Effect of Camphor Incorporation on the Material and Antibacterial Properties of Soy Protein Isolate Films

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Abstract: Seeking for green alternatives to synthetic plastics, soy protein based plastics are becoming quite a popular choice. Soy protein isolate (SPI) is a plant derived protein that holds fair film forming abilities. It demonstrates evenness of film surface, decent tensile strength and interacts easily with a wide range of additives. Additives are generally added to improve the material properties and antibacterial nature of the film. In this study, we have explored the effect of camphor incorporation on the material properties and overall performance of the film. SPI based films (having 7% SPI (w/v)) were prepared by solution casting method. The concentration of camphor was varied from 0.5 to 3% w/w of 7% SPI. The films prepared by camphor addition were characterized for their transmittance, Fourier Transform Infrared (FTIR) spectroscopy and mechanical properties. The FTIR spectra confirm the incorporation of camphor as a major change in the band intensity was seen compared to neat films. However, camphor addition made the films hydrophilic and a noticeable decrease in the tensile strength was seen. The water vapour transmission rate increased upon camphor addition as compared to neat SPI films. Nevertheless, camphor- SPI film was stable as very minimum leaching occurred during the study. Unlike the neat camphor solution (1-3% w/v), the camphor modified SPI films didn't exhibit antibacterial activity against *Listeria monocytogenes* and *Escherichia coli*. Camphor was seen to significantly increase the antioxidant properties of SPI films.

Keywords: Soy protein isolate, bio polymeric films, camphor, antibacterial, antioxidant, mechanical properties.

INTRODUCTION

In the past few decades, polymers have furnished our society with a wide range of benefits and ease of use but they have brought some adverse consequences on the environment due to their non-biodegradability [1]. As an alternative to synthetic plastics, protein-based films are globally explored due to their availability, eco-friendly nature and excellent film-forming properties [2]. Soy protein derived bio plastics are modified in numerous ways for the development of edible films, coatings and packaging materials. The major storage proteins of soybeans (*Glycine max*) are globulins. There are four protein fractions that are classified according to their sedimentation properties, and they are 2S, 7S, 11S and 15S fractions. It has been reported that 2S, 7S, 11S and 15S constitute 8%, 35%, 52% and 5% of the total protein content, respectively [3]. Soy protein based films are more flexible, smoother and clearer in comparison to films prepared from other plant derived proteins. Soy protein consists of hydrophilic/hydrophobic amino acids which offer different functional groups that facilitate interaction with a wide range of additives [4].

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The SPI bio based plastics are generally prepared by solution casting and compression molding process. Soy protein derived plastics have poor mechanical strength and antibacterial properties. However, with the incorporation of suitable additives the material properties of films can be enhanced significantly. Globally, research is conducted to find suitable additives such as polyphenolic rich extract like grape seed [5], licorice extract [6], organic acids, phenolic acids [7] and many carboxylic acid compounds. Rani *et al*., have reported that curcum in incorporation leads to an increase in the mechanical strength to 7.02MPa compared to neat film having tensile strength of 4.57MPa [8]. Sivarooban *et al*., has reported the increase in mechanical strength of SPI film upon incorporation of grape seed extract [5]. Plant derived active compounds are explored for their antimicrobial activities. Several plants such as tea [9], grape seed [5], rosemary, ginkgo biloba [10] and essential oils demonstrate strong antimicrobial properties [11]. The bioactive compounds of citronella essential oil are reported to exhibit antifungal and insecticidal properties against *Fusarium* and *Penicillium* species [12]. Such polyphenolic compounds are also reported to exhibit antioxidant properties owing to the presence of several H-donor species [13]. Phenolic compounds such as red grape seed extract, licorice extract, curcumin and pine needle extract impart antioxidant activity to SPI films [14].

Cinnamomum camphora, generally known as camphor, belongs to *Lauraceae* family and is natively found in South Korea, China, and India. There are roughly 40 species of *Cinnamomum* in India, which can be found in the Andaman and Nicobar Islands, the Eastern Himalayas, and the Western Ghats. It is a waxy, white or translucent solid with a strong aromatic fragrance. It is soluble in ether, alcohol, chloroform, and other organic solvents but insoluble in water [15]. Camphor sublimates at room temperature and melts at 180°C [16]. In recent few years, essential oils derived from plant extracts have been investigated for their antimicrobial activities. Essential oils derived from thyme, lemon grass, clove etc. are used as preservative and insect repellent owing to their antibacterial and antifungal activities. The leaves and stems of camphor plants are used to extract essential oil that contains antifungal, insecticidal and anti-inflammatory properties [16]. Camphor trees and its parts have several uses including industry, cosmetics, insecticides, pharmaceuticals, wood, ornamentation, and for religious purposes [17]. The study has shown the antibacterial efficacy of camphor complexes such as camphor imine and camphor sulfonimine against *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus* [18]. The biological actions of camphor include antibacterial, antiviral, and antitussive activities [19]. The synergistic and additive bactericidal behaviour of camphor with lavender essential oil is reported against *Listeria monocytogenes* [20].

