

Supporting Information
for

***In situ* crosslinked Schiff-base biohydrogels containing *Carica papaya* peel extract: Application in packaging of fresh berries**

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S1. Materials

Chitosan (deacetylation degree > 75%), were purchased from Sigma Aldrich, India and used as received. The viscosity-average molecular weight (M_v) of chitosan has been evaluated as a function of intrinsic viscosity from Mark-Houwink-Sakurada equation given as:

$$\eta = k M_v^\alpha$$

Where η represents intrinsic viscosity and k , α are the constants for the solvent-polymer system at specific temperature. The viscosity was determined by using an Ubbelohde type glass capillary viscometer. By recording the polymer solution's flow-through time in the viscometer capillary; the relative viscosity (η_r), the specific viscosity (η_{sp}), the reduced viscosity (η_{red}) were calculated.

The intrinsic viscosity (η) of our sample of chitosan used was calculated by following the reported method [1]. Five different concentrations (0.01%, 0.02%, 0.03%, 0.04% 0.05% w/w) of chitosan in 0.01 M HCl were prepared. The measurements were performed in triplicate for each concentration and the temperature was maintained at 30 °C. In this case, $\alpha = 0.72$ and $k = 5.48 \times 10^{-4}$ as obtained from literature at 30 °C [1]. From this data, the M_v of our sample of chitosan was found to be 1.78×10^5 [2].

[1]. R.H. Chen, M. L. Tsaih (1998) Effect of temperature on the intrinsic viscosity and conformation of chitosans in dilute HCl solution. *Int. J. Biol. Macromol.*, 23, 135 – 141

[2] G. Dalei, S. Das, S. R. Jena, J. Nayak, D. Jena, L. Samanta (2023) *In situ* crosslinked dialdehyde guar gum-chitosan Schiff-base hydrogels for dual drug release in colorectal cancer therapy. *Chem. Eng. Sci.*, 269, 118482

S2. Preparation of papaya peel extract (PPE)

Green papayas were purchased from local markets in Cuttack, Odisha. The fresh peels were washed properly, manually cut and sun-dried for 7 days. The dried peels were then powdered and stored at ambient temperature. Thereafter, 50 g of this powder was dissolved in 80% (v/v) hydroethanolic solution and incubated for 48 h at 25 °C. The mixture was centrifuged and the papaya peel extract (PPE) was lyophilized to obtain the final product.

S3. Evaluation of physical properties of films

The thicknesses of the developed films were measured by digital micrometer. The thickness of film at different points was measured, and the average value of about 10 measurements was reported as the thickness of the sample.

To measure the moisture content; films were first weighed using a weighing balance and then dried in an oven until a constant weight was obtained in approximately 24 h. The test was performed in triplicate, and the moisture content was calculated using from eq. (1):

$$\text{Moisture content (\%)} = \frac{W_i - W_f}{W_i} \times 100 \quad (1)$$

Where W_i is the initial weight of the film before drying and W_f is the final weight of the film after drying.

To estimate the film solubility; the films were cut into size of $2 \times 2 \text{ cm}^2$ and weighed before being immersed in 10 mL of distilled water with constant gentle agitation (approx. 30 rpm) for 1 h at $25 \text{ }^\circ\text{C}$. Then, the films were filtered and dried to a constant weight. The water solubility was calculated from eq. (2):

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

Where W_i is the initial weight of the film before drying and W_f is the final weight of the film after drying.

For estimating the film opacity; the films were cut into a rectangle piece and directly placed in a spectrophotometer test cell, using an empty test cell as the reference. The opacity index of the films was calculated from eq. (3):

$$\text{Opacity} = \frac{A_{600}}{x} \quad (3)$$

Where A_{600} is the absorbance at 600 nm and x is the film thickness.

S4. Evaluation of water vapour permeability (WVP) and oxygen permeability (OP)

The WVP was measured gravimetrically. 10 mL of distilled water was placed in a glass bottle with an inner diameter of 30 mm. Teflon tape was used and tightly wrapped with films around the bottle mouth. The initial mass of the bottle was recorded and kept in the oven at $40 \text{ }^\circ\text{C}$ for 24 h. The bottle was taken out from the oven after 24 h and weighed once again. Mass of the bottle was recorded for 3 consecutive days. Then WVP was calculated from eq. (4):

$$\text{WVP} = \frac{W \times x}{t \times A \times \Delta P} \quad (4)$$

Where W = mass gain of the sample (g), x = thickness of the film (m), t = 24 h, A = permeation area (m^2) and ΔP = difference in partial vapour pressure between the pure water and dry atmosphere at $25 \text{ }^\circ\text{C}$. Three measurements were recorded for each film.

