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Capsicum-extract blended chitosan composite films and studying their antibacterial properties

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Abstract. Capsicum extract at different concentrations (0.5–3.0 ml) was blended with a chitosan polymer to obtain the composite films. It was compared with the pristine film to examine their antioxidant, mechanical, barrier, biodegradability, stability and antimicrobial properties. The morphology of the films was studied using a scanning electron microscope. From the obtained results, it was observed that the antioxidant, mechanical, biodegradability, stability and antimicrobial properties were enhanced when compared to the other films. The barrier properties of the composite films showed a decrease in activity when compared to the pure chitosan film. It may be due to the incorporation of a capsicum extract agent into the chitosan polymer matrix which plays a vital role in enhancing the overall properties.

Keywords. Biopolymer; chitosan; natural extract; capsicum; composite film.

1. Introduction

Food packaging is a vital part of modern society. The principal roles of food packaging are to protect food products from chemical, physical and biological influences [1]. However, synthetic polymer packaging causes many demerits like cost-effective, release of toxic gases and non-biodegradability. To overcome these problems, biopolymers like chitosan, chitin, guar gum, starch and cellulose are being used. Chitosan is chemically defined as a copolymer of á-(1,4)glucosamine $(C_6H_{11}O_4N)_n$, having different numbers of *N*-acetyl groups [2]. It is the deacetylated form of chitin and it is found to be the second most polysaccharides after cellulose. Owing to its exceptional properties like biodegradability, non-toxicity and antimicrobial and antioxidant activities, it has been used as food preservative materials in many food industries [3]. Pepper (*Capsicum annuum L.*) is rich in vitamin C (ascorbic acid), pro-vitamin A (carotene) and calcium. The daily intake of 50–100 g fresh pepper fruits could provide 100% and about 60% of the recommended amounts of vitamin C and A, respectively. It possesses excellent antioxidant and anti-carcinogenic properties [4]. In ancient times, it has been used as the food flavouring agent for human health [5]. The pungent chemical known as capsaicinoids is majorly present in capsicum. Capsaicin, dihydrocapsaicin, nordihydrocapsaicin, homocapsaicin and homodihydrocapsaicin are found to be the five natural capsaicinoids, but the profuse and effective are capsaicin and dihydrocapsaicin [6]. In this research, the properties of the capsicum extract blended chitosan composite films of different concentrations were prepared and compared with the pristine film for active food packaging application.

In the present work, C represents the pristine chitosan film, whereas C-0.5E, C-1.0E, C-1.5E, C-2.0E, C-2.5E and C-3.0E denote the different concentrations (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 ml) of natural extract blended chitosan composite films, respectively.

2. Results and discussion

The antioxidant activity, tensile strength, water vapour permeability (WVP), biodegradability and stability of the composite films are shown in table 1.

2.1 *Antioxidant activity of films*

The capsicum extract incorporated films showed enhanced antioxidant activity when compared to the pristine chitosan film (12%). Since the capsaicin present here acts as an antioxidant agent in preventing lipid oxidation. As we increase the concentration of the capsicum extract from 0.5 to 2.5%, the radical scavenging activity also increases from 19 to 59%, respectively, whereas C-3.0E showed the contrast result. The enhancement may be due to the interaction between amide compounds present in the capsicum extract and the functional group of chitosan molecules that could influence the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging ability of the films.

Table 1. Antioxidant, tensile strength, WVP, biodegradability and stability of composite films.

Film types	Antioxidant (RSA %)	Tensile strength (MPa)	WVP (mg cm^{-2} min^{-1})	Biodegradability-soil burial (30 days) Weight loss (%)	Stability-buffer solution (21 days) Weight retained (%)
C	12	28	1.75	76	71
C-0.5E	19	34	1.50	79	74
C-1.0E	25	40	1.25	84	78
C-1.5E	36	45	1.19	88	81
C-2.0E	47	50	0.97	92	83
C-2.5E	**59**	**61**	**0.75**	**95**	**88**
C-3.0E	42	55	0.84	93	85

2.2 *Tensile strength of the films*

The tensile strength result showed that as the concentration of the capsicum extract increases, the tensile strength of the film also increases from 28 to 61 MPa. It may due to the hydrogen bonding between the amino functional group present in the chitosan and the capsaicin active group present in the capsicum extract. But further addition of the extract (3.0%) to the polymer solution, the tensile strength tends to decrease due to some agglomeration of the extract on the chitosan film.