This study attempts to incorporate camphor (with inherent antibacterial nature) into SPI films with an objective to prepare films by solution casting method. The SPI films were characterized by transmittance, Fourier Transform Infrared (FTIR) spectroscopy, total leachable material, weight loss, antibacterial test, water vapour transmission rate, water uptake and antioxidant properties. The molecular mass of camphor incorporated SPI film wasalso determined by SDS-PAGE method to evaluate any change in molecular mass of SPI after incorporation of camphor.

2. MATERIALS AND METHODS

2.1. Materials

In order to make soy protein isolate films, Zhenghou Ruikang Enterprise Co., Ltd (Zhengzhou, China) supplied soy protein isolate (SPI), which included 90.27% of protein on a dry basis. Other than protein, it contains, 4.5% moisture, 4.83% ash and 0.45% fat, and remaining trace metals, as obtained from Zhengzhou Ruikang Enterprise Co. LTD. China. Anhydrous calcium chloride and sodium hydroxide pellets were purchased from Titan biotech. Glycerol as a plasticizer was purchased from Himedia. The chemicals and reagents used in our study were of Analytical Reagent grade. Camphor was purchased from a local shop store in Patna with the brand as Shri Saraswati camphor manufactured by Renu Enterprises, Patna, India.

2.2. Preparation of Sample

The SPI film was prepared from the SPI film forming solution by solution casting method. In this study, 7% SPI was used for the preparation of SPI suspension. Firstly, 1.05g of glycerol (30%w/w of 7%SPI) was added to 50ml of distilled water. The solution was continuously stirred and heated at 60^{o}C for 10min. The pH of the solution was adjusted to 9- 9.5 by 1N NaOH. In the next step, 3.5g SPI (7% w/w) was added to plasticized water solution under stirring conditions and the drop in pH was adjusted back to 9-9.5. The solution was left for stirring at 60 ^{o}C for 1h. Afterward, the SPI suspension was cooled down and kept in vacuum desiccators to get rid of air bubbles. Lastly, the resulting suspension was poured on silicon coated Petri plates of 10cm diameter. The plate was left for drying at 60^{o}C for 12h without any interference. The resulting SPI film on the petri plate was maintained at 75% RH for 4–5 h so that dried SPI film could be peeled off easily from the petri plate.

The above procedure was followed for the preparation of camphor added films with slight modifications. In 5ml of distilled water, different quantities of camphor (0.5-3% w/w w.r.t 7% SPI) with an increment of 0.5% were dissolved and heated in the microwave for a few seconds to dissolve it completely. The film solution was prepared in 45ml distilled water as mentioned above and stirred continuously at 60 ^{o}C for 30min. To this suspension, camphor solution was added and pH was maintained 9-9.5. Further, SPI-camphor solution was heated for another 30min at 60 ^{o}C. The film was casted as previously described. The films for 0, 0.5, 1, 1.5, 2, 2.5 and 3% camphor concentration (w/w of 7% SPI) were designated as SC0, SC0.5, SC1, SC1.5, SC2, SC2.5 and SC3, respectively.

2.3. Characterization

2.3.1. Transmittance

The optical transmittance of the neat and camphor incorporated SPI films were measured with an

ultraviolet–visible spectrophotometer (V-670 from Jasco, Japan, 2015). For the transmittance studies, the SPI films were cut into small stripes of specific dimensions 4cm length and 1cm breadth. The strips were kept in a glass cuvette and their transmittance was taken at a varying wavelength ranging from 200nm to 800nm [8]. Air was taken as blank.

2.3.2. Water Uptake

ASTM D570-81 were used for water uptake studies [8]. The films were cut in specific dimensions of small square pieces (1 cm x 1 cm) and preconditioned at 60 ^{o}C in a hot air oven for 24 h. Then cooling at 0% RH in desiccators, it was weighed (W_0). The film pieces were then kept into distilled water for 24 h, the excess water present in the films are dried out by the help of tissue paper and weighed (W_1).

