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Research article

# Eco-friendly natural extract loaded antioxidative chitosan/polyvinyl alcohol based active films for food packaging

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ABSTRACT

Nowadays, biodegradable antimicrobial packaging materials draw substantial attention and are considered one of the preferred emerging technologies to be used in the food industry. In this study, we have reported developing a two-component, chitosan (CS) and polyvinyl alcohol (PVA) based films loaded with natural extract of *Ocimum tenuiflorum* via solvent casting technique. Experimental results showed that all the components were well dispersed, resulting in complete homogenous films. FTIR-ATR spectral analysis indicated strong interaction between the film matrix components, which also gets reflected by the films' physical properties. Transparent biodegradable films have resulted from the mixture and incorporation of the natural agent/extracts influenced light barrier, water resistance, moisture content (9.89–9.01%), and DPPH radical scavenging properties (antioxidant value ~41.1%) of the CS/PVA films. Therefore, this natural extract containing transparent CS/PVA-based films promises to be used in the food industry as packaging material to enhance food safety.

## 1. Introduction

To ensure the quality and preserve food material from foreign contamination, food packaging is the most important and prime factor in the food industry's delivery of safe edible products. Due to scientists immense investigation and dedication, it's well-known that packaging has been far developed well beyond its primary function as merely providing protection. It also helps provide other information related to food products such as marketing, awareness, branding, product information, etc. Most of the conventional packaging materials used in the food industry are dominated by petroleum-based products and nondegradable polymers, contributing to environmental pollution or contaminating the enclosed food material. Therefore, an abrupt increase witnessed in the field of the investigation involving production and characterization of novel eco-friendly packaging materials and other contact surfaces, aiming to counter the environmental impact caused by conventional synthetic packaging materials and maintaining the quality of products, respectively (De Arruda et al., 2014; Tan et al., 2015).

In this context, natural polymers serve as the best alternative to conventional materials in this field. Several biopolymers have been investigated to develop eco-friendly, biodegradable, and smart food packaging materials. Natural polymers such as polysaccharides, proteins, and lipids-based films provide various valuable properties like eco-friendly, biodegradability, biocompatibility, edibility, and sustainability (Ali and Ahmed 2018a, 2018b). Besides these, new trends have been emerged to improve the functionality of such packaging materials further in the direction of smart materials via loading various active functional materials such as antimicrobial agents, antioxidants, flavoring agents, colorants, nanoparticles, etc., (Beigzadeh Ghelejlu et al., 2016, Dominguez et al., 2018, Irkin and Esmer 2015, Yang et al., 2016). Also, increasing consumer demands witnessed towards eco-friendly packaging materials while preserving food quality-driven researchers to think toward more sustainable, non-toxic alternatives (Shah et al., 2014). Therefore collective attention has been forwarded to develop antimicrobial and antifungal packaging films incorporated nontoxic additives to improve food quality, extend shelf-life and minimizing chemical preservatives (Vieira et al., 2011).

Chitosan (CS) a natural amino biopolymer with inherent bioactive property, good film-forming ability, nontoxic, degradability, and excellent barrier property has been extensively studied by researchers related to the food industry packaging materials. At low pH, CS attains cationic character due to protonation of amino groups, which leads to

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**Table 1.** Compositions of different film forming solutions (FFS).

| Samples* | 1% CS (ml) | 5% PVA (ml) | Extract (ml) |
|---|---|---|---|
| PVA | 0 | 20 | 0 |
| CS50PVA50 | 10 | 10 | 0 |
| CS70PVA30 | 14 | 06 | 0 |
| CS50PVA502TL | 10 | 10 | 2 |
| CS50PVA504TL | 10 | 10 | 4 |
| CS50PVA506TL | 10 | 10 | 6 |
| CS70PVA302TL | 14 | 06 | 2 |
| CS70PVA304TL | 14 | 06 | 4 |
| CS70PVA306TL | 14 | 06 | 6 |

* Total volume of film-forming solution was 20 ml containing 2–6 ml natural extract.

electrostatic interaction with other compounds of interest (Ahmed and Ikram 2015). However, films based on natural biopolymers have low mechanical strength and are extremely sensitive to environmental conditions. As a result of which, researchers have developed hybrid films containing both biopolymer and biodegradable synthetic polymers such as polyvinyl alcohol (PVA). The blending of biopolymers with synthetic polymers changes the resulting films' physicochemical properties and improves the mechanical strength and cost performance (Goonoo et al., 2013; Tanase et al., 2015).

Some synthetic polymers such as PVA which USDA has approved for meat and poultry product packaging with excellent biodegradability, water-soluble, nontoxic, and biocompatibility have shown good miscibility with CS due to hydrogen bonding between amino and hydroxyl groups in the two polymers (DeMerlis and Schoneker 2003). PVA also exhibits excellent film-forming ability with high mechanical strength, bio-adhesive, and emulsifying properties. It has been reported that the concentration of PVA does not affect the antimicrobial properties of CS (Cai et al., 2016). Therefore it can be concluded that PVA acts as the best alternative among synthetic polymers that impart appropriate mechanical strength, biodegradability, film formation and chemical resistance to the resultant films which become the prime objective of packaging materials. Furthermore, to improve the packaging films' properties, various additives such as bioactive natural extract, plasticizer, and crosslinker were also added to such films. In this perspective, usually, plant extracts have gained much attention due to their high phenolic compound concentration, which imparts strong antioxidant properties (Tiwari et al., 2009). *Ocimum tenuiflorum,* also known as holy basil or tulsi is used in this study. It is a high medicinal value plant and is distributed throughout India and other parts of the Asian subcontinent. The leaves of this plant contain an essential oil consisting of various compounds such as eugenol, carvacrol, eugenol, methyl-chavical, limatrol and caryophylline, etc., having extraordinary medicinal value and also exhibit potent antimicrobial activity against *Staphylococcus aureus* and *E. coli* (Yamani et al., 2016).

The present study's objective was to develop natural plant extract incorporated active films from a hybrid mixture of CS and PVA via the solution casting method. Fourier transform infrared (FTIR) and attenuated total reflectance (ATR) spectroscopic techniques were used to analyze the interaction in the film matrix between the components. Various properties such as transparency, water-resistant, light barrier, and the resulting films' antioxidant capacity were also investigated.