2.3 *WVP of the films*

The pure chitosan film showed a higher WVP value (1.75) because of its hydrophilic nature. The composite films namely, C-0.5E, C-1.0E, C-1.5E, C-2.0E and C-2.5E showed a decrease in values (from 1.50 to 0.75) as we increase the concentration of the capsicum extract, respectively, except for the C-3.0E film. It may be due to the hydrophobic nature of the capsaicin active group, which resists the inward bounding of water molecules from the atmosphere. Besides the good interaction of the two components, the presence of hydrogen bonds that are responsible for hydrogen bonding with water molecules will get reduced and result in a decrease in the WVP values.

2.4 *Biodegradability of the films*

The biodegradability test was carried out using the soil burial method for 30 days. It was found that as we increase the concentration of the capsicum extract, the biodegradability activity also increases and thus all the prepared films showed $> 75\%$ of weight loss in one month. It may be due to the presence of bioactive groups like capsaicin (contains the amide molecules) and $-NH_2$ functional groups (chitosan) in the composite films. However, active groups present in the composite films will undergo an enhanced reaction by penetrating the layer of microorganisms present in the soil and result in the degradation process more effectively.

2.5 *Stability of the films*

The stability measurement of the prepared films was performed under physiological conditions at regular time intervals. From the obtained results, it was examined that on the 21st day, the weight retained on each films was measured to be 71, 74, 78, 81, 83, 88 and 85% for C, C-0.5E, C-1.0E, C-1.5E, C-2.0E, C-2.5E and C-3.0E films, respectively. Overall, all the prepared films showed $> 70\%$ of stability in maintaining weight in buffer solutions. In addition to this, the stability increases when we incorporate the natural extract into the chitosan film and further, on increasing the concentration of the capsicum extract, an increase in stability was found. It may be due to the healthy bonding between the functional groups present in the capsicum extract and the chitosan molecules.

2.6 *Scanning electron microscopy (SEM) of the films*

The morphology of the pristine chitosan film (C) and the optimized 2.5% capsicum extract incorporated chitosan film (C-2.5E) are shown in figure 1. The pure chitosan film shows a spongy surface, but the natural extract incorporated films show a rough surface. It may be due to the addition of the capsicum extract to the polymer matrix. From the obtained images, it was evident that the addition of the capsicum extract had played its role in the chitosan matrix.

2.7 *Antibacterial activity of the films*

The antibacterial activities of the chitosan film (C) and the optimized composite films (C-2.5E) were tested against the Gram-negative (*E. coli*) and Gram-positive bacteria (*S. aureus*) and are shown in figures 2a (positive control) and 2b (negative control). It was observed that in both the cases, the optimized composite film showed more activity when compared to the pristine film. In addition to this, more antibacterial activity was found towards Gram-positive bacteria when compared to the Gram-negative bacteria. It may be due to the presence of the lipopolysaccharide layer (resist the entering

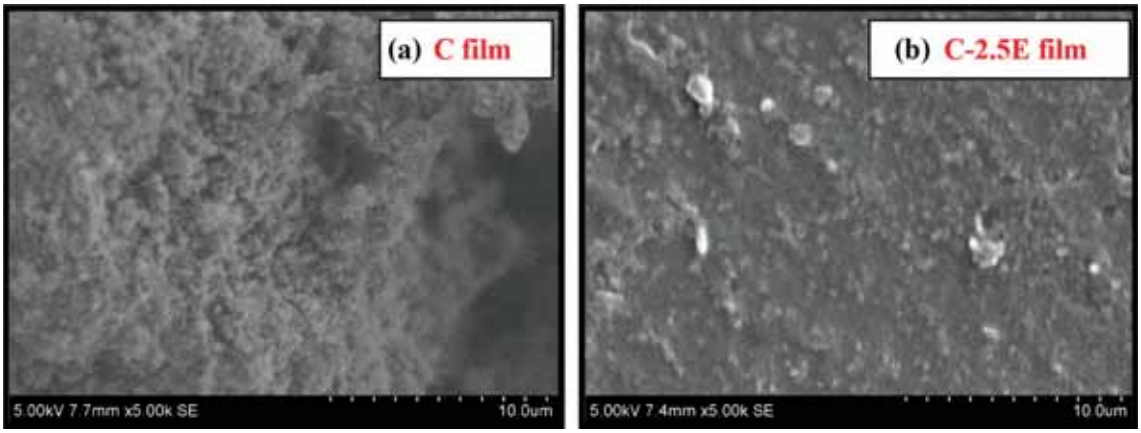

Figure 1. SEM images of (**a**) chitosan (C) and (**b**) composite (C-2.5E) films.

