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Bioactive constituents and bio-waste derived chitosan / xylan based biodegradable hybrid nanocomposite for sensitive detection of fish freshness

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ABSTRACT

The prime objective of the present study is to develop a biodegradable food packaging hybrid film materials from renewable sources. Polyvinyl alcohol (PVA), chitosan (CS), xylan (CC) and hydroxyapatite (nHA) were used as the sources of bio-degradable polymer and curcumin (Cur) as antioxidant agent to prepare an intelligent packaging PVA/CS/CC/nHA hybrid film (PCC) to assess the fish freshness. The color change response of PCC in an NH_3 environment was studied at different time intervals using electronic absorption spectrum. UV studies indicate that the higher relative humidity was more favorable for a color response. The function trials were conducted to assess the freshness of Indian oil sardine (Sardinellalongiceps) fish at room temperature. The colorimetric behavior of PCC presents the visible and sharp color changes. Hence, the colorimetric behavior of PCC can be considered and used as tool to assess the real-time fish freshness method for smart packaging.

1. Introduction

Food spoilage is not only impairing human health but also leads to an economical crisis of the nation. To overcome the above discrepancies packaging materials are essential to protect and preserve as physico-chemical and antimicrobial disintegration and to keep the food as long as in the package and it must improves and to provide the good quality. Among the sea foods, fish is one of the most widely used consumable products. It is the life supporting for fisherman and hence it is important to keep the freshness of fish. To avoid unreliable and labor intensive methods to determine the freshness of fish various methods have been attempted. Among them color, texture and smell are some of the measures to assess the extent of microbial action and are considered to check the quality of fish namely deterioration and Total viable count (TVC) (Connell, 1995).

Polysaccharides and proteins are the general basic resources to form biodegradable dynamic packaging (Liu, Meng, Liu, Kan, & Jin, 2017). They can act as the carriers for dynamic substances such as antioxidant, antimicrobial and oxygen scavenger. Even though, Polysaccharide films have lower water and gas barrier behaviour due to higher hydrophilicity while lipid films have lower tensile strength when compared to that of protein (Maryam Adilah, Jamilah, & NurHanani, 2018)

Chitosan (CS), a distinctive polysaccharide largely isolated from crustacean wastes, has considered as an active biodegradable packaging material for preservation of food (Dutta, Tripathi, Mehrotra, & Dutta, 2009). As expected, the neat chitosan (CS) film is not satisfactorily used in food packaging because of its inherent weakness and high affinity to humidity (Branca et al., 2016; Silva-Pereira, Teixeira, Pereira-Júnior, & Stefani, 2015). In order to alleviate these drawbacks, blending chitosan with other suitable polymeric materials like polyvinyl alcohol, polylactic acid and gelatin has been considered as an effective method to obtain hybrid composite films. (Bonilla, Fortunati, Atarés, Chiralt, & Kenny, 2014; Hosseini, Rezaei, Zandi, & Ghavi, 2013; Jridi et al., 2014; Wang et al., 2015). Among these films, PVA-chitosan film has been received great interest due to its outstanding mechanical properties (El-Hefian, Nasef, & Yahaya, 2010). However, the hydrophilicity of amine groups present in chitosan and hydroxyl groups in PVA will decrease the moisture barrier of film and these can be overcome by reinforcing suitable natural fillers and nanoparticles to make them suitable for food packaging hybrid composite films.

Natural fillers and reinforcements are recognized as renewable raw materials and their accessibility is abundant. These natural fillers

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possess numerous advantages including their properties namely viz., low density, renewability, biodegradability, recyclability and competitive cost. Corn (Zea mays) is Poaceae (grass family) which is one of the top most three cereal crops grown in the world. Scenario corn generates about several million tons of corn residues in every year. Corn cob is one of the most important residues obtained during processing of corn that are abundantly available around the world. Corn cob is the center core of a corn. Xylan was prepared from corn cobs. xylan–chitosan conjugates to develop a natural food preservative (Li, Shi, Wang, & Du, 2011). PVA can be blended with corn cob to obtain hybrid composite films with improved properties such as the water resistance, mechanical strength, moisture permeability and compatibility (Wang et al., 2013). In the recent past, polymer nanocomposite hybrid materials have been developed and widely used as biodegradable packaging films with improved barrier properties by incorporating nano-reinforcements into polymeric matrix.In this context, natural hydroxyappatite (HA) has been considered as an effective reinforcement for PVA polymer to obtain biocomposites for use in tissue engineering applications (Sheikh et al., 2010). However further intensive research is needed to develop new fish spoilage monitoring systems. Moreover, it is worth to mention here that the cost competitive, environmentally friendly biodegradable natural nHA can be obtained from calcinations of conch shell and used in the present work. Hence, we report here a possible system for fish freshness monitoring film developed from polyvinyl alcohol, chitosan, xylan, n-hyroxyapatite and curcumin, which has been successfully tested and found suitable as a natural pH indicator

2. Material and methods

2.1. Materials

All chemicals used in this study were of reagent grade (supplied by Sigma, Merck or Fluka) and used as supplied. Polyvinyl alcohol (PVA) (Mol. Wt – 89,000–98,000 Da), Corn cob sample collected from local market, the crab shells were collected from a local sea shore in Kilakarai, Tamil Nadu, India.