$$Water\ Uptake\ (\%) = \frac{W_1 - W_0}{W_0}$$

2.3.3. Total Leachable Material

The total leachable material properties of all the camphor incorporated SPI films were determined by the method as mentioned elsewhere [8]. The films were cut in small square pieces (1 cm x 1 cm) and are preconditioned at 60 ^{o}C in a hot air oven for 24 h. After that it was cooled in a desiccator at 0% RH. The initial weight of the test tube was recorded (M_0). The film pieces were then immersed in 2 ml distilled water for 24 h, and then the films were taken out from the test tube. Water in test tubes was dried by heating at high temperature to evaporate all water. Then the test tube weight was recorded as M_1. Total leachable material was calculated by below mentioned formula:

$$Total\ leachable\ material\ (\%) = \frac{M_1 - M_0}{M_0} \times 100$$

2.3.4. FTIR

The FTIR spectrophotometer from Jasco (Japan) was used to record the infrared spectroscopy of the unprocessed and camphor-incorporated SPI films. The solid film samples FTIR spectra were recorded at room temperature. The samples were scanned at a resolution of 4 cm^{-1} from a range of 4000 to 400 cm^{-1}. An average of 32 scans for all spectra was recorded.

2.3.5. Mechanical Properties

Universal Tensile Testing device from Zwick, Germany, was used to conduct the tensile testing in accordance with ASTM D882-91. The tensile specimens, which had dimensions of 80 mm by 10 mm and a thickness of 0.2 mm was prepared. These tests were done for the neat and camphor-incorporated SPI films to measure their tensile strength, tensile modulus, and elongation at break values. Tensile tests were run at 20 mm/min cross-head speed. Each formulation's three test samples were examined, and the average of these values were taken to plot the graph.

2.3.6. Water Vapour Transmission Rate

The water vapour transmission rate (WVTR) was determined by the water vapour permeability cup as per method described in previous literature [21]. For this, 5g of anhydrous calcium chloride was kept inside and sealed with a circular piece of SPI film. The initial weight of this cup was recorded as W_0. Further, a water vapour permeability cup with SPI film is kept in a desiccator of 75% RH. After every 24h the weight of the permeability cup was recorded (W_1) for 3 days. The weight change *G*, t is the time during which G occurred in h and A is the test area of film in m^2.

$$G = Intial\ weight\ (W_0) - Final\ Weight\ (W_1)$$

$$Water\ vapour\ transmission = \frac{(G \div t)}{A}$$

2.4. Molecular Weight Determination by SDS-PAGE

The change in molecular mass of SPI after camphor addition was determined through SDS-PAGE (sodium dodecyl sulphate-polyacrylamide gel electrophoresis). Film sample solutions of different concentrations (0-3%) were mixed with sample loading buffer and autoclave distilled water. The sample mixture was then heated at 95^{o}C for 15 min. Further, the mixed protein samples were centrifuged at 14000rpm for 4min at 4^{o}C. 5µl (10µg) of each protein sample was applied in the wells of the stacking gel. Stacking gel (pH 6.8) and resolving gel (pH 8.8) were 5% and 12% respectively. After electrophoresis, the gels were stained with 0.25% Coomassie blue, R-250 and destained until the background stain was removed [8].

2.5. Antibacterial Properties

For testing the antibacterial activity agar disc diffusion method was employed against *Listeria monocytogenes* and *Escherichia coli*, chosen as test organisms. The bacterial culture *of Listeria monocytogenes* and *Escherichia coli* were revived by inoculating a loopful bacterial isolate in Luria Bertani

broth. Overnight grown culture of log phase was used for test analysis. In agar disc diffusion method, the nutrient agar plates were used and the films were cut into small square pieces (1 cm x 1 cm). The films were placed on uniformly spread nutrient agar plates with around 1 million cells. The plates were incubated for 12-16h at 37^{o}C in a static incubator [13]. To confirm the bactericidal activity of camphor, 10ml of different concentration of camphor (0-1%w/v) was prepared separately. To this solution, 1 million cells of test organisms were added and incubated at 37 ^{o}C for 12-16h. Further, the optical density (OD) was recorded at 600nm through UV- Vis Spectrophotometer. Each test was done in triplicates to maintain the statistical significance of results.

2.6. Antioxidant Properties

For the antioxidant test, the 0.4gm SPI film, 95% ethanol and DPPH (1,1-diphenyl-2-picryl-hydrazyl) are used. 0.2g of film sample was incubated at 37 ^{o}C in 4 ml of 95% ethanol and left for 12h under shaking condition. The DPPH solution was prepared in 95% ethanol. After 12h, 2 ml of freshly prepared DPPH solution was mixed with 2 ml of sample solution and kept in the dark for 30min at room temperature [13]. The absorbance was recorded at 517nm via UV-Vis. Spectroscopy. The DPPH radical scavenging assay was calculated by the following equation:

$$Radical\ Scavenging\ Activity\ (\%) = \frac{A1 - A2}{A1} \times 100$$

Where, A_1 is the absorbance of blank DPPH solution and A_2 is the absorbance of the sample extract of the SPI films.