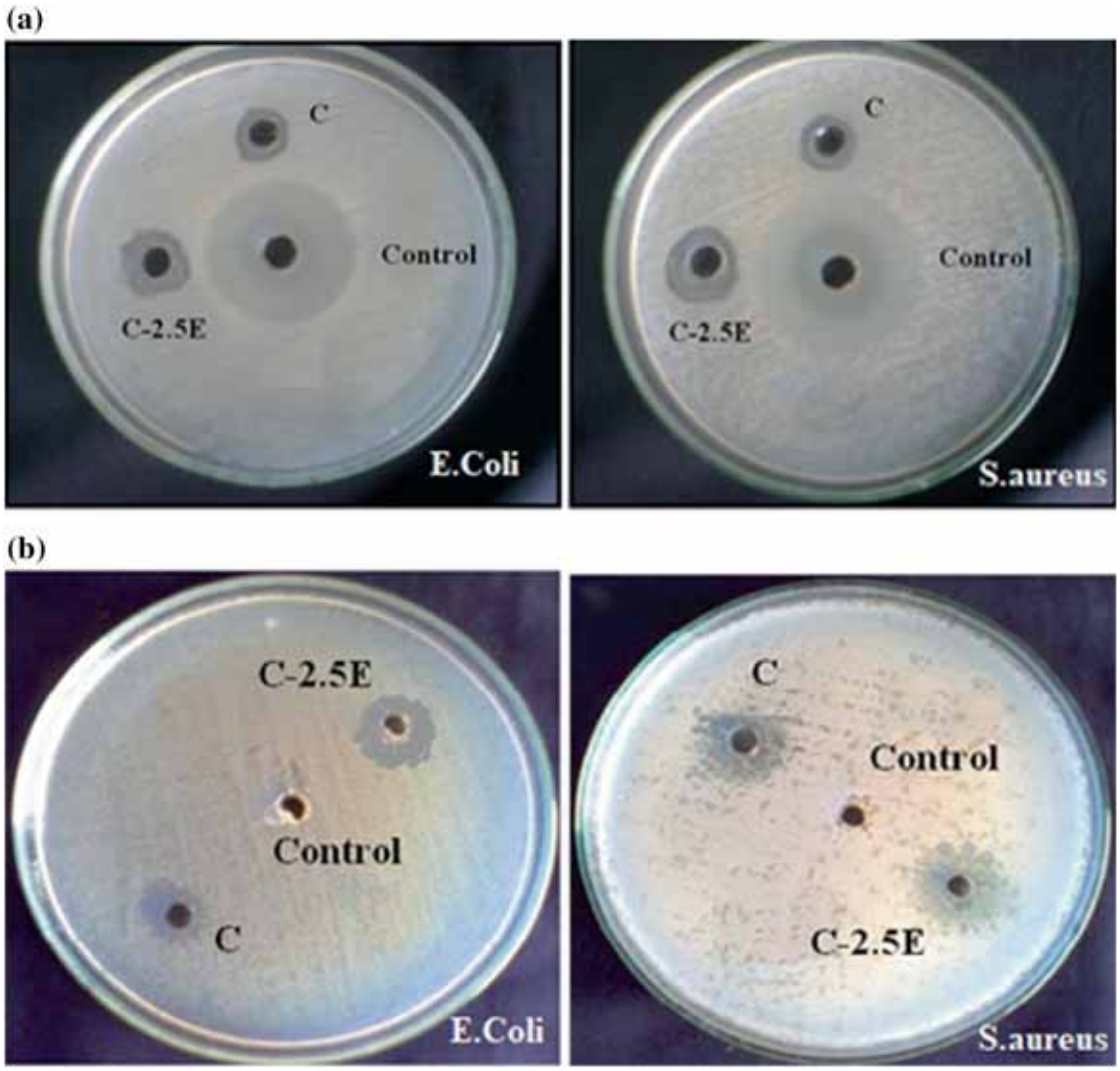

Figure 2. Antibacterial activity of the films (**a**): (C = chitosan, C-2.5E = 2.5 ml extract blended chitosan composite film, control = positive control (azithromycin)) and (**b**) (C = chitosan, C-2.5E = 2.5 ml extract blended chitosan composite film, control = negative control (DMSO)).

of foreign substances) in the Gram-negative bacteria, while this was absent in the layer of Gram-positive bacteria.

