibit enhanced mechanical strength and improved antibacterial, antioxidant, and biodegradable properties, which are essential for prolonging the shelf life of coated or packaged food products, such as strawberries. Choi *et al.* developed a ternary blend (CS/PVA/gelatin)-based composite film incorporating *Duchesnea indica* extract for the preservation of strawberries [41]. In another study, Islam *et al.* fabricated packaging films using a casting methodology with CS/PVA incorporated with zinc oxide nanoparticles and dragon fruit waste extract. The incorporation of ZnO nanoparticles and dragon fruit waste extract enhanced the thermal and mechanical strength and provided antioxidant and antibacterial potential to the films [42]. Similarly, Wang *et. al.* synthesized bilayered films with excellent biodegradable, antibacterial, mechanical, and oxygen barrier properties using CS as the outer layer and PVA/gelatin as the inner layer loaded with 2.5 % curcumin nanoparticles for the preservation of fish (bigeye tuna) [43]. However, CS/PVA/gelatin ternary films have not yet been combined with RRE, and their application as edible coatings for strawberries has not been evaluated in terms of antioxidant/antibacterial performance, barrier properties, biodegradability, and sensory acceptance. This study aims to develop and characterizes/PVA/gelatin coatings enriched with varying concentrations of RRE and evaluate their effectiveness in extending the shelf life of strawberries.

2. Experimental

2.1 Materials

The chemicals and solvents utilized in this study were employed directly as obtained from manufacturers or redistilled when necessary. Macklin Chemicals (China) supplied Chitosan (CS) with a deacetylation degree exceeding 95% (molecular weight (MW) 100–200 kDa) and viscosity was 100–200 mpa.s. Polyvinyl alcohol (PVA) (MW 72,000 with 98% degree of hydrolysis) was procured from Merck (Germany). Gelatin and glacial acetic acid ($C_2H_4O_2$) were bought from Sigma Aldrich–Germany. A taxonomist identified the plant sample as *Rosa rubiginosa* (RR), which was obtained from the University of Sahiwal, Pakistan. Strawberries were purchased from a local market in Sahiwal–Pakistan

2.2 Methodology

2.2.1 Preparation of *Rosa rubiginosa* extract (RRE)

The flower petals of *Rosa rubiginosa* were thoroughly washed with deionized water to remove any debris or impurities present on the surface, followed by air drying at room temperature (20–25 ℃) for 10–12 days. The petals were ground to make their powder, and then 5% *w/v* aqueous solution of *Rosa rubiginosa* was prepared by refluxing at 60 ℃ and centrifugal force of 125.8 g for a continuous 4 hours. The solution was filtered out with Whatman filter paper number 1, under vacuum conditions, and the filtrate was stored at 4 ℃ for further use to fabricate coatings and films.

2.2.2 Preparation of CS/PVA/Gelatin derived coatings and films loaded with RRE

The CS/PVA/Gelatin based composite coatings and then finally films were produced using blending of individual component solutions and then conventional solution casting methodology, as described by Rubilar *et al.*, [44]. A 1% chitosan (*w/v*) solution was prepared by dissolving 1 g of chitosan in a 1% acetic acid solution, with stirring at room temperature for a duration of 72 hours. Similarly, 5% PVA (*w/v*) solution was prepared by dissolving 5 g of PVA in the respective amount of deionized water by refluxing at 80 ℃ for 5 hours. 2% (*w/v*) gelatin solution was made by dissolving 2 g of gelatin in deionized water. 30% (*v/v*) glycerol solution was made by combining 30 mL glycerol with the required amount of deionized water. Then, different volume proportions of CS, PVA, gelatin, glycerol, and RRE (**Table 1**) were combined and stirred for 48 hours at room temperature to form a uniform solution. The resulting solutions were then used for dip coating of strawberries and were also used for evaluation of antioxidant and antibacterial potential. The other part of solution was cast in petri dishes and kept at room temperature, to fabricate composite films, for characterization and evaluation of certain other properties. The solution was evaporated within 2 to 3 days and films were formed, after which they were removed from the dishes.

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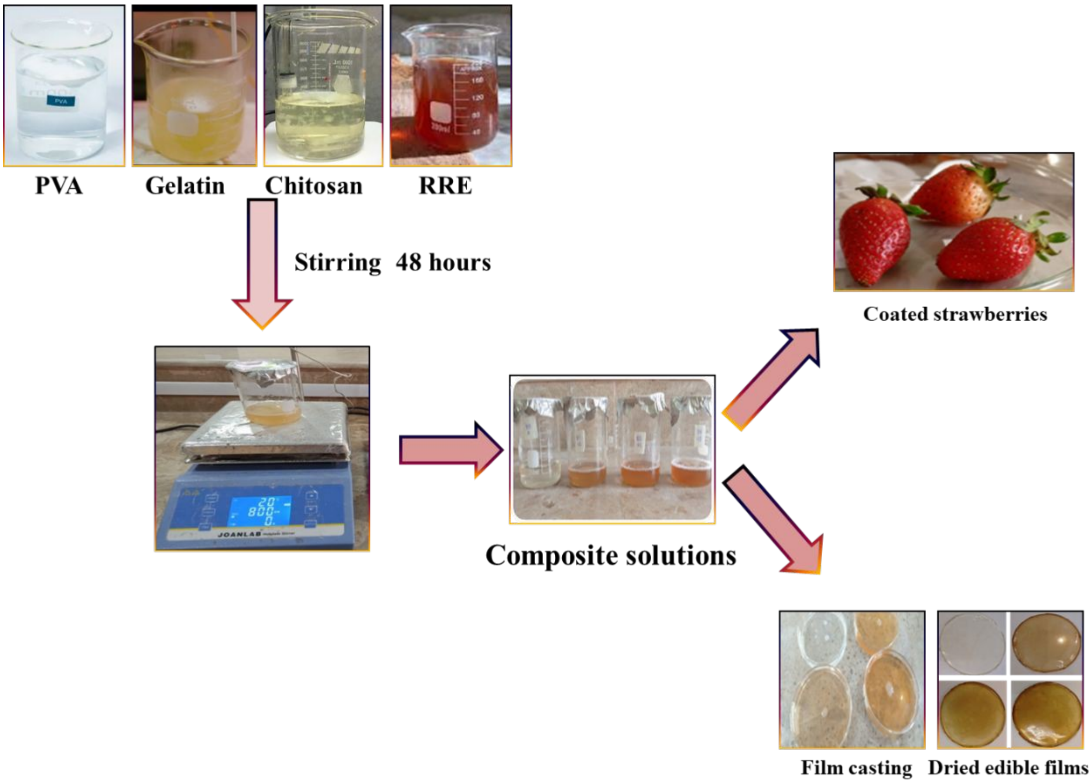

Figure 1. Preparation of composite CS/PVA/Gelatin coatings and films loaded with RRE

Table 1. Different volume ratios of all solutions for coatings and film formation

Samples codes	CS solution (mL)	PVA solution (mL)	Glycerol solution (mL)	Gelatin solution (mL)	RRE (mL)
BS-0	20	20	10	2	0
BS-01	20	20	10	2	2
BS-02	20	20	10	2	4
BS-03	20	20	10	2	6

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2.3 Characterization of synthesized film

2.3.1 FTIR analysis

All composite fabricated films were characterized by FTIR spectrometer (Thermo Nicolet 6700P, Waltham, Massachusetts, USA), to evaluate interactions among individual components and to identify peaks of prominent functional groups of films. Spectra were acquired on photo acoustic mode, with a range of 4000 –400 cm^{-1}, using carbon background and helium purging with 8 cm^{-1} resolution and 256 scan numbers

.2.3.2 Thickness of fabricated films

The thickness of fabricated films in millimetres (mm) was noted by using a digital thickness gage (manufactured by Insize company, China), with 0–10 mm range, 0.01 mm resolution and ±0.02 mm accuracy. Reported thickness values are the mean values of five random values taken at various positions, including the surroundings and centre.

2.3.3 Camera images

Camera images of synthesized films were taken to evaluate the appearance, homogeneity, colour and texture. Images were taken by the mobile camera of Infinix Hot–30 play made by Infinix Mobile China, Dual–LED flash, HDR, panorama (16 MP, f/1.8, wide, PDAF, QVGA, 1080p@30fps).

2.3.4 Scanning electron microscope (SEM) analysis

To investigate the surface and cross-sectional microstructure of the fabricated films, SEM analysis was conducted using the EmCrafts Cube II tabletop model from South Korea. The samples were sputter-coated and placed on SEM holders, with images captured at different magnifications using an accelerating voltage of 10 KV.

2.3.5 Dynamic mechanical analysis (DMA)

Mechanical properties of films were evaluated by measuring tensile strength and elongation at break using a dynamic mechanical analyzer (DMA) Q800 manufactured by TA Instruments (USA) with software version of V21.3 Build 96 using controlled force module. The films were cut in 6×3 cm size and analysed by the method equilibrate at 50 ℃, isothermal for 5 minutes and ramp force 3 Newton/minute to 18 Newton/minute.

2.3.6 X-ray diffraction (XRD) analysis

XRD analysis was performed to analyse the crystalline/amorphous nature of fabricated CS/PVA/gelatin enriched with RRE films using XRD powder system, D8 advance, model cube 10, EmCrafts manufactured by Bruker, South Korea. Films spectra were recorded in range of 10–70^{0} with 20 seconds per step and 0.09 step sizes.