## 2. Materials and methods

### 2.1. Materials

Chitosan with deacetylation degree 85% was purchased from HiMedia Pvt. Ltd., Mumbai, India, Polyvinyl alcohol MW = 85,000–1,20,000 from SD Fine Chem. Ltd., Mumbai, India. 2,2-Diphenyl 1-picrylhydrazyl (DPPH), acetic acid, and methanol were purchased from Merck specialties, Pvt. Ltd. Mumbai, India. All chemicals were used as received without further purification. Double distilled water was used throughout the experiment.

### 2.2. Methods

#### 2.2.1. Preparation of plant (Ocimum tenuiflorium) extract

Fresh leaves (~10.0 g) of the *Ocimum tenuiflorium* plant were obtained from the university (Jamia Millia Islamia, New Delhi, India) garden and thoroughly washed with tap water to remove the debris and other impurities, followed by another washing with double distilled water. The clean leaves were then refluxed in double-distilled water (300 ml) at 60 °C for about 40 min. The mixture was then cooled down to room temperature, filtered with Whatman No. 1 filter paper, and stored at 4 °C in a closed bottle.

#### 2.2.2. Preparation of films

The films were prepared using a conventional casting method in a similar way as reported by Rubilar et al. with slight modification (Rubilar et al., 2013). 1% (w/v) film-forming solution of CS was prepared in acetic acid solution (1%, v/v) at room temperature under constant stirring by using a magnetic stirrer. The solutions was then filtered through a cheesecloth. Sidewise, homogenous aqueous solution of PVA (5%, w/v) was prepared under constant stirring at 45 °C. After forming a clear solution, both CS and PVA solutions were mixed in an appropriate ratio to develop the blend solution and stirred at 50 °C for 20 min on a magnetic hotplate. The prepared plant extract solution was then added to the blend solution to reach a final concentration of 10%, 20%, and 30% (v/v) and again stirred for 15 min (different compositions are given in Table 1). The final mixture was then decanted into glass Petri dishes to prepare the films and dried in an oven at 40 °C. Finally, the dried films (CS/PVA-TL) so obtained were peeled off from the Petri dishes and kept in an airtight desiccator for 48 h. The films were then carefully washed with double distilled water twice (except pure PVA film) and again dried in an airtight desiccator. Similarly, pure PVA and PVACS films without plant extract were also prepared.

#### 2.2.3. Measurement of film thickness

The prepared film thickness was measured using a calibrated digital *micrometer* Vernier Caliper (Aerospace LCD digital Vernier Caliper-150 MM) at three different locations, and the average thickness was used to measure the film properties.

#### 2.2.4. Determination of moisture content

Moisture content (MC %) of films was determined by loss on drying method. Preweighed small film strips were dried in a hot air oven at 105 °C until a constant weight was achieved (dried sample). The total moisture content was calculated as the percentage of weight loss based on the initial weight, according to Eq. (1):

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

$M_w$ is the initial weight of films conditioned at 75% RH to moisture equilibrium, and $M_d$ is the weight of films after drying. All the experiments were repeated in triplicate, and the average value was used for calculation.

#### 2.2.5. Determination of total soluble matter

Total soluble matter (TSM) in the films was determined according to a pre-reported method by Wang et al. and defined as the percentage of dry weight solubilized in water after 24 h (Wang et al., 2013). From the oven-dried (105 ± 2 °C) films, small strips of dimension (2 × 2 cm) were cut down, weighed, and immersed in water (50 ml) at 25 °C. After the predetermined time (24 h), the strips were taken out carefully with

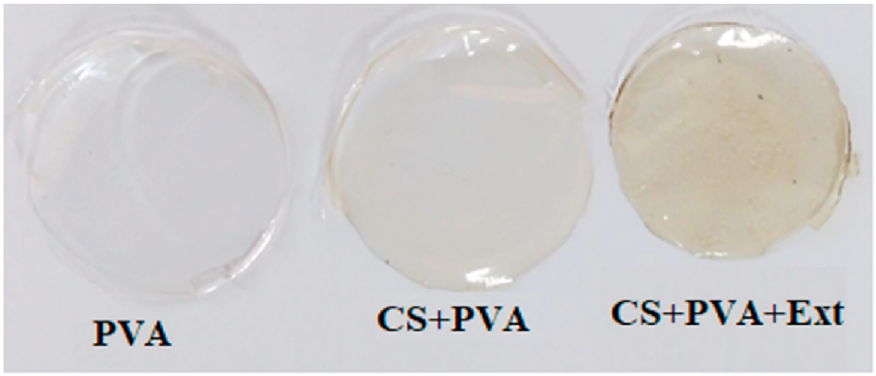



**Figure 1.** Optical images of PVA, CS/PVA, and CS/PVA with plant extracts films.

forceps and dried to constant weight in an oven at 105 ± 2 °C. The complete dried (constant weight) strips were then weighed to determine the amount of insolubilized matter and the total amount of the solubilized matter in the films were determined following Eq. (2):

$$\mathrm{TMS}(\%) = \frac{W_i - W_f}{W_i} \times 100 \quad (2)$$

Wi and Wf are the initial mass (before immersed in water) and final mass (after 24 h immersed in water) of the sample.

#### 2.2.6. Optical property

The transparency property of the prepared films was measured using UV-Vis spectrophotometer with the help of a reported method by Kanatt et al. with some modification (Kanatt et al., 2012). The method was followed by the cutdown of a small rectangular film strip (0.7 × 1.5 cm) and positioned in the test cell of UV-Vis spectrophotometer (U-3900 Spectrophotometer, Hitachi) and an empty cell was used reference cell. A UV-Vis spectrum for each of the film strips was recorded from 200 to 800 nm wavelength. The transparency at 600 nm ($T_{600}$) wavelength was obtained using Eq. (3):

$$T_{600} = -\log \frac{\% T}{b} \quad (3)$$

Where, %T is the percentage transmittance, and b is the thickness of the film (mm). Similarly, the opacity of the films was calculated according to a reported method by Gontard et al. as follows (Eq. (4)):

$$\text{Opacity} = \text{absorbance at 500 nm} \times \text{film thickness (mm)} \quad (4)$$

#### 2.2.7. Determination of DPPH radical scavenging capacity

DPPH scavenging method was used to evaluate plant extracts' antioxidant capacity loaded in the films (Hartwig et al., 2012). This method has been widely accepted for measuring the efficiency of plant extract as an antioxidant agent due to its good stability, simplicity, feasibility, and credible sensitivity. The extent of antioxidant capacity has been measured as such compounds' ability to scavenge the DPPH radical. The method follows as film extract solution was prepared by dissolving natural extract loaded film (20 mg) in 2 ml distilled water, and 1 ml of the film extract solution was mixed with 0.2 ml of methanolic (1mM) solution of DPPH. The mixture was thoroughly mixed and kept under dark for a half-hour under ambient temperature.