2.2. Preparation of chitosan

Air dried crab shell washed with distilled water were heated to boiling with 4% NaOH at 2:3 ratio (solid:liquid) in a glass vessel for 10 min. The alkali was then drained off and the shell was washed well with distilled water followed by removal of mineral by soaking with 1 N HCl. The acid was then exhausted off using several washings with distilled water and the chitin was obtained when washed-out with 10% hypochlorite for 10 min. The chitin thus obtained was converted to chitosan by deacetylation with 50% NaOH for 4 h at 100 °C.

2.3. Preparation of xylan

Extractions of xylan from agro waste of corn cob have been carried out. Initially the corn cob sample was dried and pulverized into fine particles in a ballmill. Then sieved through 40-mesh (400 mm) screen but retained on 80-mesh (177 mm) screen was acquired. After these fine particle was dewaxed using a mixture of methanol and benzene at a ratio of 2:1 (v/v) in a Soxhlet extractor for 6 h. Then dewaxed particles were delignified with acidified sodium chlorite at 70 °C for 2 h. The particle after delignification contains cellulose as well as hemicellulose and thus, it is collectively known as holocellulose. Now, the holocellulose was treated with 1 N NaOH solution at 60 °C for 1 h followed by filtration using Whatman 44 filter paper. Then the alkaline extract was neutralized with 1 N acetic acid and the sample xylan obtained was separated using methanol. The xylan sample was washed several times using methanol and was finally dehydrated at 60 °C in vacuum oven. Likewise a number of washing steps were also performed for deposit containing mainly cellulose with double distilled water followed by washing with acetone and dried at 60 °C in a vacuum oven up to a constant weight. The description processes were accepted out from the similar single bulk of polymer (Kumar & Negi, 2014).

2.4. Preparation of nano-Hydroxyappatite (nHA)

Conch shells were obtained from a local sea shore in Kilakarai, Tamil Nadu, India. The shells were washed and cleaned in distilled water, to get clear of the any probable dirt. The shells were dried and subsequently crushed down into smaller parts and then ground in a hand mortar. The fine powder was sieved over a 75 mm mesh. The obtained sample was heated to 700 °C for 3 h. With respect to the CaO content, the equivalent amount of H_3PO_4, was added drop by drop. In this process the ratio of calcium to phosphorus (Ca/P) was fixed to be 1.667 corresponding to the ratio of HA. The mixture was ultrasonicated for 2 h. After evaporation, the obtained HA was placed into hot-air oven at 100 °C overnight for further purification.

2.5. Preparation of pH indicator film (PCC)

Chitosan was dissolved in 1% (v/v) glacial acetic acid at a concentration of 1% (w/v) and was filtered to remove the insoluble impurities. Then, 0.1% (w/v) of curcumin was dissolved in the methanol solution. Then, 0.5% (w/v) of xylan was dissolved in the chitosan solution. Then, concentration of 1 mg/mL curcumin was prepared by dissolving 10 mg of curcumin in 10 mL of ethanol (90%) was dissolved in the chitosan solution. The final hybrid composite film contains Chitosan/xylan/PVA/Curcumin/nHA (PCC) in the proportion of 1/0.5/0.5/0.01/0.01respectively. 0.01 g sodium tripolyphosphate ($Na_5P_3O_{10}$) solution was added to the final hydrogel to promote cross-linking between PVA, Chitosan and Corncob. The product resulted was casting method in Petri dishes an oven for 96 h at 35 °C to remove solvent residues if any and to obtain the final pH indicator(PCC) films (Fig. 1).