3. RESULTS AND DISCUSSION

3.1. Appearance and Transmittance of Films

The neat and camphor incorporated films showed a yellow colour with a homogenous surface texture. The addition of camphor didn't lead to significant changes in the colour of films. This is due to the absence of any inherent pigmentation in camphor thus it didn't alter the physical appearance of films. However, the incorporation of camphor made the films comparatively thinner than control films. The thickness of the SC0 film was in range of 0.25 ± 0.025mm while the thickness for camphor incorporated films SC0.5 to SC3 varied between 0.13 ± 0.023mm. The reason for film thinning is sublimation of camphor during casting at high temperature.

Figure **1** shows the transmittance properties of neat and camphor incorporated SPI films. As stated earlier that camphor incorporated SPI films became thin hence, we saw an obvious increase in the transmittance of camphor added films. The SC0 film showed least transmittance value which gradually increased with an increase in concentration of camphor. The highest transmittance was recorded for SC3 films. Transmittances below 350nm were almost zero for almost all the samples. In the UV region of the wavelength, slight changes were observed. Generally, SPI exhibits zero or negligible transmittance till 350nm but upon camphor addition such films showed negligible transmittance till 400nm. This confirms the enhanced UV light barrier properties of camphor added films. Similar to polyphenols such as lignin and citronella, camphor acts to increase the UV light barrier performance of films [22-23].

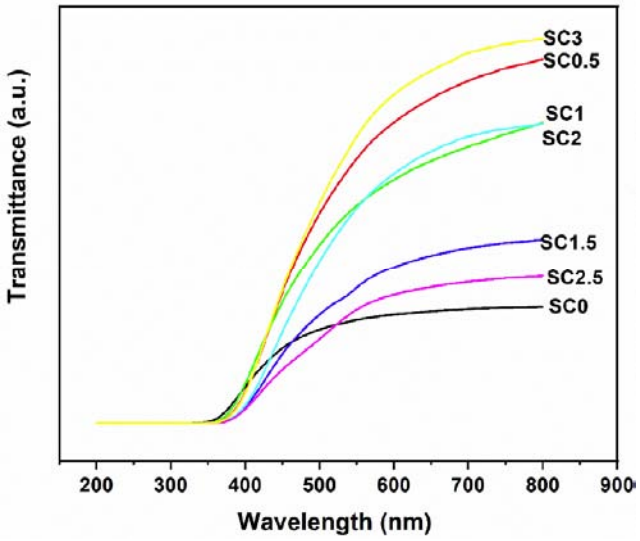

Figure 1: Transmittance of neat and camphor incorporated SPI films.

3.2. Water Uptake

It has been found that biopolymeric films remained intact even after 24h of immersion in water for all concentrations of camphor incorporated SPI films. However, all the concentrations of camphor incorporated SPI films disintegrated once it was tried to remove from water. It shows that camphor incorporated SPI films are more hydrophilic in nature than compared to neat films. However, there is the presence of ketone groups which might be interacting very loosely with the SPI side chains. Moreover, the control film didn't disintegrate even after complete immersion and showed a water uptake capacity of 384.11%. Generally, the addition of polyphenols and essential

oils are seen to increase the hydrophilicity of films as seen for curcumin and citronella essential oil. Camphor addition showed similar effects as it decreased the hydrophobicity of SPI films [8-12].

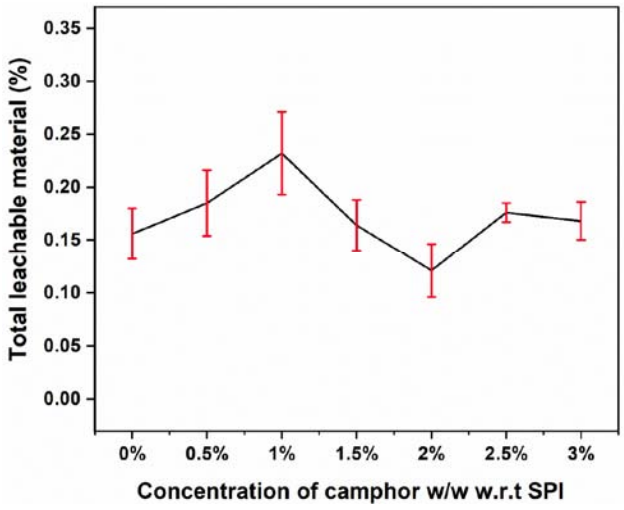

Figure 2: Total leachable material (%) of neat and camphor incorporated SPI films.

3.3. Total Leachable Material

Figure **2** shows the total leachable material of different concentration of camphor incorporated SPI films. The leaching of material increases from 0.15% (SC0) to 0.23% for SC1 and then it decreases upto SC3 films. For SC1.5, leaching was minimum and further, it increased upto 0.18 for SC3. For SC1.5 and SC2, the leaching was least that demonstrates better cross-linking and interaction between SPI and camphor at that concentration. However, overall the leaching of all biopolymeric film was seen to be less than 0.5% that ensures the better interaction between camphor and SPI that holds it tightly. The observed low value of leaching was similar to curcumin SPI conjugate film as reported earlier [8].