The mixture's absorbance was then recorded at 517 nm against the corresponding blank solution on a spectrophotometer. The analysis was repeated in triplicate, and the percentage DPPH radical scavenging activity was calculated from the Eq. (5):

$$\text{Scavenging activity (SA\%)} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100 \quad (5)$$

Where $A_{control}$ is the absorbance of DPPH solution and $A_{sample}$ is the absorbance of extract solution with DPPH.

### 2.3. FTIR-ATR

FTIR-ATR spectra were recorded on a Bruker Tensor 37 spectrophotometer (Germany) from 4000-600 $cm^{-1}$ wavelength range, via directly using the solid film sample and data acquisition with IR-solution software. The spectra obtained were used to determine the film matrix's interactions (i.e., between CS, PVA, and extract).

### 2.4. X-ray diffraction

X-ray diffraction (XRD) patterns of the films were recorded on a Rigaku Ultima IV type X-ray diffractometer (Japan) in the 2θ range from 10 to 80° scanning rate of 1°/min. Cu Kα radiation (wavelength, 1.54 Å; filament current, 30 mA; voltage, 40 kV) is used to generate X-rays.

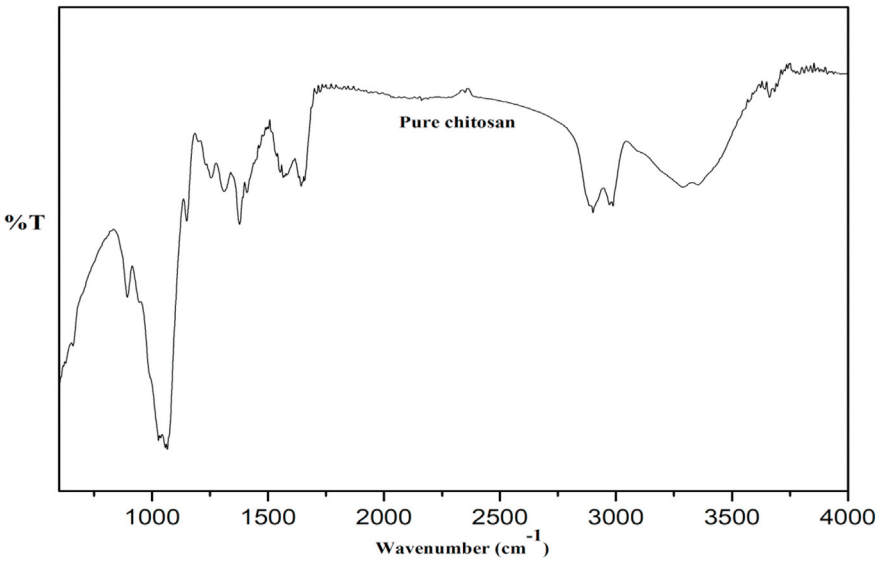
### 2.5. Statistical analysis

All the experiments were carried out in triplicates. Results are presented as the mean, standard error of means. One-way analysis of variance (ANOVA) was performed. Significant differences between different samples were analyzed using the Tukey test. A p-value less than 0.05 was considered statistically significant.

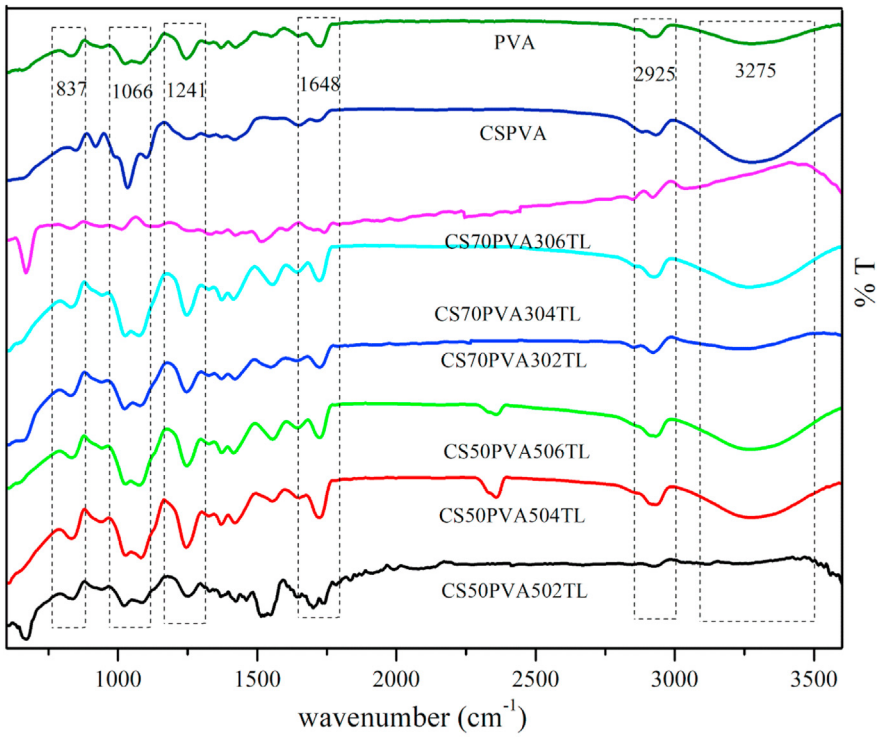
## 3. Results and discussion

### 3.1. Visual observation of film appearance and thickness

Visual observation and color of the packaging or coating material are some of the essential factors directly influencing the quality and customer attraction of the food material. The visual observation of PVA, CS/PVA, and CS/PVA loaded with the natural extract of *Ocimum tenuiflorum* was homogenous, thin, uniform, and somewhat colorless, which became slightly fainted extract loaded films as shown in Figure 1. Similar effects of natural extract addition on CS films' color were reported by other researchers also (Siripatrawan and Vitchayakitti, 2016). The homogeneity of the film revealed that the two polymeric solutions were completely miscible with each other irrespective of concentration as used in this study, which could be attributed to possible strong intermolecular hydrogen bonding between CS (-$NH_2$ and -OH) and PVA (-OH) active functional groups (Figure 2) (Naveen Kumar et al., 2010). Moreover, the films were firm and flexible after drying, which gets easily peeled off from Petri dishes without surface cracks or no bubbles were observed.