2.6. Physical properties of films

2.6.1. FT-IR studies

A Fourier transform infrared (FTIR) spectrum was collected with the samples mixed in a KBr pellet using a Shimadzu IR affinity-1 spectrometer in the region 4000-400 cm^{-1} (resolution: 2 cm^{-1}, number of scans: 25) The degree of deacetylation (DDA) was evaluated by recording absorbance at 1655 cm^{-1} for amide-I and at 3450 cm^{-1} for OH group in chitosan. The absorbance of chitosan was used to calculate the degree of deacetylation (DDA) using the following equation (Domszy & Roberts, 1985)

$$\% \text{ N-acetylation} = [1- (A1655/A3450) /1.33 \times 100] \quad (1)$$

2.6.2. UV–vis spectra

The PCC film was cut into rectangular pieces of 2 × 4 cm in size to

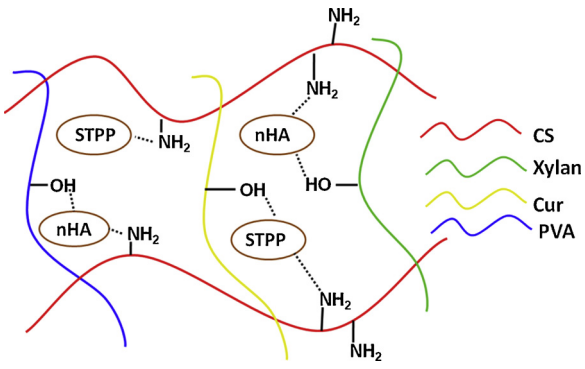

Fig. 1. Schematic mechanism of PCC film.

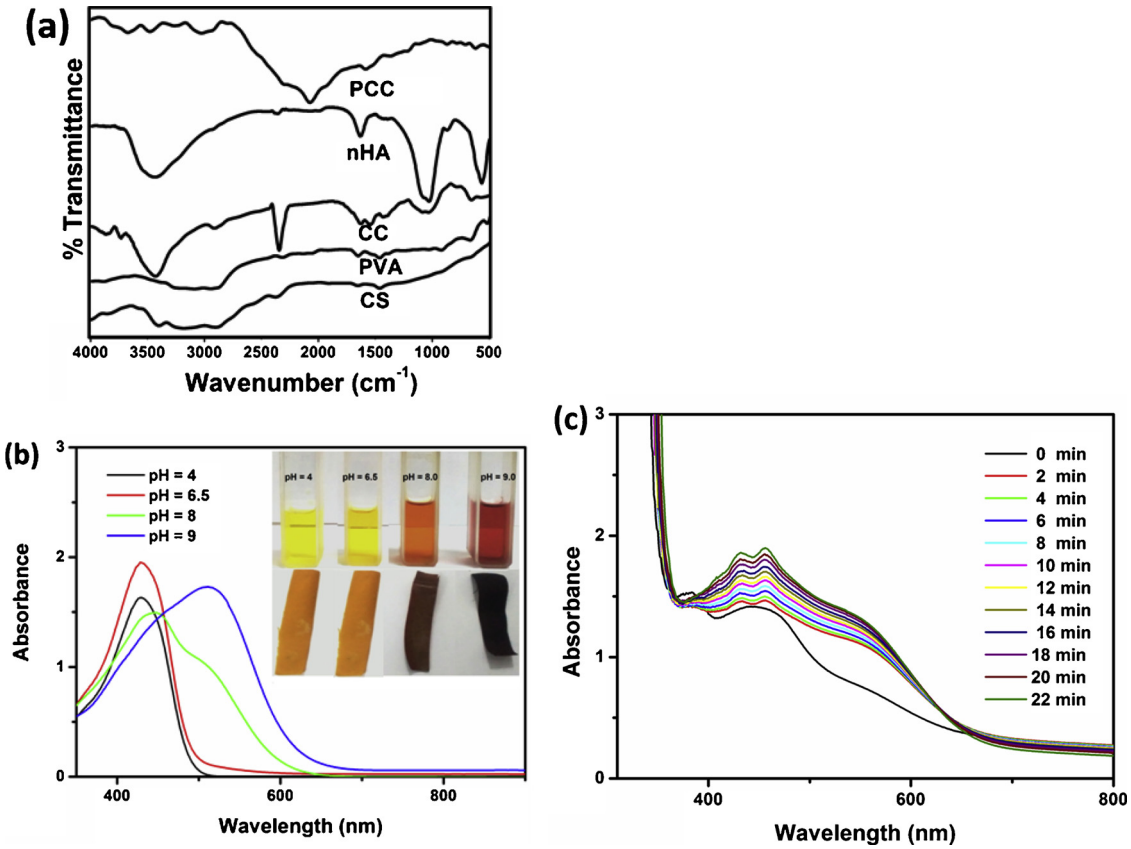

Fig. 2. (a) The FT-IR spectra of Chitosan, Xylan, PVA, nHA and PCC film **(b)** UV-vis spectra of the PCC scaffolds immersed at pH = 4, 6.5, 8 and 9 **(c)** UV-vis spectra of the PCC exposed in 8 mM ammonia solution at25 °C for 22 min.

study the light barrier characteristics at 200–800 nm using a UV–vis (UV-1800-Shimadzu, Kyoto, Japan) spectrophotometer.

2.6.3. NH_3 sensing of the colorimetric films

The ammonia (NH_3) sensing of the PCC films was recorded by earlier reported literature (Kuswandi, Jayus, Larasati, Abdullah, & Heng, 2012).