2.3.7 Percent moisture content (MC %) determination

The percentage moisture content was determined using the loss on drying method. Initially, small sections of the film samples were cut, and their weight was measured. These samples were then placed in an oven set at 80 ℃ for one hour and weighed again with precision. This process was repeated until the weight remained constant. The moisture content percentage (MC %) was calculated using equation 1 [45, 46].

$$MC(\%) = \frac{M_w - M_d}{M_w} \times 100 \ (1)$$

Where Mw stands for initial weight of film samples before drying conditioned at 53% relative humidity to moisture equilibrium and Md stands for dried weight. Average moisture content percentage was determined from three replications.

2.3.8 Water solubility percent (WS %)

Small pieces of films were cut and placed in oven at 80 ℃ for 1 hour and then their dried weight was measured. The films were then introduced into petri dishes containing 25 mL of distilled

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water for 24 hours for their maximum solubility in water. The films were removed from petri dishes and were dried in oven at 80 ℃ until to attain a constant weight. The water solubility percent (WS %) was evaluated by following formula [47].

$$WS\,\% = \frac{Wi - Wf}{Wi} \times 100 \qquad Equation\ 2$$

Where Wi stands for initially dried weight in grams and W_f stands for final/terminal dried weight (g). For each measurement, three readings were taken then WS % was evaluated by taking mean reading.

2.3.9 Water absorption pattern of films

The small pieces of films were cut and their dried weight was measured. These pieces were put into the petri dishes containing 10 mL distilled water, so that films can absorb maximum water and may swell by retaining water within their structure. The water absorption behavior of each film was observed by keeping films dipped in water for 10 minutes and then their removal and subsequently weight measurement. After weighing, the films were again immersed in water, and the process was repeated until to achieve constant weight. The percentage water absorption capacity of each film was determined by using the following formula [48].

$$\boldsymbol{Water\ absorption\ (\%) = \frac{Ws - Wd}{Wd} \times 100}$$

Where Ws stands for swollen weight and Wd stands for dried weight.

2.3.10 Water drop test

A piece of the film was placed over a support raised from the table and a water drop was poured onto the film surface. Then pass or fail judgment was given that depending on whether the drop passed through the film or not [49, 50].

2.3.11 Oxygen permeability

Winkler's method, which measures dissolved oxygen in mg/mL, was employed to assess the oxygen permeability of CS/PVA/gelatin/RRE films[51]. In this process, distilled water was placed in glass bottles sealed with composite films using a sealant, and the quantity of oxygen that permeated through them was measured.

2.3.12 Antibacterial potential of CS/PVA/gelatin/RRE coatings solutions

The antibacterial potential of CS/PVA/gelatin coating solution and CS/PVA/gelatin infused with RRE solutions was evaluated against two gram-positive bacterial strains, *S. aureus* and *B. subtilis*, as well as one gram-negative strain, *E. coli*. This assessment was conducted using the standard method previously described [52]. A suspension of 100 µL for each of the two bacterial strains, containing approximately 10^7 colony-forming units (CFU) per mL, was prepared. The bacterial cells were cultured in nutrient broth (Oxoid, UK) for around 8 hours at 37 ℃. Using a sterilized swab, the bacterial culture was evenly distributed on agar plates, which were then allowed to dry for 15 minutes. Filter discs soaked with CS/PVA/gelatin/RRE solutions were placed on the agar plates, with four discs, each containing a different sample, positioned equidistantly on each test plate. A separate petri dish served as a positive control, containing a filter disc impregnated with the commercially available antibiotic ciprofloxacin at a concentration of 10 µg/mL. Before incubating the plates at 37 ℃ for 17–24 hours, they were refrigerated at 4 ℃ for two hours. The inhibition zones produced by each sample were measured in millimetres using calipers, indicating the antibacterial potential of each sample and the positive control. The experiments were conducted in triplicate to ensure reliability.

2.3.13 Antioxidant potential of CS/PVA/gelatin/RRE coatings solutions

The antioxidant properties of samples containing CS/PVA/gelatin infused with RRE were assessed using the 2, 2-diphenylpicrylhydrazyl (DPPH) method. This technique, commonly employed to evaluate the antioxidant potential of samples with plant extracts, is highly regarded for its stability, practicality, and reliability. The level of antioxidant activity is gauged by the components' ability, particularly plant-based ones like flavonoids and polyphenolics, to neutralize the DPPH radical. In this method, a sample was prepared by mixing 20 µL of solution with 3 mL of distilled water. Then, 1 mL of this mixture was added to 0.2 mL of a methanolic DPPH solution (1 mM). The resulting mixture was kept in the dark at room temperature for about 40 minutes. The absorbance of the solution was measured at 517 nm against a blank using a UV-Visible spectrophotometer. This procedure was conducted three times, and the percentage of DPPH radical scavenging activity was calculated using a specific formula [45, 46].

$$\text{Scavenging activity (SA \%)} = \frac{Acontrol - Asample}{\text{Acontrol}} \times 100$$

Where $A_{control}$ shows the absorbance of control that is DPPH solution and A_{sample} stands for absorbance of samples by DPPH.

2.3.14 Biodegradation test

Film samples were cut into small strips for evaluation of their biodegradation behavior. The experiment utilized 200 g of dry soil collected from the Sahiwal field area, which was then moistened by spraying distilled water. Four polythene cups were filled with equal amounts of this prepared soil. The film samples were buried at a consistent depth within the soil and maintained at room temperature with 70% relative humidity. After 15 days, the film's condition was evaluated based on their visual appearance and weight loss. Subsequently, the samples were reburied in the soil and examined again after 45 days to assess their further degradation [48].

2.3.15 Total flavonoid contents (TFCs)

TFCs of RRE with a concentration of 5 mg/mL were assessed by a procedure already declared in literature with a few modifications [53]. This is the same concentration which was incorporated into CS/PVA/gelatin films. For this assay, freshly prepared reagents including 1 M NaOH, 5%

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$NaNO_2$ and 10 % Al_2Cl_3 were used. The assay involved taking 1 mL of RRE in a test tube and then adding 400 µL of $NaNO_2$ and 6 mL of distilled water. The mixture was thoroughly blended to ensure maximum homogeneity. The mixture was incubated for 10 minutes, followed by addition of 700 µL of Al_2Cl_3 (10%) and 3 mL of NaOH (1M). The absorbance was subsequently recorded at 510 nm using the STA–8100ST UV/Vis spectrophotometer from Van Nuys, Los Angeles, CA, USA, to assess TFCs as catechins equivalents per unit of dry matter.

2.3.16 Total phenolic content (TPCs)

Folin–Ciocalteu method was employed to determine the TPCs of RRE [53]. RRE solution was prepared at a concentration of 5 mg/mL. Then 200 µL of RRE was taken into test tubes and 1000 µL of Folin-Ciocalteu reagent was added, followed by vigorous blending and mixing of solutions. Subsequently, 800 µL of Na_2CO_3 (7.5%) was introduced to the solution, which was then left at room temperature for 2 hours. The mixture was thoroughly combined, and the absorbance of the resulting solution was recorded at 765 nm using an STA–8100ST UV/Vis spectrophotometer (Van Nuys, Los Angeles, CA, USA). Gallic acid served as the standard for quantifying the TPCs, which were expressed as GAE (Gallic Acid Equivalent) per dry matter. Each sample underwent analysis in triplicate.

2.3.17 Application of composite coatings on strawberries

The potential of synthesized solutions was evaluated as a coating application on strawberries to increase their shelf life using dip coating technique [54]. The synthesized composite solutions of CS/PVA/gelatin incorporating RRE encompass antibacterial and antioxidant potential, which was believed to provide better protection of strawberries against their early decomposition. The various solutions used for coatings on strawberries were coded as BS-0, BS-01, BS-02 and BS-03, with the only difference being of increasing RRE from 0 mL in BS-0 to 2 mL into BS-01, 4 mL into BS-02 and finally 6 mL into BS-03. Thus, it was believed that increasing the RRE volume ratio from 0 mL (BS-0) to 6 mL in BS-03 would also enhance their

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potential to protect strawberries. Initially, fresh and healthier strawberries were obtained, cleaned with tissue paper to eliminate dirt, and thoroughly dried. The prepared solutions were then applied to the dried strawberries using the dip-coating method. Each strawberry's weight was recorded, and they were stored at room temperature for 17 days with 50% relative humidity. The decay and deterioration of the strawberries, along with their weight loss, were regularly monitored. Thus, the study examined the extension of shelf life and preservation of their freshness. Strawberry samples were tested in triplicate, with their weight loss percent recorded for each. The average value was then used for calculations.

$$\text{Weight loss (\%)} = \frac{\text{Wi} - \text{Wf}}{\text{Wi}} \times 100$$

Where Wi stands for the initial weight of strawberries and Wf stands for the final weight of strawberries.

2.3.18 Sensory evaluation

The sensory assessment of coated strawberries was performed according to Nowacka et al. (2018) [55] for all formulations including, BS-0, BS-01, BS-02 and BS-03 and a control without any coating. The parameters such as taste, smell, color, juiciness, firmness and acceptance in general were noted on a scale of 0 to 10. A score of 8 was seen as exceptional, additional desired, juicy and acceptable whereas a score of 1 was regarded as poor, extra unwanted, dry and unsuitable.

2.3.19 Statistical analysis

Statistical analysis of data was run through analysis of variance (Two-way-ANOVA) using the software Origin Pro 2022, Origin Lab Corporation, Northampton, Massachusetts (USA). All measurements from the tests were taken in triplicate (n = 3), and the data results are presented as the mean ± standard deviation (SD). The error bars on column graphs in all figures represent the standard deviation among three replicates, which is a measure of data variability and reproducibility. Differences between means were evaluated by Tukey's multiple range test ($p < 0.05$).