(a)




(b)

**Figure 2.** FTIR-ATR spectra of (a) Pure chitosan, (b) PVA, CS/PVA, and natural extract loaded CS/PVA-based films.

Film thickness is an essential parameter that is directly allied with films' physical properties (Table 2). Pure PVA films (5%, 20 ml) have an average thickness of 0.51 mm, which almost decreases by ~43% (0.29 mm) in the blends containing 50% PVA (5%) and % CS (1%). The decrease in the film thickness upon blending could probably be due to the decreasing concentration of the blend solution, as the CS solution concentration is only 1%. Subsequent decreases in the thickness (0.18 mm, to ~38%) were observed after the addition of the natural extract solution. However, the average thickness of natural extract-loaded CS/PVA film did not vary much.

### 3.2. FTIR-ATR

FTIR-ATR analysis was carried out to study the molecular interaction between CS, PVA, and natural extract in the films. FTIR-ATR spectrum of pure chitosan film is reported in Figure 2 (a). Pure PVA films consist of a broad absorption peak at 3275 $cm^{-1}$ corresponding to stretching frequency of hydroxyl (-OH) groups, 2925 $cm^{-1}$, and 2840 $cm^{-1}$ relates to the asymmetric and symmetric stretching vibration of C–H groups, respectively (Rodrigues et al., 2007). The primary characteristic bands for chitosan include 3465 $cm^{-1}$

**Table 2.** Average film thickness, transparency, and opacity of PVA, CS/PVA, and natural extract loaded CS/PVA films, water solubility (WS), and moisture content (MC).

| Samples | Average thickness of films (mm, ±0.05mm) | Transparency | Opacity | WS (%) | MC (%) |
|---|---|---|---|---|---|
| PVA | 0.51 | 13.93 ± 0.17 | 0.05 ± 0.02 | 100.0 | 16.67 |
| CS50PVA50 | 0.29 | 10.79 ± 0.21 | 0.10 ± 0.01 | 70.0 | 13.41 |
| CS50PVA502TL | 0.18 | 11.12 ± 0.15 | 1.37 ± 0.01 | 66.6 | 9.89 |
| CS50PVA504TL | 0.18 | 10.89 ± 0.14 | 0.22 ± 0.02 | 60.9 | 9.89 |
| CS50PVA506TL | 0.18 | 11.11 ± 0.18 | 0.005 ± 0.03 | 64.1 | 9.11 |
| CS70PVA302TL | 0.18 | 10.99 ± 0.17 | 1.51 ± 0.01 | 64.8 | 9.21 |
| CS70PVA304TL | 0.16 | 10.21 ± 0.18 | 0.28 ± 0.02 | 60.2 | 9.14 |
| CS70PVA306TL | 0.12 | 10.48 ± 0.14 | 0.18 ± 0.04 | 64.3 | 9.01 |

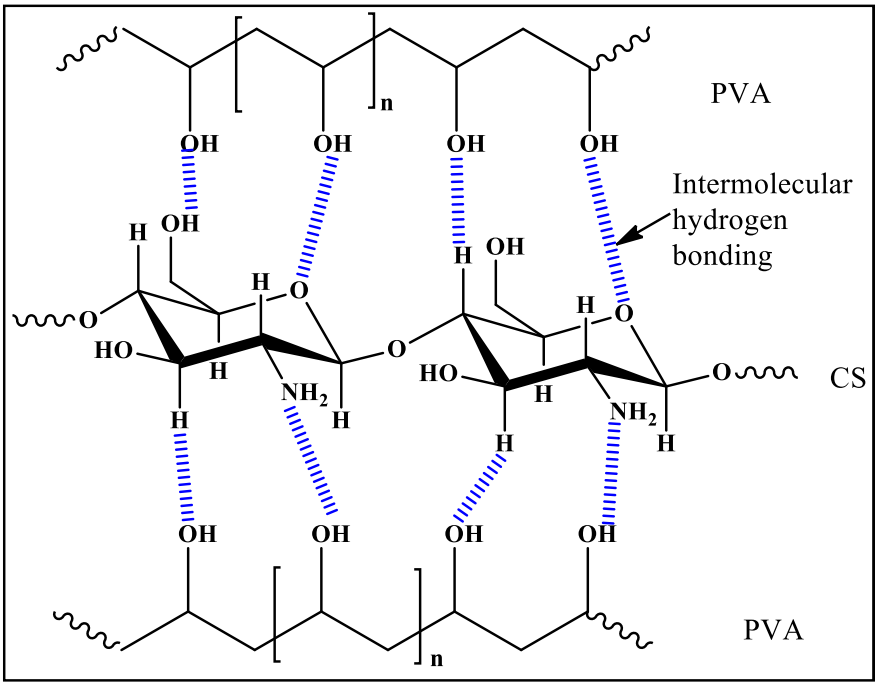



**Figure 3.** Mechanistic representation of possible intermolecular hydrogen bonding between CS and PVA.

stretching vibration of N–H and O–H bonds, 1646 $cm^{-1}$ for C=O stretching (amide I), 1580 $cm^{-1}$ N–H bending (amide II), 1545 $cm^{-1}$ secondary amide vibration, and 1035-1155 $cm^{-1}$ C–O bonds (Altiok et al., 2010). FTIR-ATR spectra of PVA, CS/PVA, and natural extract loaded CS/PVA films were shown in Figure 2 (b). FTIR-ATR spectra of CS/PVA blend films were shown some characteristic changes in the absorption pattern as well as the frequency shifting (lower range) due to hydrogen bonding interaction between reactive functional groups in CS (-$NH_2$ and -OH) and PVA (-OH) (Figure 3) (Abdelghany et al., 2019). The –CH stretching band was observed at 2925 $cm^{-1}$, C=O stretching (amide I) at 1648 $cm^{-1}$, and deformed C–O stretching vibration of hydroxyl groups at 1066 $cm^{-1}$ and C–N stretching vibration of CS at 1241 $cm^{-1}$ (Anicuta et al., 2010). Therefore, FTIR-ATR spectra confirm strong polymeric interaction in the blend film matrix between the two components.