2.6.4. Scanning electron microscope (SEM)

The SEM micrographs of the samples were analysed using a Jeol/JSM 6390 scanning electron microscope with an accelerating voltage of 10 kV. The films sputtered with thin layer of gold were frozen in liquid nitrogen and snapped immediately to prepare the cross-sections.

2.6.5. In vitro bio-degradation

In vitro bio-degration of PCC films was studied by previously reported literature (Saravanan et al., 2011). The weight loss of the PCC films was evaluated using the following equation.

$$Bio - degration\ ratio\ (weighlt\ loss) = W_0 - W_t \quad (2)$$

Where, Wo is the Initial weight of the scaffolds after 24 h, 48 h and 72 h of incubation, the scaffolds were washed in Milli-Q water to remove ions adsorbed on surface and dried. Wt is the weight of bone dried sample.

2.6.6. Swelling studies

Scaffolds were dipped in PBS (phosphate buffered saline) buffer solution (pH 7.4) at 37 °C. The procedure has been followed by Saravanan et al. (2011). The ratio of swelling was calculated using the following formula:

$$Swelling\ ratio\ = W_w\ - W_0/W_0 \quad (3)$$

Where, Ww is wet weight of PCC scaffold after 24 h. Wo is the bone dry weight of the PCC scaffold.

2.6.7. Sensing fish spoilage

To assess the goodness of films as smart packaging in the food industry, about of 1 kg of fish (shrimp) was purchased from a local sea shore in Kilakarai, Tamil Nadu, India. 25 g of fish and the sensing films were sealed in a plastic film to scrutinize the condition of the shrimp at ambient temperature. The total volatile basic nitrogen (TVB-N) of fresh and color-changed shrimp was measured using Kjeltec Distillation unit (Kuswandi et al., 2012).

2.6.8. Antimicrobial activities

The antibacterial properties of the PCC films were brought towards *Escherichia coli* (*E. coli,* Gram-negative) and *Staphylococcus aureus* (*Stap. Aureus*, Gram-positive) bacterial strains. The antifungal action of the PCC films against *Aspergillus niger* (*A. niger*) and *Aspergillus flavus* (*A. flavus*) fungal strains. Streptomycin and amphotericin was used as a control drug for antibacterial and antifungal studies. The procedures are followed by earlier reported literatures (Amirabad et al., 2018)

2.6.9. DPPH radical scavenging assay

The antioxidant behavior of the films was evaluated using DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay in accordance with Siripatrawan and Harte (2010). The DPPH radical scavenging activity of PCC films was measured using the equation is given below.

$$Radical\ scavenging\ activity\ (\%) = \frac{(Abs\ DPPH - Abs\ sample\ )}{Abs\ DPPH} \times 100 \quad (4)$$