3. Result and discussion

3.1 FTIR analysis

FTIR analyses were performed to determine the various functional groups present in the films synthesized from CS/PVA/gelatin and loaded with RRE and to check their intermolecular interactions. **Figure 2** shows the FTIR spectra of CS/PVA/gelatin films without RRE (BS-0) and with RRE (BS-01, BS-02 and BS-03). FTIR spectra of all films in **Figure 2** showed a very broad peak at 3300 cm^{-1} to 3250 cm^{-1} due to the overlapping stretching frequencies of hydroxyl O-H and amino N-H groups [46]. Alkyl C-H group asymmetric and symmetric stretching was observed between 2950 cm^{-1} and 2916 cm^{-1}. The peaks for the amide I and amide II bands appeared at 1669 cm^{-1} and 1564 cm^{-1}, respectively [56]. CH_2 bending peaks were seen at 1408 cm^{-1} and 1338 cm^{-1}. C-O and C-O-C stretching vibrations were observed at 1143 cm^{-1} and 1099 cm^{-1}. These vibration bands were also reported by Dawei Yun *et al.* [57]. Chitosan's ring stretching vibrations were appeared in the fingerprint region at 895 cm^{-1} [58].

The FTIR spectra of films with RRE exhibited an additional prominent peak at 2970 cm^{-1} due to aromatic C-H stretch[59], which was due to aromatic polyphenolic and flavonoid compounds present in RRE. Similarly, FTIR spectra of BS-01, BS-02 and BS-03 exhibited numerous small peaks from 1800 cm^{-1} to 1500 cm^{-1}, due to additional components of RRE [60]. Minor variations towards lower frequency for certain peaks were observed in FTIR spectra, possibly due to bonding interactions between CS, PVA, gelatin and RRE. Additional peaks at 1700 cm^{-1}, present in BS-01, BS-02 and BS-03 spectra and absent in BS-0 spectrum, were attributed to

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various components like secondary metabolites and polyphenols present in RRE [61]. The BS-0 film displayed O-H and N-H stretching at 3330 cm^{-1}, the band shifted towards lower wavenumber at 3267 cm^{-1} for films incorporated with RRE (BS-01, BS-02 and BS-03), due to the greater interactions, created by active functional groups O-H and N-H present within CS, PVA, gelatin and RRE. As the OH group present in polyphenols develops hydrogen bonding with the OH group present in CS and PVA, and NH_2 group in chitosan, thus, greater hydrogen bonding results in lower frequency ranges. These discussed FTIR results are also supported by literature studies [62]. The hydroxyl group (-OH) and amino group (-NH_2) stretching vibrations in the BS-0 film spectrum were appeared to be greater than those found in BS-01, BS-02, and BS-03 spectra. This was due to presence of higher number of unreacted –OH and –NH_2 functional groups in BS-0 film. The reduction in stretching vibrationsin spectra of BS-01, BS-02, and BS-03 films containing RRE suggested the involvement of active –OH and –NH_2 groups in PVA and chitosan with the functional groups present in RRE, leading to the formation of hydrogen bonds and other weak electrostatic interactions. Overall, FTIR analysis confirmed the presence of the characteristic peaks for all components under consideration.

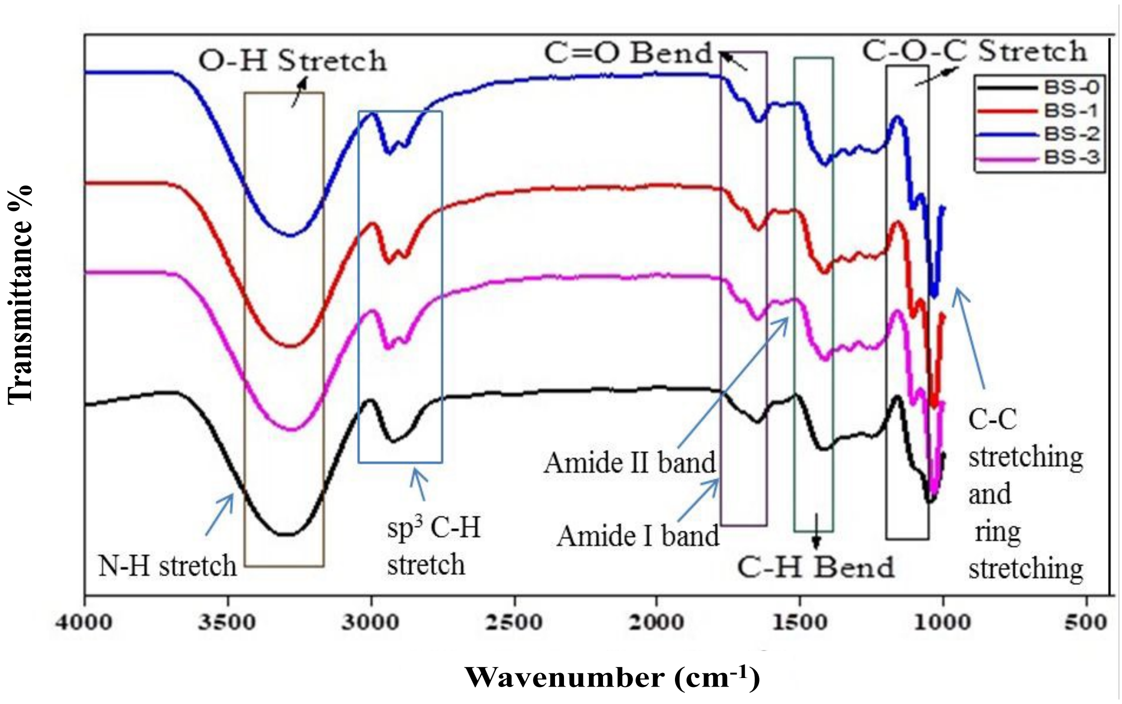

Figure 2. FTIR of films of BS-0, BS-01, BS-02 and BS-03

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3.2 Film thickness

The thickness of the films is a vital factor in attaining desirable optical properties and optimum barrier against water and vapor transfer. An increase in film thickness may reduce the diffusion rates of oxygen and water vapors of the film [63]. The addition of plasticizers, antioxidants, and antimicrobial agents can increase the thickness of the films, offering better barrier properties and improved mechanical strength [64]. The thickness results shown in **Figure 3** indicate that the BS-0 film (without RRE) showed a minimum thickness of 0.123±0.02 mm, while the maximum thickness was displayed by BS-03 (0.884±0.06 mm), followed by BS-02 (0.771±0.05 mm), and BS-01 (0.439±0.03 mm). Gradually increasing the RRE from 0 mL (BS-0) to 6 mL (BS-03) also enhanced the film thickness, which is consistent with the literature [65]. Films with suitable or greater thicknesses offer better barrier properties, thus lower water and oxygen permeability, making them ideal for prolonged packaging of perishable fruits.

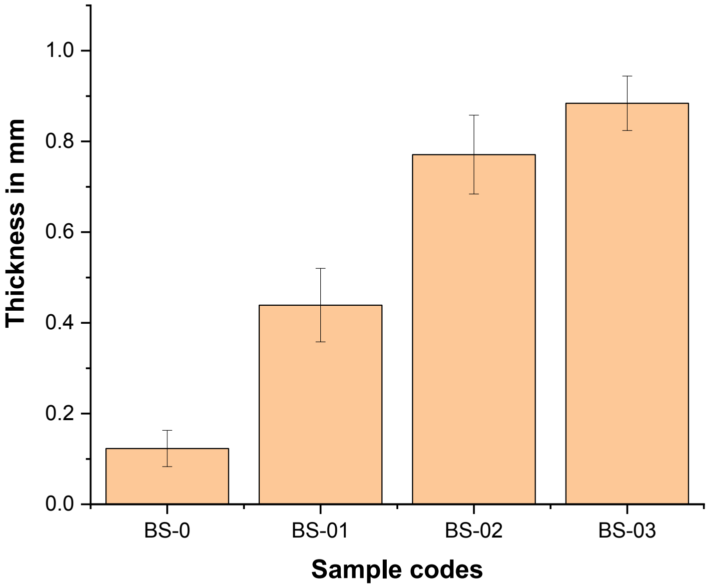

Figure 3. Film thicknesses of composite films

3.3 Visual impact by camera images

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The visual appearance and color of films are essential parameters that affect the standard and quality of food items. Colored films have more potential to wrap food items and preserve them because they reduce oxidation reactions in packaged foods [66]. **Figure 4** shows the camera images of the CS/PVA/gelatin films and CS/PVA/gelatin films loaded with various volumes of RRE. The CS/PVA/gelatin films were completely transparent, whereas the other films incorporating RRE were yellowish-brown. It was noted that, by increasing the RRE volume from 2 mL in BS-01 to 6 mL in BS-03, the color of films became intense yellowish brown as illustrated in **Figure 4**. Additionally, the films were seen as smooth due to better mixing, blending and developing certain types of new interactions between film components. The dried films were observed to be smooth, homogenous, stretchable, bubble free, mechanically strong, flexible and were peeled off from the petri dishes swiftly without cracks.