3.4. FTIR Studies

Figure **3** shows the FTIR spectra of camphor incorporated SPI films. The peak intensity at 3266 cm^{-1} corresponds to the -OH stretching of SPI. As we can see in the graph that this peak becomes more intense as we move from SC0 to SC3. The more intense peak of OH reflects the increased hydrophilicity of films as reported in water uptake studies. In the control film, there was a very weak intensity peak at 2962 $cm^{-1}$for medium C-H stretching which became more prominent with incorporation of camphor. This signifies that CH_3 groups of camphor are involved in interaction with SPI amino acid residue which leads to significant stretching in these C-H bonds. Hence, these bonds became more intense as we moved towards a higher concentration of camphor. For, neat films, there was no noticeable peak observed around 800 cm^{-1} but can be easily seen for all camphor incorporated SPI films. This peak corresponds to C-H bending.

Similarly, there was a weak intensity peak present at 1046 cm^{-1} that became stronger upon incorporation of camphor. The peaks at 1639cm^{-1} and 1535cm^{-1} are associated with amide I and amide II, respectively. The Amide II bands showed a slight shift from 1535cm^{-1} (SC0) to 1546cm^{-1} (SC3) as a result of interaction between -CN stretching and -NH bending of SPI. The β-sheet of SPI has a range of peaks at approximately 1639cm^{-1}. However, this peak was more intense at SC1 and weakened as the concentration of camphor increased from 1 to 3%. At high content of the camphor, a new peak at around 1735cm^{-1} is formed

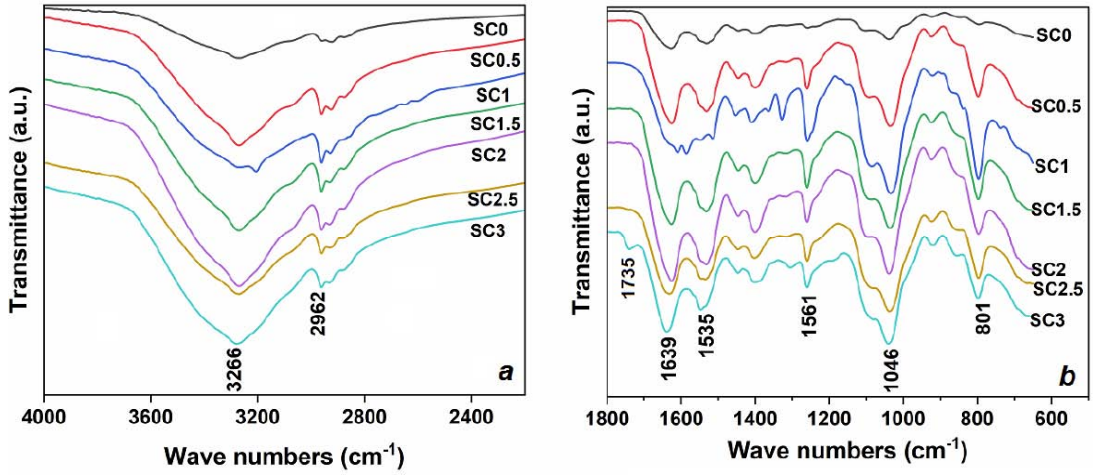

Figure 3: FTIR spectra of neat and camphor incorporated SPI films (**a**) Wavelength from 4000-2300 (**b**) Wavelength from 1800-600.

and it may be due to ester formation and this peak is not present in other camphor incorporated SPI films.

3.5. Mechanical Properties

Figure **4** depicts the mechanical properties of neat and camphor incorporated SPI films. The control films showed a tensile strength of 4.1MPa with 37.0% of elongation. After the incorporation of camphor, the tensile strength decreased as compared to SC0 for all mentioned concentration of camphor added films. However, when we compare the values of tensile strength among camphor added films, a gradual increase was observed from SC0.5 to SC2.5 films. For example, the tensile strength of SC0.5 was observed to be 1.95MPa with 11.54% of elongation. For SC2.5 films, the tensile strength and elongation at break increased to a maximum of 3.23MPa and 27.78%, respectively. However, after SC2.5 films, both tensile strength and elongation at break decreased to 2.84MPa and 23.07%, respectively for SC3 film. The observed decrease in tensile strength was probably due to thinning of films because of camphor addition.