To determine the OP; bottles with inner diameter 15 mm were wrapped with film of 30 mm diameter and sealed using Teflon tape. The bottle was then placed in a desiccator (with silica gel) at room temperature. The mass of the bottles was then recorded after 24 h for 3

consecutive days. The plot of mass change against time was taken. From the slope obtained the OPTR (Oxygen Permeability Transmission Rate) and OP were evaluated from eqs. (5) and (6):

$$OPTR = \frac{Slope}{Film\ area} \quad (5)$$

$$OP = \frac{OPTR \times x}{\Delta P} \quad (6)$$

Where ΔP = difference in partial vapour pressure, x represents the average film thickness. Measurements were determined in triplicate for each sample.

S5. Characterization

FTIR analysis: The samples were triturated with dry KBr, compressed to pellets and scanned in the range of 4000–400 cm^{-1} in a Thermo Scientific Nicolet iS5 FTIR spectrophotometer. The spectrum was read by an average of 64 scans.

TGA analysis: The thermogravimetric analysis (TGA) of samples was measured in a NETZSCH STA 449F3. The films (5 mg) were accurately weighed into hermetically sealed aluminum pans and then subjected to heating from 30 to 1000 $^{\circ}\text{C}$ at a rate of 10 $^{\circ}\text{C}/\text{min}$ under N_2 atmosphere (purging rate: 10 mL/ min).

FESEM analysis: The morphology of the samples was visualized on a Carl Zeiss-Gemini FESEM 300 with an acceleration voltage of 5 kV. The films were cut into small pieces, sputtered coated, placed on metal grids and then observed for their microstructures.

Mechanical analysis: For the mechanical tests; the hydrogel films of dimensions (50 mm \times 15 mm) were equipped with a 1 kN load cell and a strain rate of 10 mm/ min was employed in an Instron 5567 (Instron, USA) at room temperature 25 $^{\circ}\text{C}$.

S6. Biodegradation study

Biodegradability study was done by soil burial method for 4 weeks. The films were cut into rectangular pieces 20 mm x 20 mm and kept vertically in 100 g soil in a 500 mL beaker at a distance of 10 cm from top soil. The beakers were placed in a humidity chamber with temperature and humidity maintained at 25 $^{\circ}\text{C}$ and 68–70 % RH respectively. The change in weight loss was calculated as per eq. (7):

$$Biodegradation\ (\%) = \frac{\Delta W}{W} \times 100 \quad (7)$$

Where ΔW is the change in weight of the films at different buried times and W is the initial weight of the films.

S7. Antibacterial study

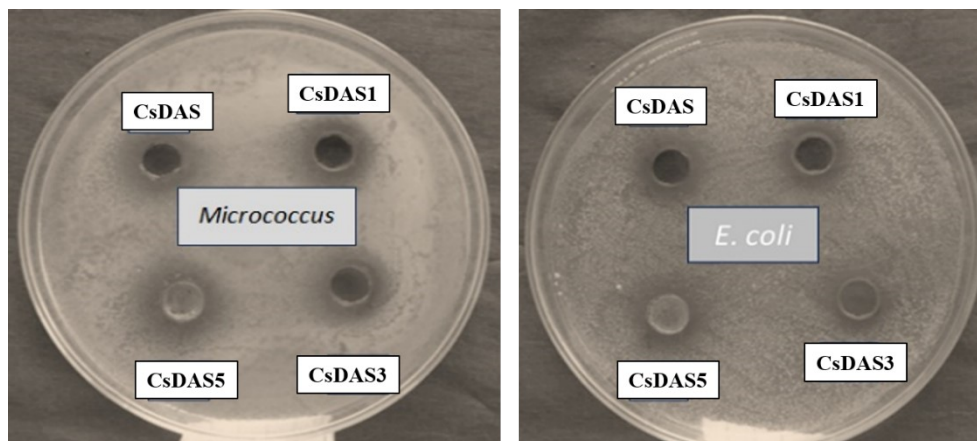


Fig. S1. Photographs of zone of inhibition of hydrogel films against *E. coli* and *M. luteus* bacteria