FTIR-ATR spectra of natural extract loaded films were shown some new peaks which are characteristic of natural extract, medium to strong absorption bands were observed in the region of 1250 to 857 $cm^{-1}$ which probably due to C–C and C–O stretching of the pyranoid ring and C–O–C glycosidic bond. A new peak at 837 $cm^{-1}$ appeared in all extract-loaded films, which become well relate to the ring vibration of carvacrol which is a chemical constituent of *Ocimum tenuiflorium* (Altiok et al., 2010; Abdelghany et al., 2019; Schulz et al., 2005). However, not many changes were observed in CS/PVA films' characteristic spectra after extract addition, which confirms that natural extract is dispersed in the polymer matrix. A similar, observation was made by other researchers (Perdones et al., 2016; Wang et al., 2013).

### 3.3. Optical properties

Optical properties such as light transmission, transparency, and opacity level are important packaging materials. It is understood that the packaging material is the primary preventive fence for the protection of food material against various foreign influences. UV light-induced photo-oxidation of food material (especially fat-rich such as meat, cheese, fried food, etc.) is a significant factor leading to spoilage of food material (Lu and Xu 2009). Therefore, the UV light barrier protective packaging material could handle this problem to some extent. Light transmittance properties of pure PVA, blend CS/PVA, and natural extract loaded films were determined over wavelength ranging from 200 to 800 nm. The percentage transmittance at the selected wavelength was given in Table 3, and the values were calculated by using Eq. (3). It is clear from the transmittance value that the blend and natural extract loaded films exhibited low transmission in the UV light region compared to pure PVA films. The reduction of light transmission can be attributed to phytochemical constituents of *Ocimum tenuiflorium* having several polyphenol

**Table 3.** Light transmittance (%T) of PVA, CS/PVA, and natural extract loaded CS/PVA films at different wavelength.

| Sample | Light transmittance (%) at different wavelength (nm) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 200 | 250 | 300 | 350 | 400 | 450 | 500 | 550 | 600 | 650 | 700 | 750 | 800 |
| PVA | 89.9 | 93.2 | 95.2 | 96.7 | 97.4 | 98.6 | 98.8 | 99.5 | 100.6 | 101.8 | 103.0 | 104.4 | 105.9 |
| CS50PVA50 | 59.1 | 73.1 | 83.5 | 85.9 | 86.2 | 89.7 | 90.7 | 91.8 | 93.1 | 93.9 | 94.4 | 95.0 | 96.1 |
| CS50PVA502TL | 24.3 | 18.3 | 19.4 | 20.7 | 37.8 | 52.3 | 56.2 | 58.2 | 60.2 | 62.3 | 64.7 | 66.6 | 68.7 |
| CS50PVA504TL | 23.7 | 29.0 | 36.3 | 38.9 | 77.8 | 87.4 | 89.1 | 90.1 | 91.2 | 92.6 | 93.9 | 95.2 | 96.6 |
| CS50PVA506TL | 19.9 | 23.4 | 29.5 | 30.0 | 71.7 | 92.2 | 96.3 | 98.1 | 100.2 | 102.0 | 104.2 | 105.9 | 107.3 |
| CS70PVA302TL | 38.0 | 45.3 | 54.2 | 58.7 | 80.3 | 91.2 | 93.1 | 94.1 | 95.4 | 96.6 | 97.9 | 99.5 | 100.6 |
| CS70PVA304TL | 23.1 | 26.8 | 33.2 | 35.2 | 67.1 | 83.9 | 86.8 | 88.5 | 89.9 | 91.6 | 93.3 | 95.4 | 97.0 |
| CS70PVA306TL | 18.8 | 22.3 | 59.7 | 65.0 | 92.0 | 92.0 | 93.3 | 95.0 | 95.0 | 96.3 | 97.7 | 99.3 | 100.2 |

compounds, benzene rings, and carbonyl compounds which improves the n→π* absorption in the UV region (Dammak et al., 2017). Therefore, it can be concluded that blend (CS/PVA) and natural extract loaded (CS/PVA-TL) films have an excellent barrier to UV light, a powerful lipid oxidizing agent in the food system. The previous findings of other researchers support these results; Hu et al. reported improvement in UV light barrier of gelatin films by incorporation of *Ginkgo biloba* extract, while Vilela et al. reported enhancement of chitosan films UV light barrier property by ellagic acid (Hu et al., 2019; Vilela et al., 2017).

Furthermore, the light transmission from the films varied with the blend composition. The higher concentration of CS in the films enhances the UV light barrier compared to PVA. The opacity decreases with increasing the concentration of natural extract (Table 2), Eq. (4) shows the relationship between film thickness and opacity, the opacity values were calculated using this equation. Moreover, the film transparency at 600 nm was comparable to some synthetic polymer films such as polyethylene (86.9%), polyester (83.5%), polyvinylidene chloride (90.0%) and polypropylene (89.1%), etc., which were typically used for packaging purpose (Shiku et al., 2003; Tan et al., 2015). Hence, it is clear from the optical properties result that the natural extract loaded CS/PVA-based films have adequate transparency and UV light barrier to be used as packaging material.

### 3.4. Film solubility and moisture content

Film solubility and moisture content are other essential parameters of coating and packaging material, especially in a humid environment and directly related to films' hydrophilic properties. Table 2 also shows the percentage of water solubility (WS %) and moisture content (MC %) of pure PVA, CS/PVA, and CS/PVA-TL films. Eq. (1) relates the film weight with moisture contents, and this equation was used to calculate the moisture content in the films. Pure PVA films completely (100%) dissolve in water; however, blending with CS decreases its solubility, which may be due to the low solubility of CS in water. The incorporation of natural extract in the hybrid (CS/PVA) films further decreases their solubility. However, the change is not significant, as revealed from the graph. Figure 4 shows the graph of total soluble matter (%) present in different films. The solubility decrease may be due to strong interaction between reactive functional groups in CS/PVA blends and extract, which lower the extent of hydrophilicity in the films and consequently decrease its solubility (Ghelejlu et al., 2016). A similar phenomenon in reducing chitosan films solubility after incorporating tea extract, *Zataria multiflora*,