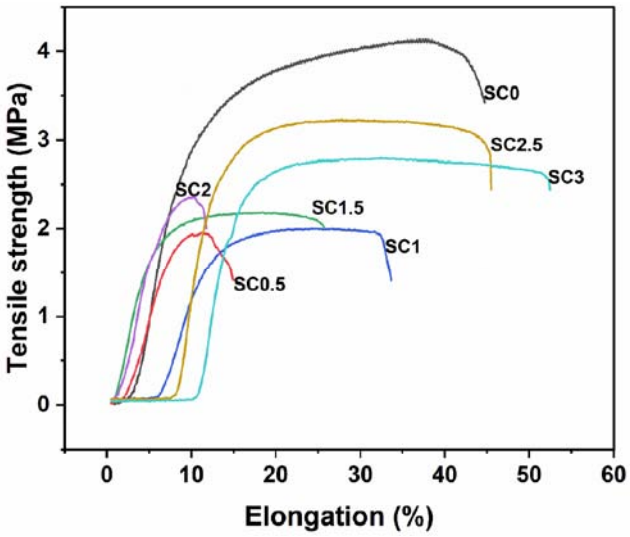

Figure 4: Mechanical properties of neat and camphor incorporated SPI films.

3.6. Water Vapour Transmission Rate

The water vapour transmission rate (WVTR) in the initial 24h was observed to be 17.11%, 15.11%, 14.77% and 25.85% for SC0, SC1, SC2 and SC3 films respectively. As evident from Figure **5** that initially camphor addition decreased the WVTR but at higher concentration, it increased the WVTR. It shows that camphor incorporation in SPI creates a barrier for water transmission. The cross-linking between SPI and camphor is more intimate at 1-2% concentration i.e., for SC1 and SC2 films, as also depicted in SDS-PAGE gel image where band intensities were minimum for these films (discussed later). This enhanced cross-linking provides resistance for water molecules to pass through it. Hence, fewer water droplets could transmit through the film matrix thereby decreasing the WVTR value. The maximum decrease in water vapour transmission rate was reported for SC2 films. However, for SC3 it increased even more than control films. This may be due to poor cross-linking between SPI and camphor at higher concentrations of additives which can also be supported by low tensile strength for SC3 films.

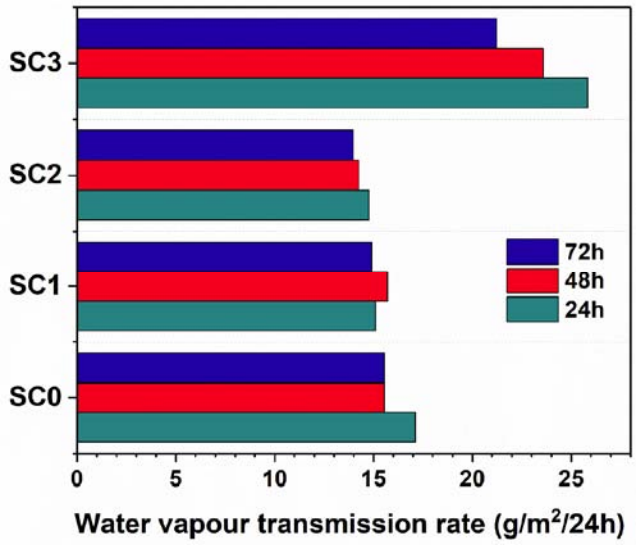

Figure 5: Water Vapour Transmission Rate (g/m^2/24h) of neat and camphor incorporated SPI film for different time intervals.

3.7. Molecular Mass Determination by SDS-PAGE

The incorporation of camphor leads to significant change in intensity of the molecular weight bands of SPI as evidenced from SDS-PAGE. The decrease in intensity of the bands may indicate cross linking as reported in previous literature [7]. The maximum cross linking was observed in SC1.5, SC2, SC2.5 samples as evident from the decreased intensities of bands at mentioned concentration. Hence, the least TLM% for SC1.5 and SC2 is validated by the least intense band for them as appeared on SDS-PAGE gel. SPI contains different molecular masses having major components of 7S and 11S. The band at 22KDa denotes the basic subunits and 35-39KDa indicate the acidic subunit of glycinin (11S). At 50-52 KDa, 79 KDa, and 85-89 KDa, three distinct bands were seen that correspond to the β, α, and α' subunits of the beta-conglycinin (7S) fraction.