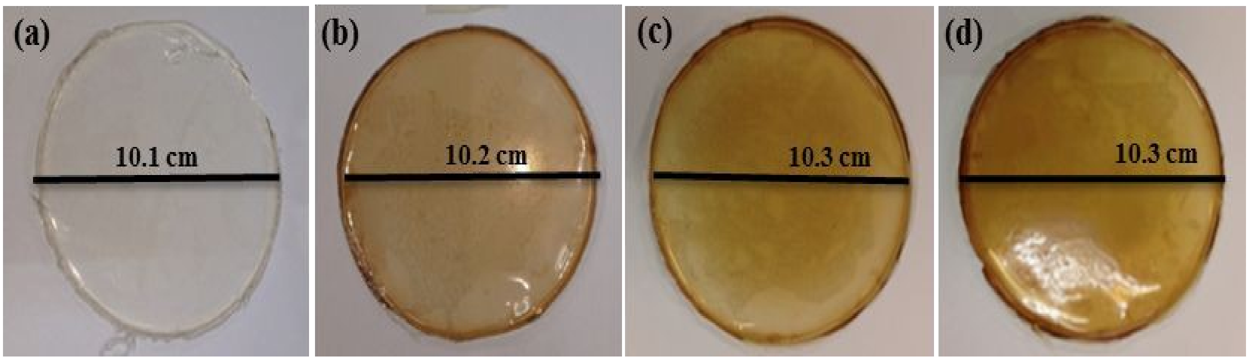

Figure 4. Visual appearances of composite films; (a) BS-0, (b) BS-01, (c) BS-02, (d) BS-03

3.4 Morphological analysis using SEM

SEM analysis was used to examine the surface morphology of the prepared CS/PVA/gelatin film and CS/PVA/gelatin loaded films with RRE. The SEM images of composite films are shown **Figure 5**. The SEM images in **Figure 5 (a, b, c)** of film BS-0 (composed of only CS/PVA/gelatin) appeared clearer and more homogenous without cracks and crystalline particles/aggregates on their surface, indicating the amorphous nature of this film. The micrographs in **Figure 5 (d, e, f)** of film BS-01 showed that all components were uniformly distributed and appeared in one phase and homogeneous. The images showed the presence of certain aggregates/crystals within the structure, which were due to the addition of 2 mL of RRE into the CS/PVA/gelatin matrix. The addition of RRE into CS/PVA/gelatin matrix has induced certain intermolecular interactions, thus producing a few aggregates of some components. The micrographs in **Figure 5 (g, h, i)** represent BS-02 film incorporating CS/PVA/gelatin loaded with 4 mL of RRE. Here, the images also revealed the uniformity, consistency and homogeneity of all the added components. Additionally, BS-02 film also showed many white crystals, which might have been formed due to the interactions between active functional groups in CS, PVA, gelatin and RRE. Thus, it was evident from the images that increasing the RRE ratio from 2 mL (BS-01) to 4 mL (BS-02) had altered the morphology of films to some extent. The film with a higher volume ratio of RRE (4 mL in BS-02) exhibited more crystalline molecules/aggregates within its structure, as already reported in the literature [67]. One potential explanation is the interaction between the active components of RRE and the active groups in CS, PVA, and gelatin, which leads to the partial release of bioactive compounds such as polyphenols, alkaloids, and amino acids onto the film's surface [68]. A similar type of interaction was also reported by Han *et al.* in Noni (Morinda citrifolia) fruit polysaccharide enriched with blueberry leaf extract [69]. It is believed that bridging phenomena occurred inside the bio-composite films in which some molecules of RRE bonded to the CS chain while the rest of the functional groups attached to another CS chain. Thus, the overall surface morphology showed that RRE was effectively incorporated into the CS/PVA/gelatin solution matrix. In contrast to the above results, **Figure 5 (j, k, l)** represents BS-03 film (incorporating 6 mL of RRE), which showed film structure as clearer, smoother and more homogenous, without a higher number of crystalline particles/aggregates on its surface as compared to BS-02 film. Thus, it can be concluded that increasing RRE to 6 mL, into CS/PVA/gelatin matrix would again make the film texture smooth [70].

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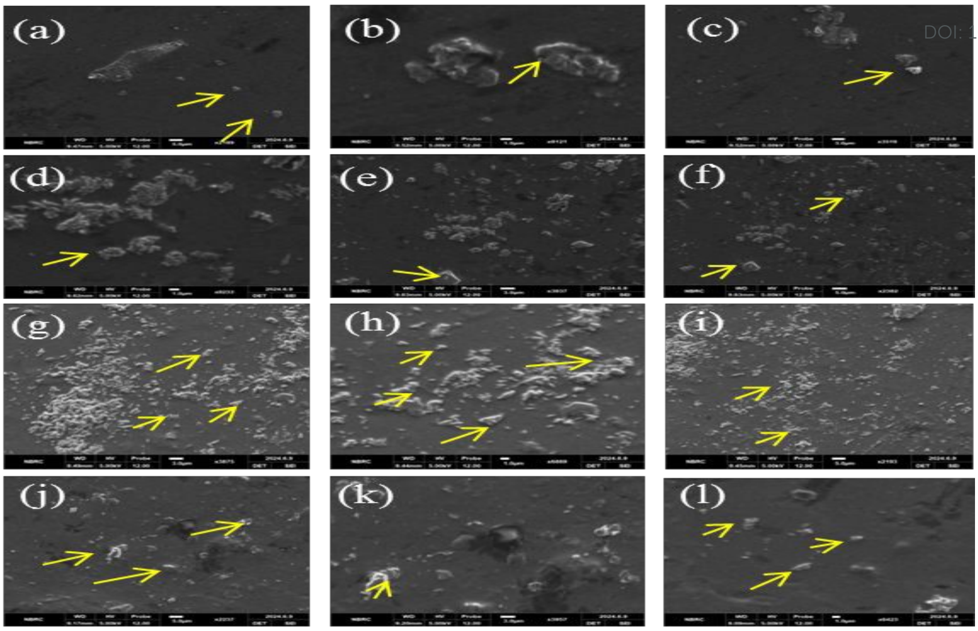

Figure 5. SEM micrographs of BS-0 (**a, b, c**), BS-01 (**d, e, f**), BS-02 (**g, h, i**) and BS-03 (**j, k, l**)

The cross-sectional analysis of film BS-0 is shown in **Figure 6 (a),** which shows that the film was homogeneous, uniform, thick, and had no cracks in its structure. Similarly cross-sectional SEM image of BS-01 film was more homogeneous, uniform and without any pores as compared to BS-02 film, which had certain cracks in its structure.

Further cross-sectional images in **Figure 6 (c, d)** revealed that BS-02 and BS-03 films incorporating RRE addition to CS/PVA and gelatin were smoother, and their thickness increased, respectively. The increase in thickness was primarily due to the increasing volume ratios of RRE. The film BS-02 cross-sectional image in **Figure 6 (c)** showed some cracks and irregular cavities, which showed its changed morphology as compared to other films. This indicated the presence of certain types of intermolecular interactions between functional groups of RRE active constituents and CS, PVA and gelatin.

The BS-03 film also appeared smooth and without cracks/pores. The homogenous distribution of bioactive components within the matrix of films ensures that their antibacterial and antioxidant activities remain unaffected.

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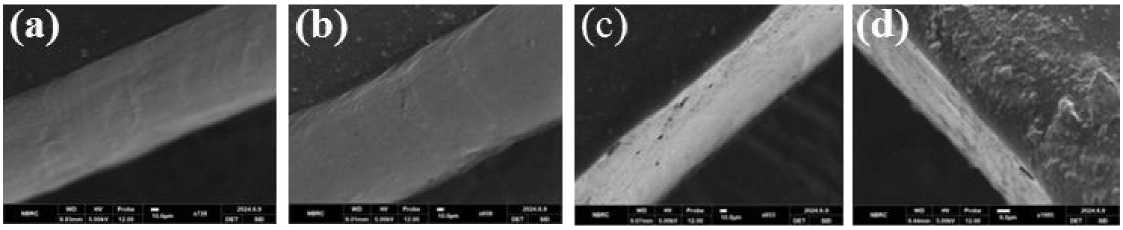

Figure 6. Cross-sectional film images: BS-0 (a), BS-01 (b), BS-02 (c) and BS-03 (d).

3.5 Mechanical strength

The mechanical properties of films are crucial for their use in packaging. To assess the mechanical strengths of these films, stress-strain curves were plotted and are depicted in **Figure 7**. Two sample films, including BS-0 (incorporating CS/PVA/gelatine) and BS-03 (incorporating CS/PVA/gelatine and RRE) were selected for the determination of mechanical properties of films and to evaluate the effect of incorporation of RRE into CS/PVA/gelatin matrix. The BS-0 film stress strain curve was shown by the green curve, while the BS-03 stress strain curve was shown by the red colour. It was noted that the addition of RRE had slightly reduced the mechanical properties of films. The stress value for BS-0 film was noted as 0.32 MPa, while the stress value for BS-03 film was 0.21. Similarly, the strain % for BS-01 film was noted as 56 %, while for BS-03 film it was nearly 25 %. This phenomenon can be explained as RRE contains many polyphenolic and flavonoid components, which have active functional groups. These active functional groups had created certain interactions with polymeric chains of CS, PVA and gelatin. Thus, polymeric interactions among CS, PVA and gelatin chains were reduced, which ultimately altered the internal structure of films. It is well known that chitosan has low mechanical strength; thus, it is blended with synthetic polymers like PVA to enhance its mechanical strength. The introduction of aqueous RRE into CS/PVA/gelatin film (BS-03) has reduced its deformation, made the film stiffer and more rigid, and less flexible. The

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Young's modulus for film BS-0 was calculated as 0.57 MPa, while Young's modulus for film BS-03 was calculated as 0.86 MPa. Thus BS-03 film was observed to be less deforming, stiffer and more rigid as compared to the BS-01 film. Thus BS-03 film is suitable for applications which require more strength and dimensional stability such as packaging applications. While the BS-01 film was deformed more under higher stress, which exhibits its more flexibility and better suitability for those applications where flexibility and stretchability are required.