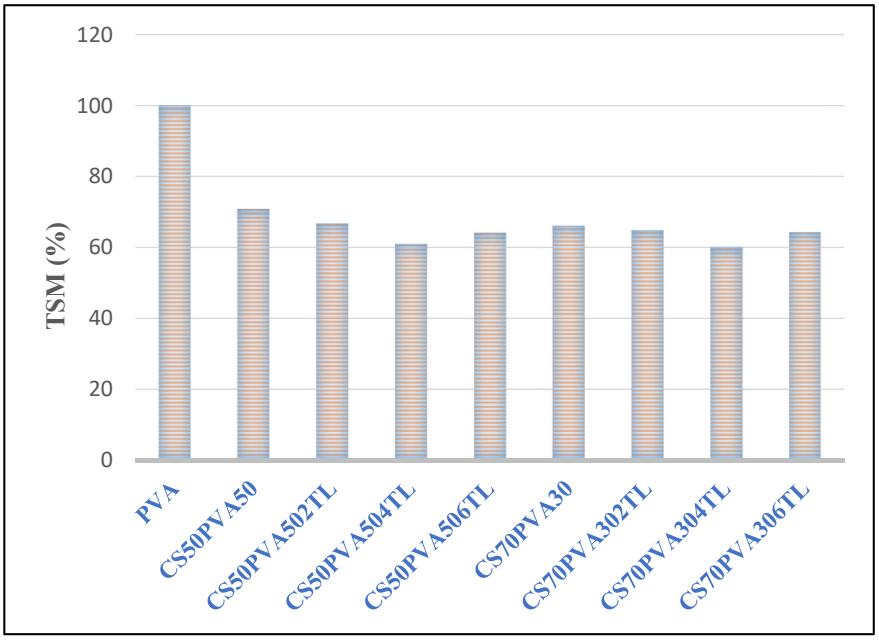



**Figure 4.** Total soluble matter (TSM, %) in the films.

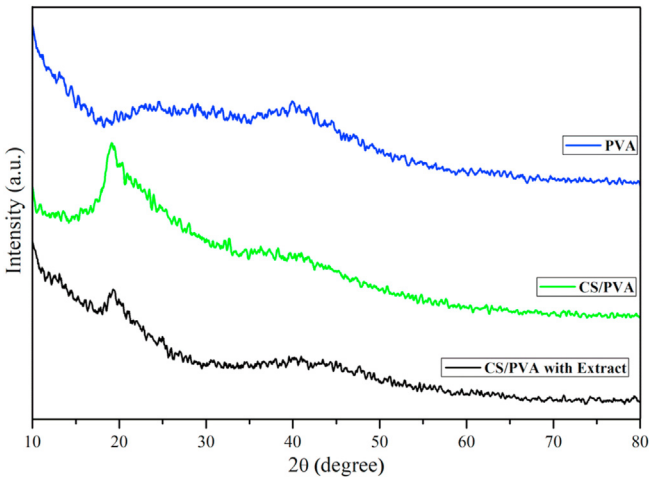



**Figure 5.** X-ray diffraction patterns of PVA, CS/PVA, and CS/PVA with extract films.

and *Eucalyptus globulus* was reported by different researchers (Hafsa et al., 2016; Peng et al., 2013; Shojaee-Aliabadi et al., 2014).

Moisture content (MC %) of the films was also significantly affected by natural extract addition (Table 2). Pure PVA film has the highest moisture content (16.67 %), which can be attributed to its hydrophilic nature. The blend film's moisture content decreases to 13.41 % when the concentration of PVA is 50%, and further decreases to 11.91 % after the PVA concentration reduces to 30%. The reduction in moisture content further supports the interaction between PVA and CS. The addition of natural extract to blend films adds to more moisture content reduction (9.89–9.01%), The values were calculated by using Eq. (2), emphasizing more interaction in the film matrix and dropping the availability of reactive hydrophilic groups to interact with moisture (Wang et al., 2013).

### 3.5. X-ray diffraction studies

XRD analysis was carried out to analyze the nature of prepared films, and the X-ray diffraction patterns of pure PVA, CS/PVA, and CS/PVA with natural extract are shown in Figure 5. Pure PVA film exhibited a single broad peak at 2θ~39.9°, indicating a small number of crystalline PVA aggregates. The CS/PVA film showed two characteristic peaks, which are the crystalline phase of chitosan 2θ~19.3° and the amorphous phase as the shoulder with a lower intensity at 2θ~39.6°. Natural extract-loaded CS/PVA films showed the peak's reduced intensity at 2θ~19.3° and slightly shifted the shoulder. Therefore, it can be concluded that the XRD pattern confirms the interaction between extract and CS/PVA in the film matrix.

### 3.6. Antioxidant activity or DPPH radical scavenging capacity

Antioxidant activity of control (PVA), blend (CS/PVA), and natural extract loaded (CS/PVA-TL) films were determined through DPPH assay. Figure 6 compares the antioxidant activities of PVA

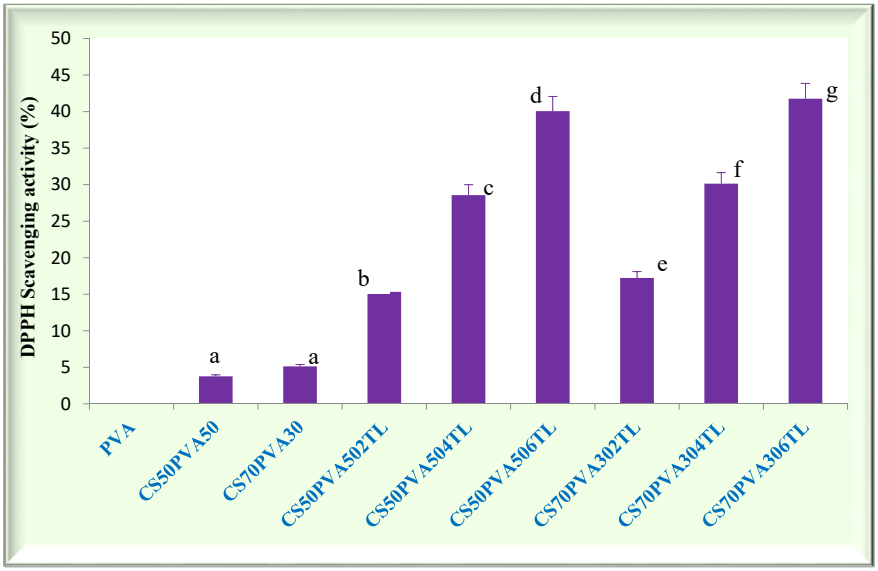



**Figure 6.** DPPH radical scavenging activity of PVA, CS/PVA, and CS/PVA-TL films. (Values with different superscripts are significantly different among samples, $p < 0.05$).