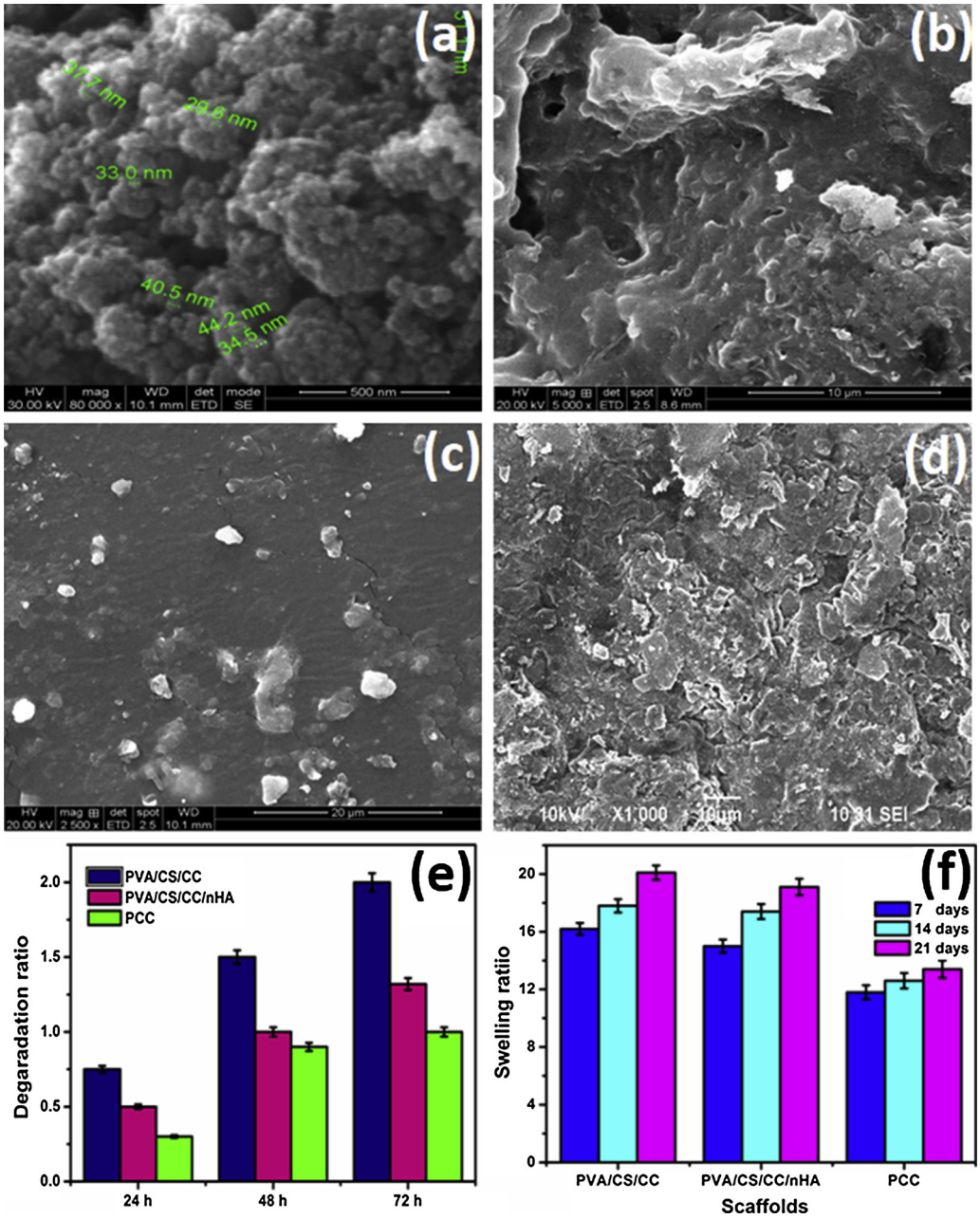

Fig. 3. (a) SEM images of **(a)** nHA **(b)** PVA/CS/CC **(c)** PVA/CC/CS/nHA **(d)** PCC **(e)** *in vitro* bio-degradation studies of PVA/CS/CC, PVA/CS/CC/nHA and PCC scaffolds. **(f)** Swelling behavior of the scaffolds in PBS (at pH = 7.4, 37 °C).

3. Results and discussion

3.1. FT-IR studies

FT-IR spectroscopy was used to assess the possible interactions of CS, PVA, CC, nHA and Curcumin with cross linking agent Fig. 2**a**. The FT-IR spectra of Chitosan, Xylan, PVA, nHA and PCC film. The spectrum for CS shows peak at 1072 cm^{-1} due to saccharide structure. The amide peak there were sharp absorption peaks at 1655 and 1381 cm^{-1} with the hydroxyl absorption band (3450 cm^{-1}), which are characteristic of chitosan and the broad peaks at 1031 and 1098 cm^{-1} describes the C—OCs——tretching vibration in chitosan respectively. The two peaks at 2854 and 2923 cm^{-1} are the owing to the C—H stretching vibrations. The degree of deacetylation was calculated as per the Eq. (1). The ratio of absorbance of amide-I at 1655 cm^{-1} to that of hydroxyl group at 3450 cm^{-1} in chitosan depends upon the degree of deacetylation the measured degree of deacetylation (DDA) of chitosan was 64%.

The spectrum of Xylan at 1645 cm^{-1} was essentially combined with absorbed water. The broad intense bands between 1012 and 1185 cm^{-1} were in xylan and C—O, CC or CO——H stretching vibrations (Buranov & Mazza, 2010). The b-glycosidic linkages between the sugar units furnish the sharp band at 885 cm^{-1}, parallel to the C—1 group frequency (Li et al., 2011). FT-IR absorption bands at 3436 and 2923 cm^{-1} corresponds to OH stretching and CH_2 asymmetric stretches from PVA. The spectra of nHA possessed an OH stretching vibrations in the region of 3445 cm^{-1} and the bands at 1039 cm^{-1} and 569 cm^{-1} was owing to the $(PO_4)^{3-}$ vibrations, which corresponds to the stretching and bending of phosphate groups respectively. The spectrum of PCC shows the interaction of Chitosan and xylan peak at 1706 cm^{-1} which indicates the amide I group (C—O stretching along the N—H deformation). Furthermore, the peak at 3436 cm^{-1} (OH stretching vibration) was shifted to lower values (3386 cm^{-1}) and becomes more broadened which can be

accredited to the hydrogen-bonding interaction of nHA with CS and PVA. In addition to the above new sharp peak was appeared at 1689 cm^{-1}. It could be offered that curcumin was linked with chitosan. The crosslinking between tripolyphosphate and chitosan is confirmed that the new peak appeared at 1631 cm^{-1} indicating the bending vibration of NH and NOP——— stretching vibration at 1551 cm^{-1}.