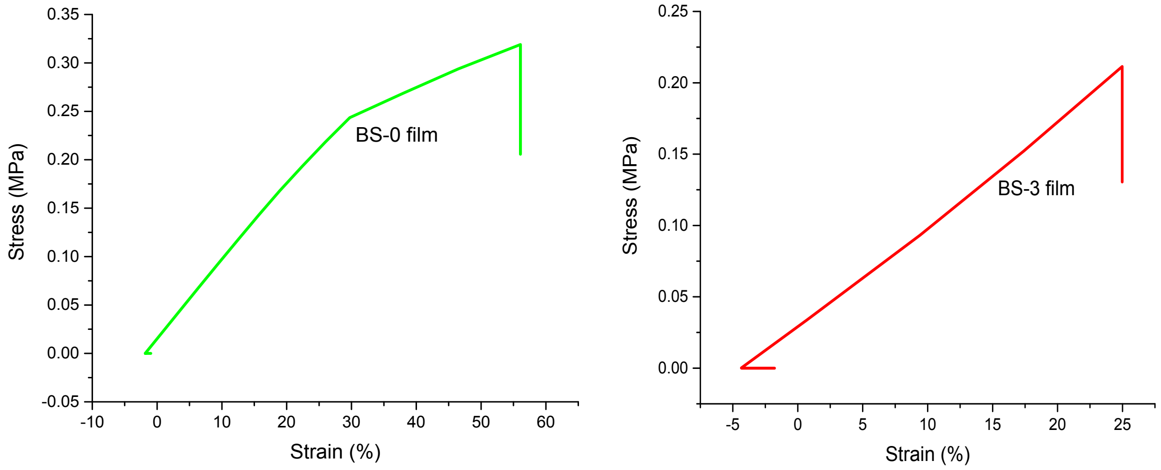

Figure 7. Mechanical properties of CS/PVA/gelatin and CS/PVA/gelatin/RRE films

3.6 XRD analysis

XRD analysis was performed to determine the crystalline or amorphous nature of composite films. Literature studies of PVA and CS XRD spectra exhibited that PVA had a prominent peak at 2θ = 39.9°, while CS exhibited a sharp peak at 2θ = 20°. The BS-0 coded film demonstrated two notable peaks, one at 2θ = 19.59° corresponding to the crystalline phase of chitosan, and another at 2θ = 39.9° representing the amorphous phase as a lower intensity shoulder. The CS/PVA film BS-01 containing RRE also showed two amorphous peaks, slightly shifted to 2θ = 20° and 2θ = 41.9°. The BS-02 film exhibited peaks at 19.9° and 39.6°, while the BS-03 film displayed intensified peaks at 20.2° and 42°. Additional peaks were observed in BS-01, BS-02, and BS-03 films due to the inclusion of RRE, as illustrated in **Figure 8**. The XRD values obtained for CS/PVA/gelatine films and CS/PVA/gelatine loaded with RRE were found to be

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aligned with previously reported values [71]. These XRD results confirmed the various interactions between CS, PVA, gelatin and RRE within the film matrix.

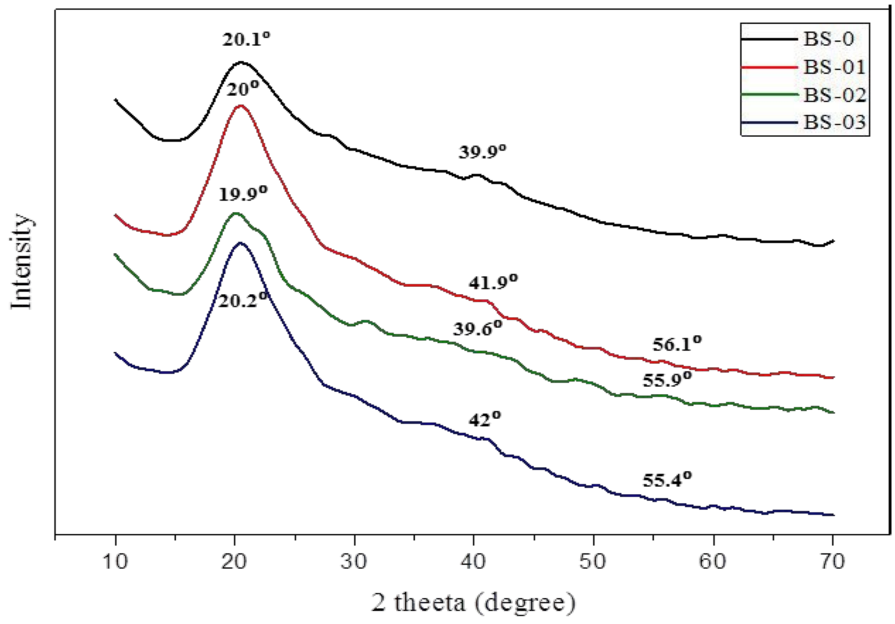

Figure 8. XRD patterns of composite films

3.7 Moisture content percent of films

The moisture content of polymer-based films is the crucial parameter to determine the quality of films, as the purpose of films is to preserve the food from microbial spoilage. Here, CS/PVA/gelatin and CS/PVA/gelatin enriched with RRE composite films were analyzed for their ability to absorb moisture, and the results are displayed in **Figure 9 (A),** and the values are mentioned in **Table 2**. The outcomes of this assay disclosed that all the films absorbed moisture content from 15 % to 17 %. Thus, a small variation was observed in moisture content results, which could be attributed to their different compositions. The moisture absorption phenomenon could be explained based on interactions such as hydrogen bonds between active amino and hydroxyl groups of CS, hydroxyl groups of PVA and water. In addition to this, the presence of glycerol had also contributed to moisture absorption, as glycerol is a well-known humectant. Furthermore, it was noted that the incorporation of RRE into CS/PVA/gelatin

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matrix had slightly lessened the percent moisture content of the films. As the ratio of RRE was increased in CS/PVA/gelatin matrix, the moisture content percent was decreased to a small extent. The reported results were in accordance with literature studies, as it has been mentioned in literature that CS and PVA produced more interactions with active components of RRE and ultimately, their interaction with water was decreased, which led to hydrophobicity and a slight reduction of moisture content [72]. The decreased percent moisture content is most favourable for food packaging applications as it offers better water barrier properties by lowering the microbial growth and improves the texture of packaged food [73].

3.8 % Water solubility

The percent solubility of composite films of CS/PVA/gelatin and CS/PVA/gelatin loaded with RRE in water is the measure of weight loss of films when fully immersed in water for 24 hours. Solubility percent determination is important to measure as it depicts the stability of films as and when they come in contact with moisture in the environment for a longer time. The solubility percent test results were presented in **Figure 9 (B)**, and their exact values were also given in **Table 2**. It was noticed that CS/PVA/gelatin film BS-0 had shown a maximum solubility % of 73%, which is mainly due to the presence of hydrophilic polymers of PVA and gelatin. However, CS is hydrophobic, and it makes interactions with both PVA and gelatin within films to decrease their solubility. The addition of RRE into CS/PVA/gelatin films had decreased their solubility, as BS-01 code film solubility was 70.2 %. Furthermore, increasing the volume ratio of RRE into CS/PVA/gelatin films further decreased the solubility of films, as observed in BS-02 (63.2 % solubility) and BS-03 films (60.2 % solubility). The intense interaction between CS and other polymers such as PVA, gelatin, and RRE led to increased intermolecular bonding, which in turn reduced their solubility [74]. The films with lower solubility rates are more beneficial for food packaging applications, as these films are more likely to compromise with the moisture present in the surroundings, and their dissolution rate slows down. However, a balance between solubility and biodegradability is important, as excessively low solubility may slow down the degradation process post-use. A similar phenomenon of reducing the solubility of films by incorporating tea extract was also reported by Hafsa *et.al* [75].

3.9 Water absorption

Water absorption percent of films is a vital indicator to check their quality to absorb and retain water within their structure without being dissolved or solubilized. The degree of water absorption was found to be inversely proportional to the degree of interactions or crosslinking among different constituents of films. More interactions or crosslinking between polymeric chains show lesser water absorption and vice versa. The films with lower water absorption percent are frequently opted for food packaging applications. The results of the water absorption percent test are shown in **Figure 9 (C)** and listed in **Table 2**. The results exhibited that the BS-0 film absorbed maximum water up to 225.3 % for 30 minutes. The BS-01, BS-02 and BS-03 films showed 97.7 %, 71.4 % and 58.23 % of water absorption, respectively. The higher absorption by BS-0 film can be attributed to the presence of hydrophilic polymers such as PVA and gelatin, which, in the absence of RRE, had made more interactions with water and thus absorbed more. The other films showed less absorption due to the presence of RRE and more interactions/crosslinking of polymeric chains with RRE instead of water molecules. This is evident as RRE volume ratio is enhanced; % water absorption is decreased, due to apparently development of higher degree of cross linking which resulted in lower % water absorption [76].

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Table 2. Film thickness, % moisture content, % solubility and % water absorption mean values ± standard deviation were presented.

Sr. No.	Sample codes	Film thickness (mm)	Moisture content (%)	Water solubility (%)	Water absorption (%)
1	BS-0	0.123±0.04	17.42±1.97	73±3.6	225.3±5.25
2	BS-01	0.439±0.081	16.51±2.52	70.2±2.52	97.7±3.44
3	BS-02	0.771±0.087	16.41±2.35	63.2±3.07	71.4±1.97
4	BS-03	0.884±0.06	15.14±1.46	60.2±4.35	58.23±2.31

3.10 Water drop test

Water drop test was performed on composite films to check their ability to pass water or not. The films that hold water appeared to be decent for their application in food packaging. The test results for all films were displayed in **Figure 9 (D),** and the results showed that the films did not allow the water drop to pass through them. The films or other materials which show superior water barrier properties are often opted for in food packaging applications [77].