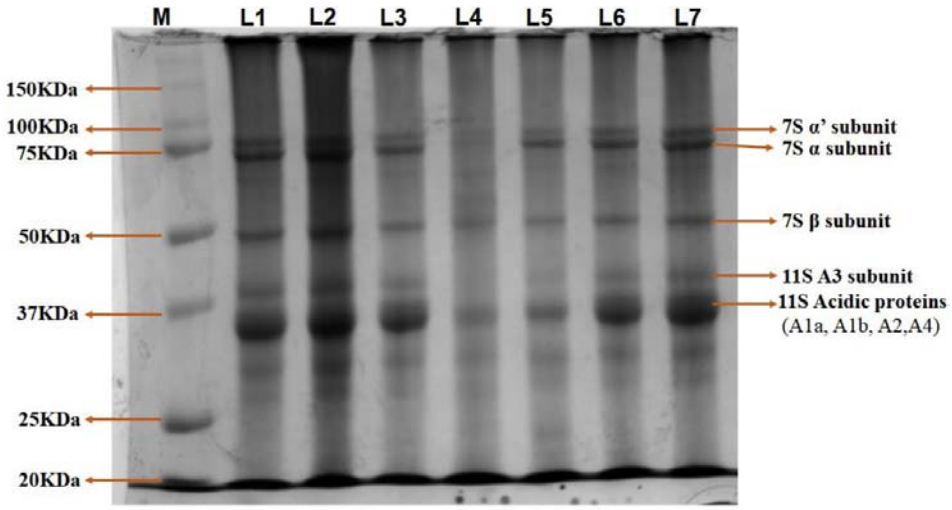

Figure 6: SDS-PAGE analysis for 3µl of marker (Precision plus dual standard wide range protein marker) in lane M, and 5µl (10µg/well) of neat and camphor incorporated samples are in increasing concentration in respective lanes. Lane 1, lane 2, lane 3, lane 4, lane 5, lane 6 and lane 7corresponds to SC0, SC0.5, SC1, SC1.5, SC2, SC2.5 and SC3 respectively. Numbers on the left are the molecular masses of marker proteins in KDa. Numbers on the right side show molecular weight of different protein fractions of SPI.

3.8. Antibacterial Properties

The nutrient agar plates showed the growth of bacteria on all over the films after 12-16h of incubation. Hence, the films didn't contain significant antibacterial activity against *Listeria monocytogenes* and *Escherichia coli.* For neat and camphor incorporated films, there was no zone of inhibition formed. Thus, the prepared films lack bactericidal activity as shown in Figure **7**.

For neat camphor concentration, in *Listeria monocytogenes* the optical density (OD) for 1% camphor decreased from 0.8 to 0.7, the approximate reduction of 12.5% as compared to control (Figure **8**). Additionally, for 2% and 3% the OD reduced as compared to control. For neat camphor, OD for 1% camphor decreased from 0.8 (control) to 0.3 for *Escherichia coli.* This is approximately a reduction of 62% as compared to control. Additionally, for 2% and 3% the OD decreased significantly. This signifies the antibacterial properties of camphor against *E. coli* and *L. monocytogenes*. However, camphor was seen to be more potent against gram-negative bacteria as evident from the significant reduction in OD for *E. coli*. Similar studies on grape seed extract and green tea that are rich source of polyphenols have shown significant antibacterial activity against *E. coli*, *S. aureus* and *L. Monocytogenes* [5].

3.9. Antioxidant properties

1,1 Diphenyl 2- Picryl Hydrazyl (DPPH) is a stable free radical having a blue colour which converts into

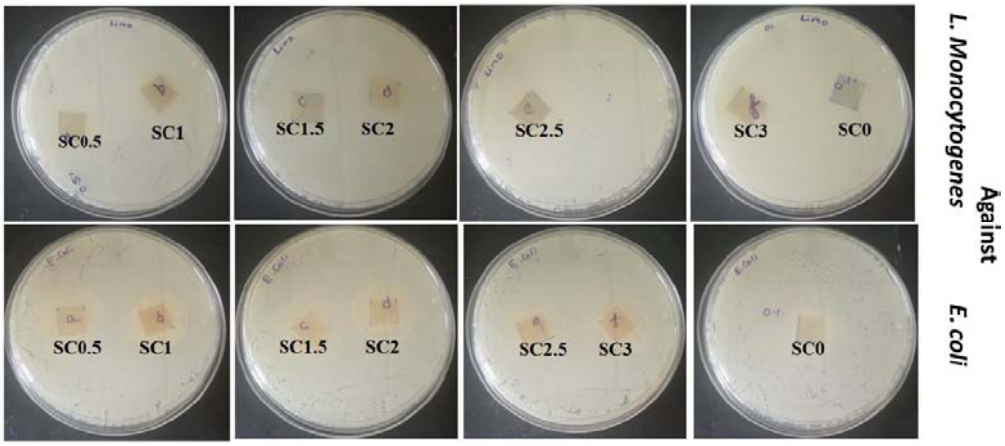

Figure 7: Antibacterial study of neat and camphor incorporated SPI films against *Escherichia coli* (top) and *Listeria monocytogenes* (bottom).