3.2. UV–vis spectroscopy

In the present study, PCC film was immersed in the buffer solution, the color of the solution was changed immediately into different colors at diverse pH levels (pale yellow, pH 4.0; yellow, pH 6.5; orange, pH 8.0 and wine pH 9.0) The electronic absorption spectra of the different colored buffer solutions with different pH range (pH 4.0, pH 6.5, pH 8.0 and pH 9.0) are shown in Fig. 2**b**. The solutions with pH 4, 6.5, 8 and 9 exhibit an absorption peak at around 429 nm, 430 nm, 443 nm and 513 nm respectively. The pH 4 and 6.5 possess the same absorption due to the presence of phenolic moiety and unsaturated linkages and the effects of curcumin on the light absorption of films in the UV range of 400–520 nm, the PCC film exhibited excellent UV barrier properties, which are expected to protect the food materials from light oxidative deterioration (Martins et al., 2012). Moreover, the UV barrier properties were increased due to the presence of phenolic compounds that were favorable for the adsorption of UV radiation (Bitencourt, Fávaro-Trindade, Sobral, & Carvalho, 2014).

3.3. NH_3 sensing analysis

To assess the NH_3 sensing capability of the PCC film were fully dipped in certain volume of strong aqueous ammonia. NH_3 can be easily released and can be used to study the sensing ability of the PCC films. Fig. 2**c** shows that peak intensity gradually increases with increases time at 0–22 min. These results suggest that PCC film has better sensing ability towards NH_3 solution due to the phenolic hydroxyl group could easily form an acid–base reaction with OH– to form phenolic oxygen anion in the curcumin structure (Ma, Du, & Wang, 2017).

3.4. The morphology of the films

The SEM micrograph surfaces of then nHA, PVA/CS/CC, PVA/CS/CC/nHA and PCC films are presented in Fig. 3. The SEM study indicates the amorphous nature of the materials with narrow particle size distributions. A representative nHA sample indicates the average particle dimensions of approximately 50 ± 20 nm (Fig. 3a) The PVA/CS/CC films exhibit a heterogeneously-fractured layer near the film surface, as shown in Fig. 3**b**. This layer shows some abnormalities due to presence of semi-crystalline nature, which are related to the presence of intermolecular hydrogen bonding as well as the regular arrangement and orientation of PVA molecules in the hybrid composite systems (Cano, Chafer, Chiralt, & Gonzalez-Martínez, 2015).

The PVA/CS/CC/nHA (Fig. 3c) films exhibit a compact and smooth surface, although a micro-phase separation was occurred between PVA, CS and CC. The PCC films (Fig. 3d) of PVA/CS/CC hybrid blends were moderately compressed and dispersed with fine particles of nHA. It was observed that the incorporation of Cur did not significantly alter the surface of the film because of the presence of non- porous and split structure in the PCC film (Fig. 3d).

3.5. In vitro bio-degradation

Lysosyme vital enzymes liable for in vivo degradation of scaffolds where as other proteolytic enzymes show only inferior degradation activity. These enzymes simply be targeted the bio polymer of β-1, 4-glycosidic bonds of xylan and N-acetyl glucosamine (NAG) groups of chitosan chains. The PVA/CS/CC/nHA scaffolds prepared at 24 h, 48 h, and 72 h illustrated lower degradation by the enzyme owing to their superior cross linked network structure when compared to that of PVA/CS/CC scaffolds (Fig. 3**e**) (Saravanan et al., 2011). The result obtained infers that the incorporation of Cur in the PVA/CS/CC/nHA scaffold possesses an upgraded resistance to biodegradation and improved longevity of these scaffolds for longer last.

3.6. Swelling studies

Swelling studies indicated that the PVA/CS/CC scaffolds retained water more than that of the initial weight after 24 h retention period in PBS. The PVA/CS/CC scaffold raises the swelling percentage (Fig. 3f). Rise in swelling expected to influence the decreased mechanical properties of the scaffold. However, the PVA/CS/CC/nHA scaffold showed reduced swelling when compared to that of PVA/CS/CC scaffold thus indicating to raise the gel formation. The combination of Cur into PVA/CS/CC/nHA has reduced swelling percentage due to the preloading of Cur into the polymer matrix, which make a barrier to the diffusion of water. The increase in the swelling percentage at number of day's increases may be credited to the disturbance of hydrogen bond existing between the amino group of chitosan and hydroxyl group of PVA macromolecules. No significant alteration in percentage of swelling was observed in the cases of PCC scaffold prepared with different time intervals and these scaffolds can be suitably used as packaging material for food materials with enhanced longevity.

