Vanillin reinforced cationic starch/poly(vinyl alcohol) based antimicrobial and antioxidant bioactive films: Sustainable food packaging materials

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Number of Pages: 04

S.1. Characterization methods of control CPVN and bioactive films

S.1.1. Optical properties

The optical characteristics of the control CPVN and bioactive film samples were examined using a UV-vis spectrophotometer (PerkinElmer LAMBDA 365). In short, 1×4 mm^2 films were exposed to UV-vis radiations (200 to 800 nm), and the obtained findings were represented as a result of % transmittance. The transparency at 600nm (T_{600}) and opacity were calculated by using the following equations [1,2]

$$T_{600} = \frac{-\log \%T}{b} \quad \text{.....(1)}$$

$$Opacity = \frac{A_{600}}{b} \quad \text{..... ...(2)}$$

Where %T is the percentage of transmittance, A_{600} is the absorbance at 600 nm and b is the thickness of pristine CPPA and active film samples.

S.1.2. Thickness and mechanical parameters

The thickness of prepared films was measured by using a digital micrometre (Mitutoyo, Japan) with a precision of 0.001 mm. In addition, the mechanical properties of control CPVN and bioactive films were analysed by the use of a Universal Testing Machine (DAK System) as per ASTM D 882-91 standard [3]. Briefly, each film sample of size 2.5×10 cm^2 was placed between the gauges of UTM with an initial grip distance of 50 mm and stretched at a crosshead speed of 1 mm/min. The mechanical parameters such as tensile strength (TS), elastic modulus (EM) and elongation at break (EB), were evaluated by preinstalled software.

S.1.3. Spectroscopic analysis

The intermolecular interactions between CT/PVA and VN were analysed by ATR-FTIR spectroscopy (PerkinElmer, version 10.5.4, USA) within a range of 4000-400 cm^{-1} with a spectral resolution of 4 cm^{-1}. Similarly, the crystalline nature of prepared active films was determined by using X-ray Diffraction studies. In short, 2×2 cm^2 CPVN films were subjected to X-ray Diffractometer and recorded the spectra between 2θ =5° to 80°. The % crystallinity was calculated by using the following equation [4],

$$Crystallinity\ (\%) = \frac{A_C}{A_T} \times 100 \quad \text{.....(3)}$$

Where A_C and A_T are the area within crystalline peaks and the total area of all peaks, respectively.

S.1.4. Surface morphology

The surface morphology of control CPVN and bioactive films was studied by the use of scanning electron microscope (SEM) (JSM-IT500) with an accelerating voltage of 10kV and atomic force microscopy (AFM) (Nanosurf Easyscan 2, Switzerland) with an aluminium coated cantilever, at ambient temperature.

S.1.5. Water contact angle (WCA) measurements

WCA (degree) of control CPVN and active films is measured with WCA analyser (DMs-401 Kyowa Interface Science Co. Ltd) *via* the sessile drop method.

S.1.6. Moisture adsorption capacity (MAC)

The MAC of control CPVN and bioactive films were evaluated as per ASTM standards D644–94. In concisely, the film samples (2×2 cm^2) were dried at 90±2 ^{0}C for 5h and weight was noted (W_i). To determine MAC, the dried samples were kept outside for 24h and the final weight was recorded as W_f.

$$\%MAC = \frac{W_i - W_f}{W_i} \times 100 \quad .....(4)$$

where W_i is the initial weight and W_f is the final weight of film samples.

S.1.7. Water solubility (WS)

The WS of control CPVN and bioactive films were evaluated as per ASTM standards D570-98 [5]. WS is determined by transferring pre weighed control CPVN and active film samples (2×2 cm^2) into beakers containing Millipore water (20cc) for 24 h at room temperature. After 24 h, the film samples were taken out and dried completely, and the final weight (W_f) was noted.

$$\%WS = \frac{W_i - W_f}{W_i} \times 100 \quad .....(5)$$

where W_i is the initial weight and W_f is the final weight of film samples.

S.1.8. Water vapour barrier properties

The water vapour barrier properties were measured in terms of water vapour transmission rate (WVTR) as per previous reports [6]. In concise, 4×4 cm^2 film samples were sealed around a vial containing 20cc milli-Q water and initial weight (W_i) is recorded. The vial is then kept in a hot

air oven at 40°C for 24 h, and the final weight (W_f) is recorded. WVTR of film samples was determined by using the following equation,

$$WVTR(\%) = \frac{W_i - W_f}{A \times T} \text{.....(6)}$$

where T is the time period (24 h) and A is area of the mouth of the vial

S.1.9. Soil burial test (SBT)

The biodegradability of control CPVN and bioactive films was studied using a soil burial approach [7]. The film samples (2×2 cm^2) were dried and initial weight was recorded (W_i) before being buried 50 mm beneath the surface of soil. The soil was kept moistened by sprinkling water every day. After 30 days, the film samples were taken out from the soil, washed with deionized water, dried in an oven at 90±2 ^{0}C, and the final weight (W_f) was recorded. The % weight loss was calculated using the equation below,

$$\%\, Weight\ loss\ = \frac{W_i - W_f}{W_i} \times\ 100\ \text{.....(7)}$$

Where W_i and W_f are the initial and final weights of the film sample.

S.1.10. Antimicrobial activity

The antimicrobial effectiveness of control CPVN and bioactive films was tested against food-borne microbes like *Bacillus subtilis* (*B. subtilis*)*, Escherichia coli* (*E. coli*)*, Staphylococcus aureus* (*S. aureus*), *and Candida albicans* (*C. albicans*). The 0.1mL film solutions (1mg/mL) was dispersed into agar medium plates containing microorganism cultures. Finally the plates were incubated at 37 °C for 24h. The inhibitory zone (mm) values were measured by using ImageJ software [8].

S.1.11. Antioxidant assay

The antioxidant potentiality of control CPVN and bioactive films was evaluated by DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method. In brief, all the film solutions were prepared in 1.5 mL ethanolic extract and DPPH in 0.5mL ethanol (0.5mM) were mixed thoroughly and incubated for 30 min. Further, the absorbance was measured at 517nm by using UV–Vis spectrophotometer (LMSP UV-1200, Labman). Further, % of free radical scavenging activity of composite films was calculated by using the following formula [9],

$$\% \text{ Free radical scavenging activity} = \frac{AC - AS}{AC} \times 100 \quad .....(8)$$

where A_c and A_s are the absorbances of the control and sample respectively.

S.1.12. Statistical analysis

OriginPro 9.0 software was implemented to perform statistical analysis through ANOVA One Way Analysis of Variance. All the experiments were carried out in triplicates. The results were expressed as mean ±SD.

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