3.11 Oxygen permeability

Oxygen permeability of films has a noteworthy effect on the quality and shelf life of packaged food items. When oxygen is entered through films, it causes spoilage of food items by oxidation, microbial growth and colour changes. Thus, lower rates of oxygen permeability can inhibit the oxidative spoilage and growth of aerobic microbes, thus leading to extended shelf life of perishable food products like strawberries. The oxygen present in the atmosphere can also weaken the bond energy of polymers, and thus unstable peroxides are formed. These unstable species slow down the degradation rate of polymeric films and thus make them unsuitable for food packaging applications. The oxygen permeability of composite films was evaluated by the method already discussed by Wrinkler, and the results were presented in **Figure 9 (E)** [78].

The O_2-permeability test results for an airtight flask (negative control) and an open flask (positive control) were 4.7±0.43 mg/L and 5.21±0.22 mg/L, respectively. The oxygen

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permeability values calculated for each film were 5.08±0.23 mg/L (BS-0), 5.05±0.31 mg/L (BS-01), 4.69±0.57 mg/L (BS-02) and 3.03±0.43 mg/L (BS-03). The data demonstrated that incorporating RRE reduced oxygen permeability, due to increasing RRE volume, which caused free volumetric holes in films to contract, making it more difficult for oxygen molecules to penetrate. Thus, lower oxygen permeability is a desirable characteristic for polymers, particularly in food packaging applications [79].

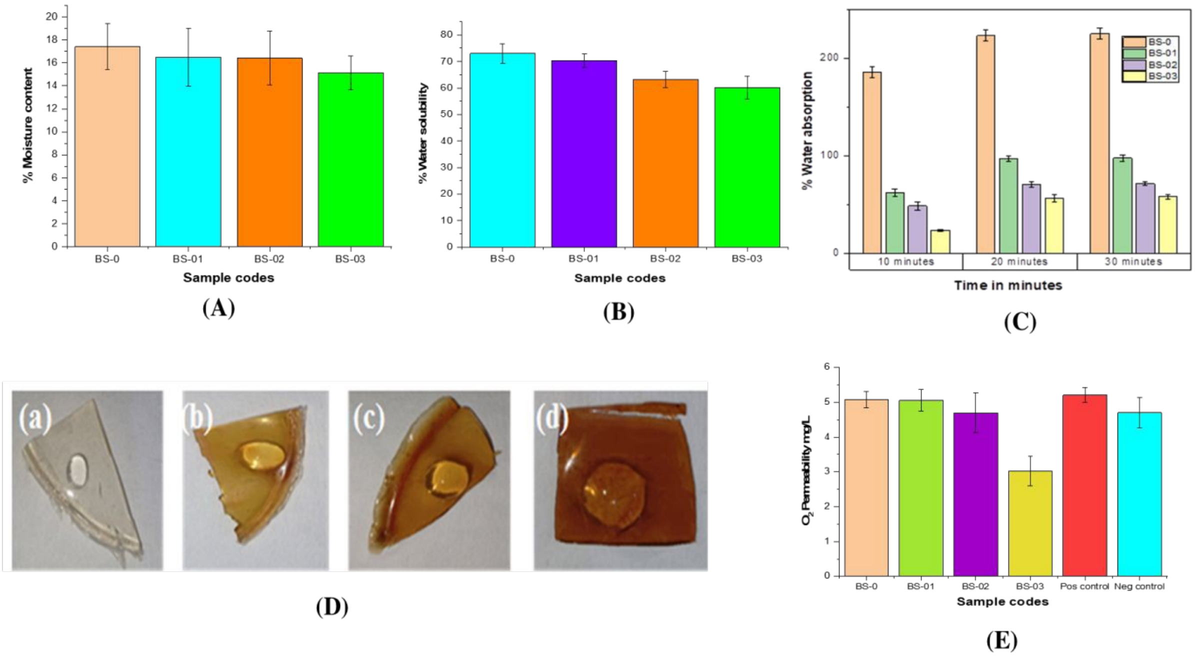

Figure 9. (**A**) % moisture content (**B**) % water solubility (**C**) % % water absorption (**D**) water drop test on films (a) BS-0 (b) BS-01 (c) BS-02 (d) BS-04 and (**E**) oxygen permeability results of the prepared films

3.12 Antibacterial potential

Natural plant extracts incorporating antibacterial components are more sterile, eco-friendly, and biodegradable. They are regarded as excellent materials to be used in food packaging against food pathogens [80]. The plant extracts contain bioactive phenolic and flavonoids components, and the hydroxyl groups in these compounds provide the basis for their antibacterial activity. The hydroxyl groups develop certain interactions, such as hydrogen bonds, with the bacterial cell membrane. Based on their hydrophobic nature, these phenolic compounds may accumulate on the surface of cells or may invade the cytoplasm of microbes. After reaching the cytoplasm, these components interact with the other cellular organelles and cell's components; thus, they change the pH inside the cell membrane [81]. The bacterial cell wall has negative carboxylate groups ($-COO^-$) and the chitosan carry positive amino groups ($-NH_3^+$). The electrostatic interactions of these groups with each other provide the mechanism for antibacterial activity [82]. CS/PVA/gelatine and CS/PVA/gelatine incorporating RRE solutions were assessed for antibacterial potential against *S. aureus*, *B. subtilis* and *E. coli,* and results are shown in **Figures 10 (A) and 10 (B) and Table 3**. The effectiveness of each coating solution was determined by measuring the inhibition zone in mm. All the coating solutions exhibited fair to good antibacterial potential against all three bacterial strains in comparison to the control. The results demonstrated that in most tests, the BS-03 sample showed better results than other samples. This was due to the presence of a higher volume ratio of RRE in the BS-03 sample. When we compared the results of all samples, BS-03 showed higher % inhibition against *S. aureus* with the lowest IC_{50} value of 78.65 ± 0.13. Similarly, the BS-02 sample exhibited higher activity against *E. coli* with the lowest IC_{50} value of 118.6 ± 0.021; while the BS-03 sample exhibited the highest potential against *B. subtilis* with the lowest IC_{50} value of 128.75 ± 0.65. Overall, BS-02 and BS-03 samples incorporating higher volume ratios of RRE showed better antibacterial potential against all three bacterial strains as compared to BS-0 and BS-01, attributed to the presence of various active components (phenolic compounds, flavonoids, and others) in the RRE. Notably, the increasing volume of RRE correlated with improved antibacterial activity.

Based on these findings, the synthesized CS/PVA/gelatine/RR films, especially BS-02 and BS-03, show promise for food packaging applications. Similar antibacterial results were also reported by Dan *et al.* [83] for chitosan-gelatin film incorporating hop plant extract. The natural

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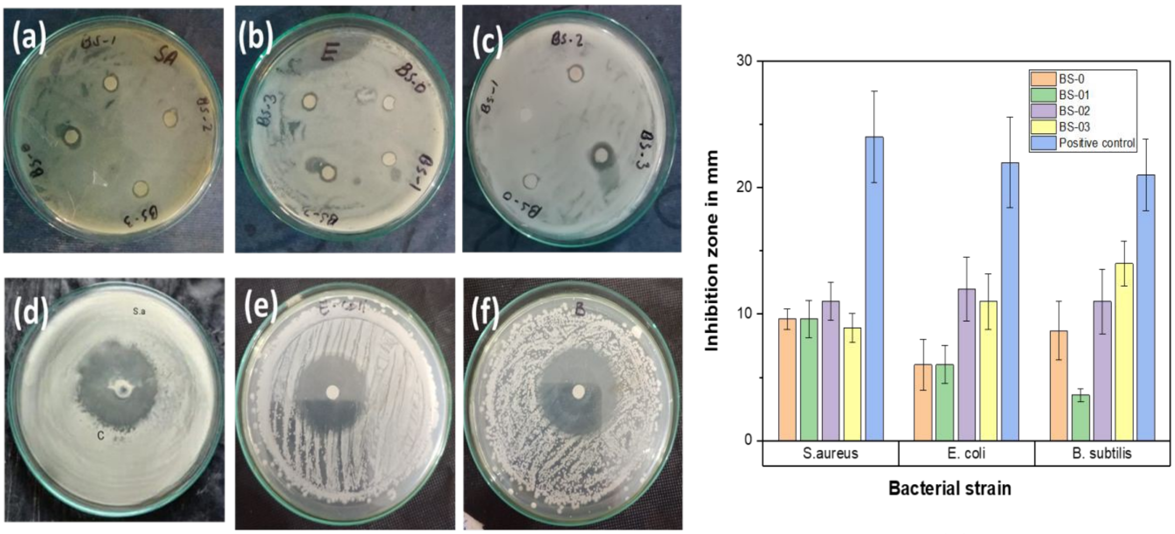
compounds found in plants are considered ideal candidates for food packaging applications due to their antibacterial properties. The two-way ANOVA demonstrated that treatment type significantly influenced antibacterial activity ($p < 0.001$), and its effect varied among bacterial species. The positive control produced the strongest inhibition zones, BS-02 and BS-03 had moderate effects, while BS-0 and BS-01 showed minimal inhibition. These results suggest that specific treatments possess promising antibacterial potential depending on the bacterial species tested.