3.7. Fish spoilage trial

The decay of the common seafood products is primarily due to microorganisms. Most of the microorganisms are liable for decay & cause the food to have unpleasant off-flavors (Kuswandi et al., 2012). TVB-N is a product of microbial squalor including ammonia, dimethylamine, trimethylamine, etc. TVB-N levels enhance usually owing to the formed by NH_3 and other unstable amines. From this point of view, the sensing film prepared in the present analysis can be used to detect the decay of Indian oil sardine fish freshness. The PCC film's starting color was straw yellow, and after 10 h it alters to orange-red owing to the increasing pH brought by enhanced concentration of TVB-N rising from 14.8 mg/100 g to 54.5 mg/100 g and presented Fig. 4**a**. After the analysis, the PCC film was peeled off from the petri dish. In the current study, TVB-N values exceeded the limit of acceptability on the day of sensory rejection (10 h) for untreated. However, a value of 25-30 N_2 100 g^{-1} for TVBN was recommended as the acceptability limit by Ababouch et al. (1996) for Atlantic sardine. The color variation was clearer as display in Fig. 4**b**. From the results obtained, it is suggested that the hybrid composite films developed in the present work could be used for an intelligent packaging to assess the spoilage of fish through direct image inspection.

3.8. Antimicrobial activity

Antimicrobial studies indicated that the PCC scaffolds for the zone of inhibition of PVA/CS/CC/nHA was higher than that of PVA/CS/CC towards *E. coli* and *S. aureus* bacterial strains (Fig. 5a). The zone inhibition of the PVA/CS/CC scaffolds was found to be 7.00 ± 0.35 mm and 5.00 ± 0.25 mm against *E. coli* and *S. aureus* respectively due to the presence of chitosan and xylan. The zone of inhibition against *E. coli* and *S. aureus* for the PVA/CS/CC/nHA scaffolds was comparatively higher in the order as 11 ± 0.55 mm and 9 ± 0.45 mm and that could be due to the presence of nHA which possesses the natural antibacterial activity. The PCC results suggested that the higher zone of inhibition 14.00 ± 0.7 mm and 13.00 ± 0.65 mm against *E. coli* and *S.aureus* was due to the antibacterial activity of Cur. Observation similar to this was also reported from previous studies with regard to the antimicrobial activity of PVA/CS films against Gram negative and Gram positive microorganisms (Tripathi, Mehrotra, & Dutta, 2009).The standard Streptomycin was found to be 15 ± 0.75 mm and

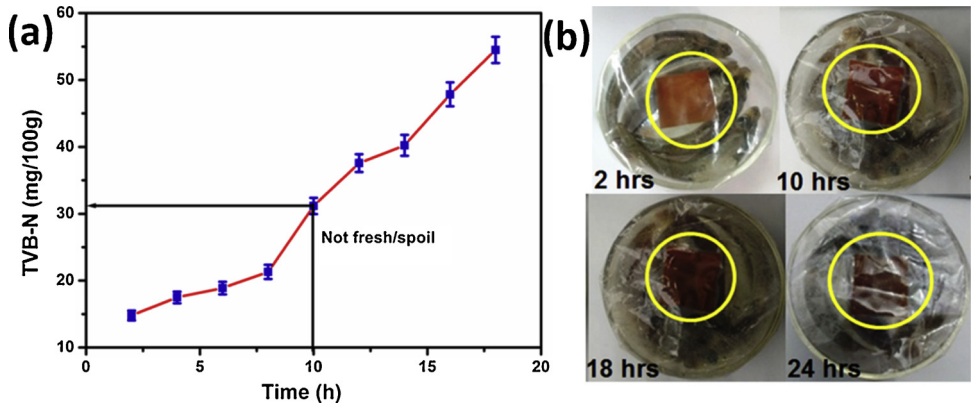

Fig. 4. (a) The change of TVB-N level of Indian oil sardine fish at room temperature **(b)** Application of sensing film as a sensor for Indian oil sardine fish freshness.