**Table 4.** Antioxidant activity (%) of PVA, CS, CS/PVA, and some natural extract loaded CS/PVA film reported in the literature.

| Film composition | Extract | Antioxidant activity (%) | Refs |
|---|---|---|---|
| PVA | - | 0 | Gaikwad et al. (2016) |
| PVA | *Apple pomace* | 39.8 | Gaikwad et al. (2016) |
| CS | Grape seed | 39.0 | Moradi et al. (2012) |
| CS | Grape seed + *Zataria multiflora Boiss* oil | 54.0 | Moradi et al. (2012) |
| CS/PVA | - | 27.42 ± 0.2 | Hajji et al. (2016) |
| CS/PVA | *Oryza sativa L.* | 29.15 ± 4.10 | Thanyacharoen et al. (2017) |
| CS/PVA | *Ocimum tenuiflorum* | 41.1 ± 1.17 | Our work |

Standard deviation (0.05–0.30), source of variation within groups (0.79), $p < 0.05$.

control films, hybrid and natural extract loaded hybrid films. Eq. (5) shows the relationship between scavenging activity and absorbance of the solution, and hence this equation is used to calculate the antioxidant values. Pure PVA film did not show any scavenging activity; however, it is clear from the graph that the antioxidant properties significantly increased with increasing *Ocimum tenuiflorum* extract concentration in the films. This can be ascribed to the high phenolic content in the extract, thereby enhancing its hydrogen-donating ability, which is the basic phenomenon of the DPPH scavenging method (Gaikwad et al., 2016; Sankhalkar and Vernekar 2016). The film's total phenolic content directly correlates with the antioxidant activity because phenolic compounds possess an ideal structure for scavenging free radicals and acting as suitable hydrogen donors. Table 4 presented the antioxidant values (%) of pure PVA and CS film, extracted loaded PVA and CS films, and natural extract loaded CS/PVA films reported in previous literature. This confirms that pure PVA film exhibits no scavenging effect; however, the addition of natural extract and blending with chitosan induce scavenging activity. In this case the maximum value of scavenging activity obtained is around 41.1 ± 1.17 % (phenol content 19.2 $g^{-1}$ dm) at a concentration of 30 % natural extract, which is much higher as compared to previously reported data. The scavenging activity of natural extract loaded pure CS film was reported to have much higher value (54.0 %) as compared to CS/PVA films as reported earlier varies between 27.42 to 29.15 % (Table 4). This shows that PVA addition has a negative impact on scavenging activity of composite films. Considering these results, it can be concluded that the aim of developing hybrid bio-synthetic (CS/PVA) based active antioxidant film for food packaging was successfully achieved by incorporating *Ocimum tenuiflorum* extract.

## 4. Conclusions

Hybrid bio-synthetic antioxidant active films were successfully prepared from a mixture of natural polymer (CS) and a biodegradable synthetic polymer (PVA) incorporating *Ocimum tenuiflorum* extract by applying a simple solution casting method. The resulting hybrid films were flexible, homogenous, and transparent, with an active UV light barrier and excellent antioxidant property. The antioxidant activity and UV light barrier properties of the films were found to be in direct correlation with the concentration of extract solution in the films. Therefore, the current results revealed the possibility of such hybrid films as a possible alternative to conventional plastic-based materials as food coating and packaging applications in the food industry. However, further work needs to be done to investigate these hybrid films' mechanical and shelf-life properties to extend their applications in commercial sectors.

## Declarations

### *Author contribution statement*

Annu, Akbar Ali, Shakeel Ahmed: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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### *Declaration of interests statement*

The authors declare no conflict of interest.

### *Additional information*

No additional information is available for this paper.

## References

Abdelghany, A.M., Menazea, A.A., Ismail, A.M., 2019. Synthesis, characterization and antimicrobial activity of Chitosan/Polyvinyl Alcohol blend doped with Hibiscus Sabdariffa L. extract. J. Mol. Struct. 1197, 603–609.

Ahmed, S., Ikram, S., 2015. Chitosan & its derivatives: a review in recent innovations. Int. J. Pharm. Sci. Rev. 6, 14–30.

Ali, A., Ahmed, S., 2018a. Recent advances in edible polymer based hydrogels as a sustainable alternative to conventional polymers. J. Agric. Food Chem. 66, 6940–6967.

Ali, A., Ahmed, S., 2018b. Development of hydrogels from edible polymers. In: Gutiérrez, T.J. (Ed.), Polymers for Food Applications. Springer International Publishing, Cham, pp. 551–589.

Altiok, D., Altiok, E., Tihminlioglu, F., 2010. Physical, antibacterial and antioxidant properties of chitosan films incorporated with thyme oil for potential wound healing applications. J. Mater. Sci. Mater. Med. 21, 2227–2236.

Anicuta, S., Dobre, L., Stroescu, M., Jipa, I., 2010. Fourier transform infrared (ftir) spectroscopy for characterization of antimicrobial films containing chitosan. Analele Univ din Oradea Fasc Ecotoxicologie, Zooteh si Tehnol Ind Aliment 1234–1240.

Beigzadeh Ghelejlu, S., Esmaiili, M., Almasi, H., 2016. Characterization of chitosan–nanoclay bionanocomposite active films containing milk thistle extract. Int. J. Biol. Macromol. 86, 613–621.

Cai, J., Chen, J., Zhang, Q., et al., 2016. Well-aligned cellulose nanofiber-reinforced polyvinyl alcohol composite film: mechanical and optical properties. Carbohydr. Polym. 140, 238–245.

Dammak, I., de Carvalho, R.A., Trindade, C.S.F., et al., 2017. Properties of active gelatin films incorporated with rutin-loaded nanoemulsions. Int. J. Biol. Macromol. 98, 39–49.

De Arruda, Q., Stefani, R., Aniceto, V., et al., 2014. Active chitosan/PVA fi lms with anthocyanins from Brassica oleraceae (Red Cabbage) as Time e Temperature Indicators for application in intelligent food packaging. Food Hydrocolloids 43, 1–9.

DeMerlis, C.C., Schoneker, D.R., 2003. Review of the oral toxicity of polyvinyl alcohol (PVA). Food Chem. Toxicol. 41, 319–326.

Dominguez, R., Barba, F.J., Gomez, B., et al., 2018. Active packaging films with natural antioxidants to be used in meat industry: a review. Food Res. Int. 113, 93–101.

Gaikwad, K.K., Lee, J.Y., Lee, Y.S., 2016. Development of polyvinyl alcohol and apple pomace bio-composite film with antioxidant properties for active food packaging application. J. Food Sci. Technol. 53, 1608–1619.

Ghelejlu, S.B., Esmaiili, M., Almasi, H., 2016. Characterization of chitosan-nanoclay bionanocomposite active films containing milk thistle extract. Int. J. Biol. Macromol. 86, 613–621.