3. Materials and methods

3.1 *Preparation of capsicum natural extract*

For the preparation of capsicum extract, 5 g of capsicum was chopped and ground in a blender well. Further, it was mixed with 250 ml of distilled water and stirred while heating for 24 h. Next day, the extracted solution was filtered and stored until use [7].

3.2 *Preparation of chitosan–capsicum composite films*

In the preparation of composite films, 1 g of chitosan was mixed with 1% of glacial acetic acid solution. To the prepared chitosan solution, different concentrations (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 ml) of the capsicum extract were added along with few drops of glycerol to obtain the composite films by the solution cast method [8–11].

3.3 *Antioxidant activity*

Antioxidant activity was determined using the DPPH assay method. At 517 nm of absorption, the DPPH solution and sample were mixed and measured. The above step was repeated several times and the mixture was left in the dark at room temperature. Their absorption was monitored after a time interval of 20 min [12].

3.4 *Tensile strength*

The tensile strength was measured for the synthesized films and expressed in MPa using the Hounsfield Universal Testing Machine of 50 kN.

3.5 *WVP*

WVP was carried out using the WVP tester. The testing method is based on an aluminium oxide capacity sensor, which measures the relative humidity in the upper chamber and the lower chamber is saturated with water. The transmission of water from the lower to the upper chamber through the prepared films will give the transmission rate [13].

3.6 *Biodegradability activity*

The soil burial method was followed to test the biodegradability of the prepared composite films. The soil parameters such as temperature, moisture and pH were tested and found to be around 28−30°C, 65–75% and 6–7, respectively. At a particular interval of time, the buried films were taken out, washed with water and dried at room temperature for 24 h [14].

3.7 *Stability of the films*

The stability measurement of the prepared chitosan composite films (5 × 5 cm) was performed by incubating the films in the pH range of 7.4 in 5 ml of phosphate buffered saline. All the samples were incubated on a shaker at 45 rpm around 37°C. At the regular time intervals (3, 6, 9, 12, 15, 18 and 21 days), the films were taken out of the medium and washed with distilled water, dried in an oven (45°C) and the weight of this films was measured. The stability was expressed as the accumulated weight retained by the films [15].

3.8 *SEM*

The VEGA3 TESCAN instrument with an accelerating voltage of 5–20 kV was used to study the morphology of all the synthesized materials.

3.9 *Antibacterial activity*

Antibacterial activities of the films were tested using the well diffusion method against Gram positive and Gram negative bacteria. The bacterial cultures were swabbed onto the sterile Petri-dish containing the nutrient agar (solidify). Wells with a diameter of 10 mm were drilled by using a cork borer. Later, 100 µl concentration of the sample, one positive (azithromycin) and one negative control (dimethyl sulphoxide, DMSO) were swabbed. The plates were incubated for 24 h, then the diameter of the inhibition was measured [16].

4. Conclusion

In summary, natural extract incorporated composite films were successfully synthesized using the solution cast method. From the obtained results, it was observed that there was an increase in antioxidant, mechanical, biodegradability, stability and antimicrobial activities and a decrease in WVP. It may be due to the presence of the capsicum natural extract in the chitosan matrix. Here, the capsaicin functional group interacts well with the amino functional group of the chitosan polymer and enhances the properties of the materials. In addition, the hydrophobic nature of the capsaicin group plays a vital role in resisting the incoming moisture to the composite film. In addition to this, the C-2.5E film was found to be the optimized film when compared to the other films. Therefore, these films are found to be an ideal material for active food packaging applications.

5. Future work

Further, the prepared films can be used to test the different kinds of food samples (vegetables and fruits) and in investigating the variations in the colony forming unit, nutrition content, etc., of the food materials that were treated using control films and composite films.

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