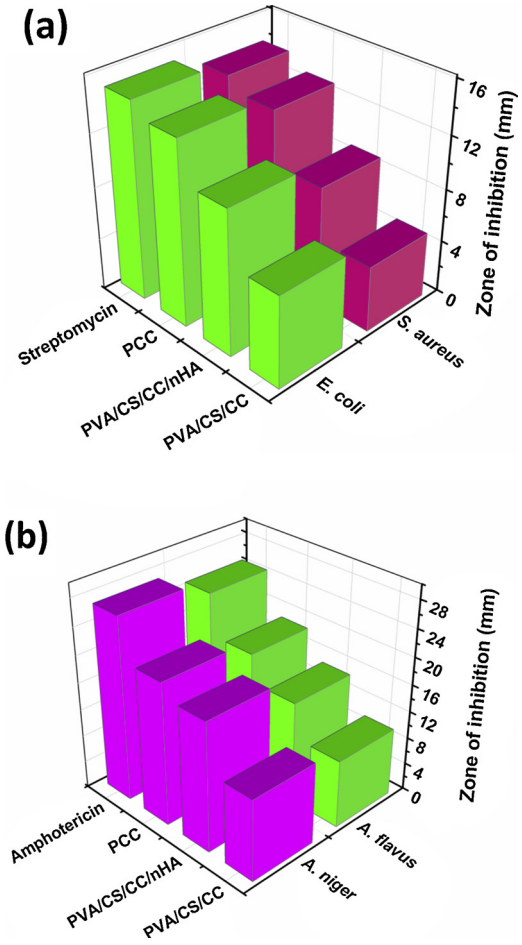

Fig. 5. Antimicrobial activities of PVA/CS/CC, PVA/CS/CC/nHA and PCC Scaffolds.

14 ± 0.7 mm against *E. coli* and *S. aureus* respectively was due to the antibacterial activity

Furthermore, results obtained from antifungal studies of PCC scaffolds with regard to the zone of inhibition, the PVA/CS/CC/nHA hybrid composite films exhibit higher than that of PVA/CS/CC towards *A. niger* and *A. flavus* fungal strains (Fig. 5b). The zone inhibition of the PVA/CS/CC scaffolds was found to be 12.00 ± 0.6 mm and 10.00 ± 0.5 mm against *A. niger* and *A. flavus*, respectively, whereas the zone of inhibition against *A. niger* and *A. flavus* obtained for the PVA/CS/CC/nHA scaffolds was comparatively higher in the order as 19 ± 0.95 mm and 15 ± 0.75 mm. The higher efficiency observed for PVA/CS/CC/nHA scaffolds maybe owing to the presence of nHA, which possesses inherent natural antifungal activity. The data on antifungal studies of PCC suggest that the greater zone of inhibition 21.00 ± 1.05 mm and 19.00 ± 0.95 mm against *A. niger* and *A. flavus* obtained was due to the antibacterial activity imparted by Cur (Liu, Cai, Jiang, Wu, & Le, 2016). The results of antifungal activities of PCC are comparable to those of standard amphotericin, which exhibits the value of 27 ± 1.35 mm and 25 ± 1.25 mm against *A. niger* and *A. flavus*.

3.9. DPPH radical scavenging assay

The radical scavenging ability of PPC film was tested against DPPH free radical. Upon insertion of PCC film into DPPH solution, the color of the solution was changed from purple to yellow in color (Fig. 6). The color change infers that the PCC film possesses the radical scavenging ability Moreover, the ability of radical scavenging behavior in terms of percentage (RSA%) of PCC film was calculated by Eq. (1). PCC film exhibited the better antioxidant activity (85.84%) due to the presence of hydrogen donor groups in PCC films.

4. Conclusion

In the present work renewable sources based hybrid composite film (PVA/CS/CC/nHA) packaging materials have been developed using corncob, chitosan, polyvinyl alcohol and hydroxylapatite and characterized using different analytical techniques. Data resulted from morphological, UV studies, radical scavenging ability, ammonia sensing analysis, fish spoilage trial, swelling studies, Invitro bio-degradation, antimicrobial and antifungal studies indicate that the hybrid composite films developed can be used as intelligent food packaging material with

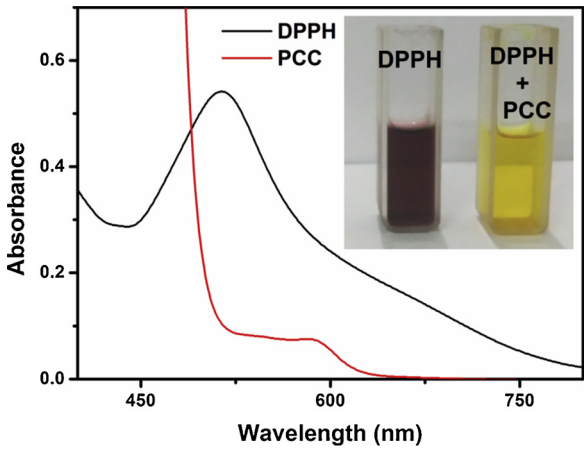

Fig. 6. Antioxidant properties of PCC Scaffolds immersed in DPPH solution.

improved longevity. Results obtained from antimicrobial and antifungal studies are comparable to those of standard Streptomycin and Amphotericin and this further supports the utility these hybrid films for freshness of fish sensing and packaging.

The author(s) declared no potential conflicts of interest with respect to the research, authorship or publication of this article.

Declaration of Competing Interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship or publication of this article.

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