Goonoo, N., Bhaw-Luximon, A., Bowlin, G.L., Jhurry, D., 2013. An assessment of biopolymer- and synthetic polymer-based scaffolds for bone and vascular tissue engineering. Polym. Int. 62, 523–533.

Hafsa, J., ali Smach, M., Ben Khedher, M.R., et al., 2016. Physical, antioxidant and antimicrobial properties of chitosan films containing Eucalyptus globulus essential oil. LWT - Food Sci. Technol. 68, 356–364.

Hajji, S., Chaker, A., Jridi, M., et al., 2016. Structural analysis, and antioxidant and antibacterial properties of chitosan-poly (vinyl alcohol) biodegradable films. Environ. Sci. Pollut. Res. 23, 15310–15320.

Hartwig, V.G., Brumovsky, L.A., Fretes, R.M., Boado, L.S., 2012. A novel procedure to measure the antioxidant capacity of yerba maté extracts. Food Sci. Technol. 32, 126–133.

Hu, X., Yuan, L., Han, L., et al., 2019. Characterization of antioxidant and antibacterial gelatin films incorporated with Ginkgo biloba extract. RSC Adv. 9, 27449–27454.

Irkin, R., Esmer, O.K., 2015. Novel food packaging systems with natural antimicrobial agents. J. Food Sci. Technol. 52, 6095–6111.

Kanatt, S.R., Rao, M.S., Chawla, S.P., Sharma, A., 2012. Active chitosan-polyvinyl alcohol films with natural extracts. Food Hydrocolloids 29, 290–297.

Lu, L.-X., Xu, F., 2009. Effect of light-barrier property of packaging film on the photo-oxidation and shelf life of cookies based on accelerated tests. Packag. Technol. Sci. 22, 107–113.

Moradi, M., Tajik, H., Razavi Rohani, S.M., et al., 2012. Characterization of antioxidant chitosan film incorporated with Zataria multiflora Boiss essential oil and grape seed extract. LWT - Food Sci. Technol. 46, 477–484.

Naveen Kumar, H.M.P., Prabhakar, M.N., Venkata Prasad, C., et al., 2010. Compatibility studies of chitosan/PVA blend in 2% aqueous acetic acid solution at 30 °C. Carbohydr. Polym. 82, 251–255.

Peng, Y., Wu, Y., Li, Y., 2013. Development of tea extracts and chitosan composite films for active packaging materials. Int. J. Biol. Macromol. 59, 282–289.

Perdones, Á., Chiralt, A., Vargas, M., 2016. Properties of film-forming dispersions and films based on chitosan containing basil or thyme essential oil. Food Hydrocolloids 57, 271–279.

Rodrigues, I.R., de Camargo Forte, M.M., Azambuja, D.S., Castagno, K.R.L., 2007. Synthesis and characterization of hybrid polymeric networks (HPN) based on polyvinyl alcohol/chitosan. React. Funct. Polym. 67, 708–715.
Rubilar, J.F., Cruz, R.M.S., Silva, H.D., et al., 2013. Physico-mechanical properties of chitosan films with carvacrol and grape seed extract. J. Food Eng. 115, 466–474.
Sankhalkar, S., Vernekar, V., 2016. Quantitative and qualitative analysis of phenolic and flavonoid content in moringa oleifera lam and Ocimum tenuiflorum L. Pharmacogn. Res. 8, 16–21.
Schulz, H., Özkan, G., Baranska, M., et al., 2005. Characterisation of essential oil plants from Turkey by IR and Raman spectroscopy. Vib. Spectrosc. 39, 249–256.
Shah, M.A., Bosco, S.J.D., Mir, S.A., 2014. Plant extracts as natural antioxidants in meat and meat products. Meat Sci. 98, 21–33.
Shiku, Y., Hamaguchi, P.Y., Tanaka, M., 2003. Effect of pH on the preparation of edible films based on fish myofibrillar proteins. Fish. Sci. 69, 1026–1032.
Shojaee-Aliabadi, S., Mohammadifar, M.A., Hosseini, H., et al., 2014. Characterization of nanobiocomposite kappa-carrageenan film with Zataria multiflora essential oil and nanoclay. Int. J. Biol. Macromol. 69, 282–289.
Siripatrawan, U., Vitchayakitti, W., 2016. Improving functional properties of chitosan films as active food packaging by incorporating with propolis. Food Hydrocolloids 61, 695–702.
Tan, Y.M., Lim, S.H., Tay, B.Y., et al., 2015. Functional chitosan-based grapefruit seed extract composite films for applications in food packaging technology. Mater. Res. Bull. 69, 142–146.
Tanase, E.E., Popa, M.E., Rapa, M., Popa, O., 2015. Preparation and characterization of biopolymer blends based on polyvinyl alcohol and starch. Rom. Biotechnol. Lett. 20, 10306–10315.
Thanyacharoen, T., Chuysinuan, P., Techasakul, S., et al., 2017. The chemical composition and antioxidant and release properties of a black rice (Oryza sativa L.)-loaded chitosan and polyvinyl alcohol composite. J. Mol. Liq. 248, 1065–1070.
Tiwari, B.K., Valdramidis, V.P., O'Donnell, C.P., et al., 2009. Application of natural antimicrobials for food preservation. J. Agric. Food Chem. 57, 5987–6000.
Vieira, M.G.A., Da Silva, M.A., Dos Santos, L.O., Beppu, M.M., 2011. Natural-based plasticizers and biopolymer films: a review. Eur. Polym. J. 47, 254–263.
Vilela, C., Pinto, R.J.B., Coelho, J., et al., 2017. Bioactive chitosan/ellagic acid films with UV-light protection for active food packaging. Food Hydrocolloids 73, 120–128.
Wang, L., Dong, Y., Men, H., et al., 2013. Preparation and characterization of active films based on chitosan incorporated tea polyphenols. Food Hydrocolloids 32, 35–41.
Yamani, H.A., Pang, E.C., Mantri, N., Deighton, M.A., 2016. Antimicrobial activity of tulsi (Ocimum tenuiflorum) essential oil and their major constituents against three species of bacteria. Front. Microbiol. 7, 681.
Yang, H.-J., Lee, J.-H., Won, M., Song, K Bin, 2016. Antioxidant activities of distiller dried grains with solubles as protein films containing tea extracts and their application in the packaging of pork meat. Food Chem. 196, 174–179.