(A) (B)

Figure 10. (A) Anti-bacterial images of samples against (a) *S. aureus* (b) *E. coli* (c) *B. subtilis,* and (d, e, f) representing positive controls for *S. aureus*, *E. coli* and *B. subtilis,* respectively; **(B)** Anti-bacterial potential of samples in terms of inhibition zone in mm

Table 3. Antibacterial properties of all synthesized sample solutions

Sample code	Inhibition zone (mm)	IC$_{50}$ at 100 mg/ml	Percent inhibition
S. aureus			
BS-0	9.6	172.91±0.0542	17.12±0.0542
BS-01	9.6	122.91±0.047	31±0.047
BS-02	11	93.6±0.049	38.57±0.049
BS-03	8.9	78.65±0.13	38.57±0.13

Control	24	50±0.9	100.714
E. coli			
BS-0	6	120±0.014	40±0.014
BS-01	6	115±0.09	40±0.09
BS-02	12	118.6±0.021	33.6±0.021
BS-03	11	120±0.028	23±0.028
Control	22	50±0.8	100
B. subtilis			
BS-0	8.7	138±0.604	40±0.234
BS-01	3.6	225±0.234	39.47368421
BS-02	11	189.64±0.28	26.10526316±0.28571
BS-03	14	128.75±0.65	37.89473684±0.65
Control	21	50.85±0.714	100.714

3.13 Antioxidant potential

The enhancement in food shelf life and its preservation applications depend upon the antioxidant potential of synthesized coating solutions and films. Oxidation reactions cause discoloration of food and reduce its flavor. Antioxidant materials are required to deactivate and scavenge free radicals. The antioxidant potential of solutions was determined by the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay (**Table 4**). DPPH is a nitrogen-free stable radical of deep purple color, which turns yellow after reacting with antioxidants. Antioxidant capacity was determined using the IC_{50} values, which represent the sample mass per mL of DPPH required to reduce the DPPH concentration by 50%. The results in **Figure 11** indicated that the BS-03 film demonstrated superior antioxidant properties (78.8±1.01with IC_{50} value of 0.3 mg/mL) attributed to the presence of plant extract (RRE) in excess. Similarly, BS-02 sample showed antioxidant potential of 70.5±0.57with IC_{50} value of 0.5 mg/mL. The BS-01 film exhibited 58.23±1.3 with IC_{50} value of 0.7 mg/mL. The lowest antioxidant activity was exhibited by BS-0 sample having value of 47.0±0.51with IC_{50} value of 1.1 mg/mL. The enhanced antioxidant activities of BS-03, BS-02 and BS-01 as compared to BS-0 were clearly

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attributed to the numerous active components found in RRE, including flavonoids, phenolics, citronellol, nerol, quercetin, catechin, epicatechin, rutin and phenethyl alcohol. These compounds prevent oxidative degradation, thus preventing food from microbial spoilage and resulting in prolonging freshness. The results were also in accordance to the literature analysis [84]. The statistical analysis reveals that both concentration and treatment type have highly significant effects on antioxidant activity. Furthermore, there is a significant interaction between these factors, indicating that the effect of each treatment depends on the concentration used. Thus, higher concentration significantly increases antioxidant activity ($p < 0.0001$).

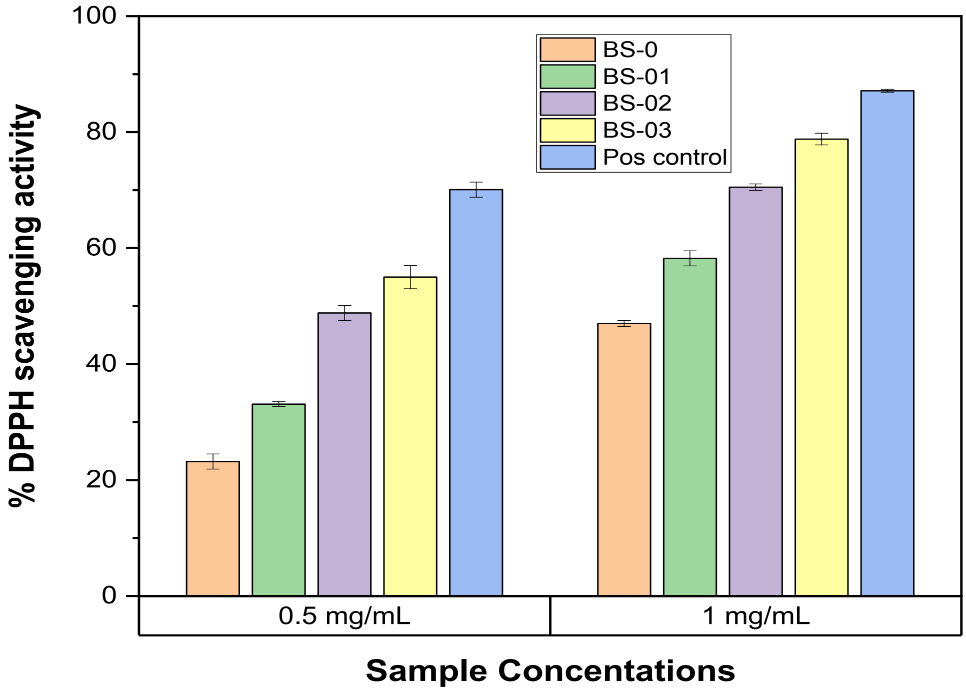

Figure 11. Antioxidant potential of sample films and positive control

Table 4. Antioxidant activities of all samples

Sample code	**DPPH assay**		
	Percent inhibition of DPPH free radicle		**IC$_{50}$**
	0.5mg/ml	1mg/ml	mg/ml
BS-0	23.20±1.3	47.0±0.51	1.1
BS-01	33.1±0.4	58.23±1.3	0.7
BS-02	48.8±1.3	70.5±0.57	0.5

BS-03	55.0±2.0	78.8±1.01	0.3
Positive control (Ascorbic acid)	70.08+1.3	87.14+0.26	0.36

3.14 Biodegradation of composite films:

A soil burial degradation test was conducted to evaluate the decomposition rate of the prepared films. The weight loss percentage with respect to the number of days for BS-0, BS-01, BS-02, and BS-03 films was measured. This method effectively indicated film biodegradability due to the presence of microbes and moisture in soil. The biodegradation process is initiated with water absorption, which disrupts hydrogen bonding between polymeric chains, followed by microbial invasion from bacteria, algae, and fungi. Biodegradation is a natural process and is also influenced by the chemical composition of composite films. Various factors affect polymeric film degradation rates, including pollutant quantity, microbial presence, and environmental conditions (oxygen, humidity, pH, and temperature). **Figure 12 (A)** displays the degraded film images after 18 and 45 days, while **Figure 12 (B)** shows the % biodegradation after 18 and 45 days. The results data showed that BS-02 film demonstrated the highest % degradation of 91.29 %, followed by BS-03 film 86.79 % and BS-01 film 85.91 %, while BS-0 film showed % degradation of 81.48%. It was observed that incorporation of RRE into CS/PVA/gelatin has improved % biodegradation of films. The biodegradation rates were higher as compared to already reported results by Riaz *et al.*, [85]. The increased % biodegradation, due to the incorporation of RRE, was attributed to the presence of carbon, nitrogen, and oxygen in many components of RRE, which served as food sources for microorganisms. Continuous water addition activates soil bacteria and weakens polymeric interactions between components, thus increasing degradation rates [86]. The incorporation of RRE into CS/PVA/gelatin films improved their biodegradability, transforming them into a rapidly degradable and eco-friendly material [85]. The faster degradation of these biopolymer films in soil environments provides a sustainable alternative with reduced environmental

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impact.

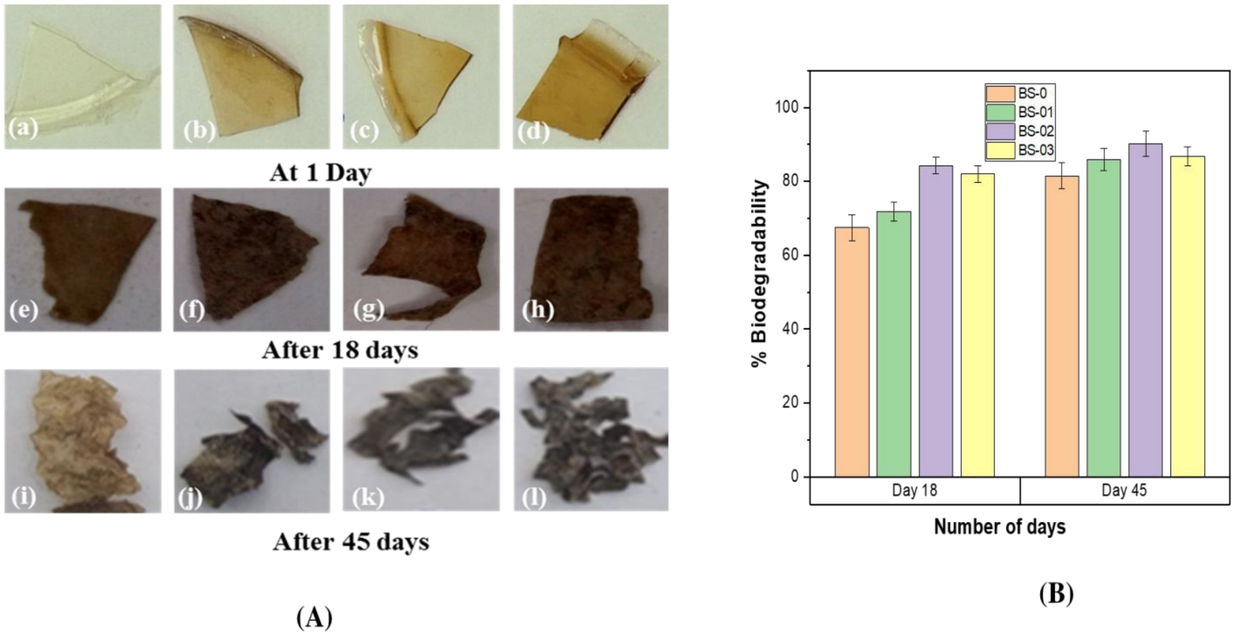

Figure 12. (A) Images of composite films (a) BS-0; (b) BS-01 (c) BS-02 (d) BS-03 before experimentation; (e) BS-0 (f) BS-01 (g) BS-02 (h) BS-03 after day 18; (i) BS-0 (j) BS-01 (k) BS-02 (l) BS-03 after day 45; (**B**) % biodegradability at day 18 and day 45 of prepared films