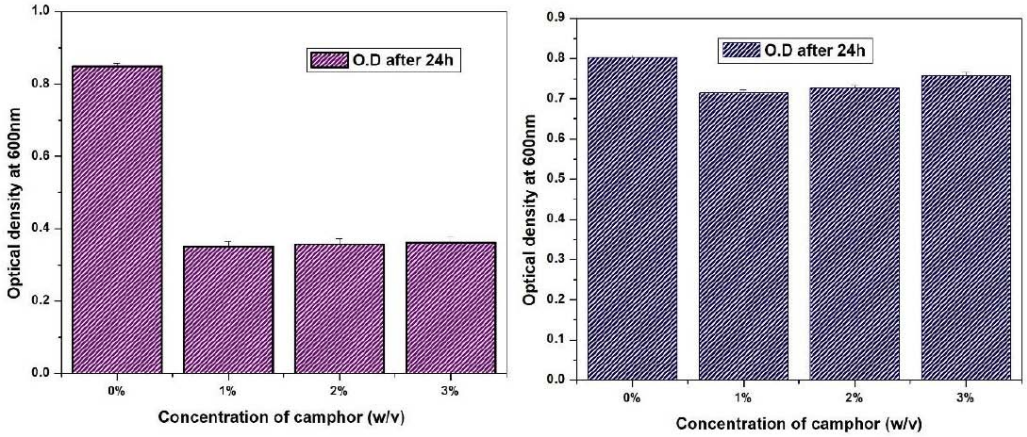

Figure 8: Antibacterial activity of camphor at different concentrations (0-3% w/v) against *Escherichia coli* (left) and *Listeria monocytogenes* (right).

yellow when scavenged. Antioxidant compounds having hydrogen donor when react with DPPH, reduces it into DPPH-H. To determine the film's level of antioxidant activity, DPPH radical scavenging assay was used. Upon oxidation the native blue colour of the solution changes to light yellow. The formation of light yellow colour signifies the antioxidant activity. Figure **9** indicates that camphor incorporated SPI has antioxidant properties. Due to more hydrogen atom donor in camphor, the additives incorporated films showed more antioxidant capacity. While increasing the concentration of camphor, the antioxidant property increases gradually. The control film had antioxidant activity of 50.3% that increased to 65.5% and 67.3% for SC2 and SC3 films. Previously, the addition of licorice residue extract, ferulic acid and curcumin are reported to increase the DPPH antioxidant capacity of SPI based films owing to their hydroxyl groups and other H-donor species [6-24].

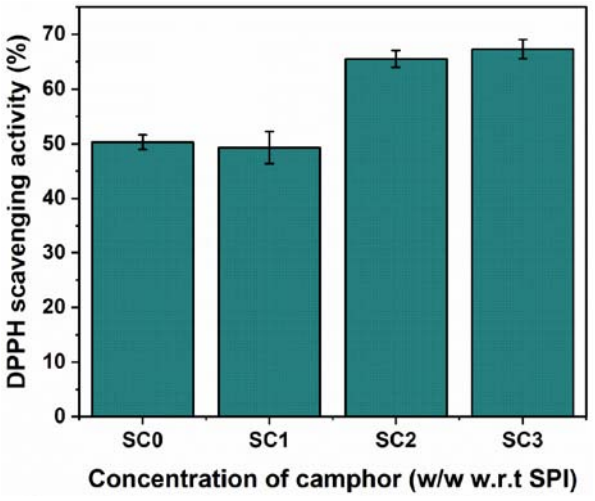

Figure 9: Antioxidant activity of camphor incorporated SPI films of SC0, SC1, SC2 and SC3 by DPPH radical scavenging assay.

CONCLUSION

In this study, we have successfully prepared camphor incorporated SPI films that exhibit UV barrier and strong antioxidant properties. It also reduces the damage caused by UV radiation such as oxidation and rancidity of fatty foods. The SDS-PAGE analysis confirms the better cross-linking of camphor and protein till 2.0 % of camphor. The neat camphor showed antibacterial activity against *Escherichia coli* and *Listeria monocytogenes* but films lack antibacterial activity. This may be due to the lower concentration of camphor used in the films as well as due to the presence of protein substrate that is prone to attack by bacteria. The camphor addition acted as a barrier for the transmission of water molecules through it as evident from the decreased water vapor transmission rate. This improved anti oxidative capacity of camphor-SPI films enhances its applicability as a food packaging material as it will prolong the shelf-life of packaged foods.

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CONFLICT OF INTEREST

Authors declare no known conflict of interest.

CREDIT AUTHOR STATEMENT

Priya Rani: writing original manuscript and conceptualization of experiments, Chandrakanti Kumari: investigation and methodology, K. Dinesh

Kumar: investigation of tensile properties and Rakesh Kumar: visualization, formal analysis, validation and writing of manuscript. All authors have read and agreed to prepared manuscript.

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