3. 15 Total phenolic contents (TPCs) and total flavonoid contents (TFC)

Polyphenols constitute the most frequent and broadly distributed group of compounds in the plant kingdom, with more than 8000 currently known compounds [87]. The rose flower has high polyphenolic compounds and essential oil, and thus serves as a key source of many raw materials for cosmetics, herbal teas and rose oil [88]. The *Rosa rubiginosa* extracts (RRE) TPCs and TFCs were expressed in µg of Gallic acid equivalent (GAE) and µg of Quercetin equivalent

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(QE) per mg of extract, respectively. The TPCs of RRE were found to be 86.74±0.91 µg GAE/mg. These values are close to as described by Murathan *et al.* [89] and Fascella *et al.*[90] Literature studies have demonstrated that among phenolic compounds, p-coumaric acid and chlorogenic acid were present abundantly in RRE extract. It was observed that the phytochemical properties of various rose species are greatly dependent upon their type of species. The RRE was found to be a promising source of natural antioxidants, which are useful in the pharmaceutical and food industries [91]. Similarly, TFCs of RRE were found to be 26.73±0.68 µg QE/mg. These values were also in accordance with already reported values by Tabaszewska *et al.* [92]Quercetin and Letulin are key flavonoid aglycons in Rose species. Quercetin and Lutulin belong to the flavonol and flavone groups, respectively [93].

3.16 Application on strawberries

Coatings offer an effective solution to extend the shelf life of fruits and vegetables. Strawberries, being delicate and water-rich, have a limited shelf life and are prone to spoilage after harvesting due to pathogen attacks. Unpackaged or uncoated foods also deteriorate due to water evaporation and cellular respiration. All the composite coating solutions of BS-0, BS-01, BS-02 and BS-03 were used for application on strawberries to evaluate their potential to protect and enhance shelf life of strawberries. Uncoated strawberries were used as a negative control for comparison of results. The weight loss % was monitored after 1st, 2nd, 6th, 7th, 8th and 17th day. **Figures 13 (A)** and **13 (B)** were used to represent results in terms of images of strawberries and weight loss % over time (in days), respectively. Results indicated that coating solutions with higher RRE ratios exhibited better protection of strawberries and provided greater shelf life, as indicated by both images and weight loss % graphs. Similar results were also discussed in literature by Mukhtar *et al.,* [94]. The results showed that uncoated strawberries (control) started to deteriorate slowly after the 2nd day and experienced a 60 % weight loss after 7th days. For coated strawberries using BS-0, BS-01, BS-02, and BS-03 coating solutions, the weight loss percentages on day 6th were 39.82 %, 34.87 %, 32.12 %, and 29.28 %, respectively, compared to 45.55 % for uncoated strawberries. Similarly, after 7th day, the weight loss % was 45.35 % for BS-0, 39.09 % for BS-01, 35.05 % for BS-02, 32.23 % for BS-03 and 60.59 % for the negative control. It can be inferred from **Figure 13 (A)** that initial weight loss up to 6 days was mainly due to the loss of water content from strawberries, and then after 6 or 7 days, fungal attack began, which started to degrade the strawberries.

By day 17th, weight loss % reached 71.09 % for BS-0, 66.32 % for BS-01, 60.07 % for BS-02, 57.2 % for BS-03 and 89.03 % for the uncoated negative control. It was noted that all coating solutions preserved strawberries well and enhanced their shelf life up to 8 days without being deteriorated by fungal attack. After 8 days, the strawberries coated by BS-0, BS-01 and BS-02 started to deteriorate by fungal attack more rapidly as compared to BS-03 coating solution, which remained intact up to 10 days. Thus, it can be concluded that increasing the volume ratio of RRE in CS/PVA/gelatin-based coating solutions has enhanced the shelf life of strawberries up to 10 days. The BS-03 coating solution demonstrated the best performance in reducing % weight loss, suggesting its highest potential for preserving delicate food items. The results of fabricated coatings on strawberries were also compared with literature studies, and the results are presented in **Table S1** in the supplementary information. The literature studies showed the strawberries were stored for 5 days at room temperature and for 15 days at 4 °C, but our fabricated coatings protected strawberries for 10 days at room temperature.

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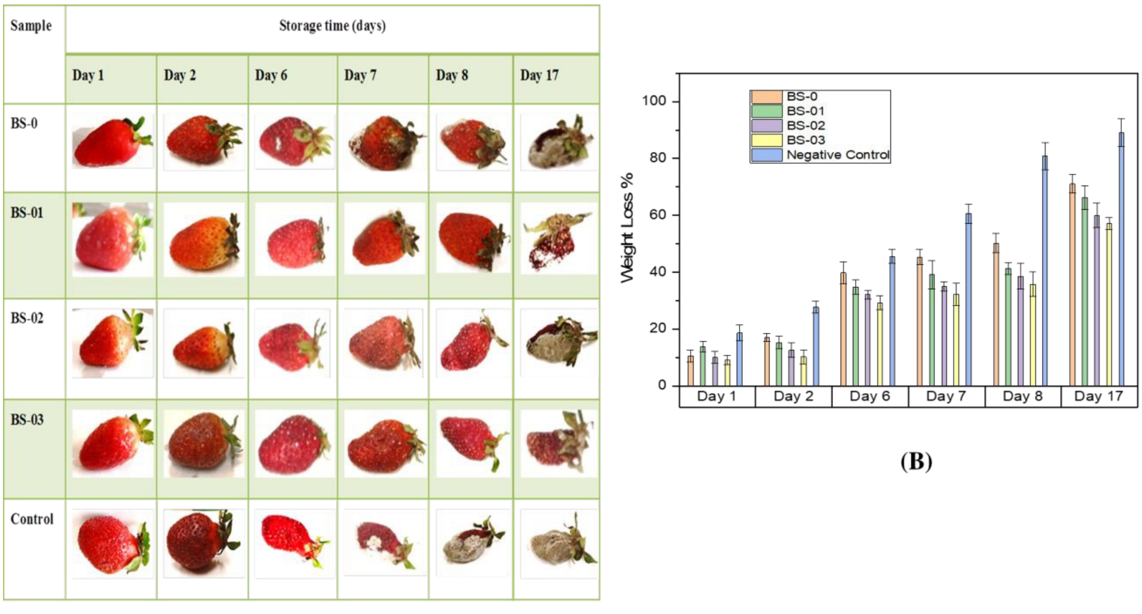

(A)

(B)

Figure 13. (A) Images representing application of coatings on strawberries and their deterioration with respect to the number of days; Control represents uncoated strawberries **(B)** Weight loss % of strawberries from day 1 to day 17

3.17 Sensory evaluation

The results of sensory evaluation for each formulation coded as BS-0, BS-01, BS-02 and BS-03 and a control (without any coating) are presented in **Tables S2 to S-6.** The results exhibited that strawberries coated with BS-03 formulation showed the most favourable and better results in terms of taste, smell, color, juiciness, firmness and acceptability in general as compared to BS-0, BS-01 and BS-02 formulations.

This might be due to the presence of highest amount of RRE in this formulation, which provided enhanced antioxidant and antibacterial properties to this formulation. The BS-0 formulation exhibited results of parameters such as taste, smell, color, juiciness, firmness and general acceptability mostly in neither good nor bad and like somewhat categories. Similarly, BS-01 showed most of the studied parameters in like somewhat category. The BS-02 coating exhibited parameters in like somewhat and moderate category. The BS-03 coating showed most attractive and convincing results of studied parameters in similar to moderate and greatly admire category. The control strawberries without any coating were subjected to the highest degradation and changes, thus their study parameters mostly fall under mildly disliked and mildly unfavourable categories.

5. Conclusion

This study demonstrated that CS/PVA/gelatin composite coatings enriched with RRE enhanced the preservation of strawberries. Increasing RRE volume ratio into CS/PVA/gelatin coatings (from BS-01 to BS-03) thickened the films and improved their barrier performance, while % moisture content, water solubility water absorption, and oxygen permeability were decreased. Antimicrobial tests showed that higher volume of RRE containing coatings, particularly BS-02 and BS-03, exhibited stronger activities against *S. aureus*, *B. subtilis*, and *E. coli* than the RRE free coating (BS-0). Antioxidant capacity also increased with enhancing RRE ratio, with BS-03 reaching 78.8 ± 1.01% DPPH inhibition (IC_{50} = 0.3 mg/mL). Soil burial trials confirmed that all films were biodegradable; the highest 45-day weight loss was observed for BS-02 (91.29%), followed by BS-03 (86.79%). Although the incorporation of RRE reduced ductility and increased stiffness, the mechanical properties of BS-03 film would be more suitable for handling and packaging applications. When applied to strawberries, all coatings helped reduce weight loss compared to the uncoated control and prolonged their shelf life. Among them, BS-03 was the most effective, preventing visible spoilage for up to 10 days and achieving the highest sensory scores. Overall, the BS-03 formulation emerges as a promising bio-based coating for the short-term extension of berry shelf life. Future research should focus on optimizing composition through response surface methodology, conducting antifungal tests,

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evaluating performance during refrigerated storage, and examining component migration for applications involving direct contact with food.

Author Contributions

B.S.: Conducted experiments, investigations, data analysis and writing original draft. M.Z.: Conceptualization, writing original draft, reviewing and editing. A.Y.: performed antibacterial assessment. F.A.: Reviewing. S.S.: Conceptualization, supervision, editing and reviewing. A.U.: Conceptualization, supervision, editing and reviewing. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

There are no conflicts to declare.

Data availability

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

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Data Availability Statement

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