



Antimicrobial and enzymatic antibrowning film used as coating for bamboo shoot quality improvement



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ABSTRACT

Edible films were prepared with varying proportion of alginate and starch in the ratio of 2:0(F₁), 2:1(F₂), 1:1(F₃), 1:1.5(F₄), 1:2(F₅), 0:2(F₆) with added carboxymethyl cellulose (15%, w/w of starch). The film F₅ had superior barrier, mechanical and thermal properties over the other films. Water vapor permeability, moisture absorption, water solubility, breakage strength and elongation capacity of F₅ film were reported as 1.21×10^{-9} g/Pa h m, 9.37%, 40%, 977.3 g and 14.62 mm respectively. However, surface characteristics showed the smooth and uniform film and thermal decomposition took place above 200 °C. The film forming solution of selected F₅ film, added with antioxidant and antimicrobial extracts was coated on bamboo shoots and stored for 5 days. The film was successful in lowering the browning of bamboo shoots, and also successfully inhibited surface microbial load. Moreover, the moisture loss of coated shoot was less compared to uncoated.

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1. Introduction

Edible film and coatings can be used to help preserve fresh cut fruits and vegetables, providing a selective barrier to moisture, oxygen and carbon dioxide, improving mechanical and textural properties. Further addition of antioxidants and antimicrobial compounds, impart microbial barriers, avoiding volatile loss, etc. (Lee, Park, Lee, & Choi, 2003). Edible biological components, viz., polysaccharides, proteins and lipids or a mixture of these were successfully used for the preparation of coatings and films (Bertuzzi, Armada, & Gottifredi, 2007; Mali, Sakanaka, Yamashita, & Grossmann, 2005). Plasticizers are added in coating mixture to improve the flexibility, and reduce brittleness of coating by reducing the internal hydrogen bonds between polymer chains and increasing intermolecular spaces (Sothornvit & Krochta, 2000). Owing to their hydrophilic nature, the incorporation of a lipid substance to the coating mix might be necessary in order to improve water vapor barrier properties (Garcia, Martino, & Zaritzky, 2000; Rojas-Graü et al., 2007).

Edible films and coatings might also serve as carriers of food additives by incorporating antioxidant, antibrowning and antimicrobials agents, colorants, flavors, nutrients, and spices into them

(Baldwin, Nisperos, Chen, & Hagenmaier, 1996; Lee et al., 2003; Li & Barth, 1998; Tapia et al., 2007; Wong, Fatt, & Radke, 1996). The edible films prepared from single film-forming polymer displays good properties in some aspects but are poor in others. An alternative promising strategy to improve the properties of edible films and coatings is through blending of biopolymers. Composite or blend films are usually composed of two or three biopolymers and are prepared by varying methods (Ban, Song, Argyropoulos, & Lucia, 2006; Famá, Gerschenson, & Goyanes, 2009; Vilaseca et al., 2007).

Most of the study highlighted the use of biodegradable materials in place of petro-based plastics with similar properties and low in cost. Starch is the most important polysaccharide polymer that is used to develop biodegradable films because it has capability of forming a continuous matrix and it is a renewable and abundant resource. Starch-based films exhibit physical characteristics similar to synthetic polymers, such as being transparent, odorless, tasteless, semi-permeable to carbon dioxide and resistant to oxygen passage (Fazilah et al., 2011). However, starch exhibits several disadvantages such as a strong hydrophilic character and poor mechanical properties. This can be overcome with the addition of carboxymethyl cellulose (CMC) in starch which helps to lower the water vapor permeability and increasing the mechanical strength (Ghanbarzadeh, Almasi, & Entezami, 2010). Alginate has potential use in films or as a coating component because of its unique colloidal and gel-forming properties. However, the incorporation of calcium help reduces their water vapor permeability and making alginate films water insoluble (Olivas, Mattinson, &

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Barbosa-Cánovas, 2007). The composite film prepared from these materials could be the best film having improved barrier and mechanical properties compared to individual biopolymers.

Bamboo shoots are highly nutritious and potentially rich sources of dietary fibers, antioxidants, amino acids, minerals, vitamins and low in calories. The protein content of the shoots is also high, and contains fewer amounts of fats; however, it is rich in essential fatty acids (Kumbhare & Bhargava, 2007; Nirmala, Sharma, & David, 2008; Park & John, 2010). Bamboo shoot is a significant item of consumption in the home, while it is not significant in commercial terms due to short shelf life. Bamboo shoots deteriorate rapidly during storage and transportation which is a serious post harvest problem for traders. Enzymatic browning also poses a serious problem during the storage of post harvest bamboo shoots. Edible coatings might have the potential to inhibit the rapid quality degradation of harvested bamboo shoots. The present study was focused to develop composite film from alginate, starch and carboxymethyl cellulose with added antibrowning and antimicrobial agents and to examine the effect of coating on bamboo shoot quality.

2. Materials and methods

2.1. Materials

Potato starch, sodium alginate and carboxymethyl cellulose (CMC) was provided by HiMedia laboratories (India). Glycerol, calcium carbonate (analytical grade) were purchased from Merck (India) and refined sunflower oil (Dhara, India) from local market. Fresh bamboo shoots (*Bambusa balcooa*) and *Garcinia pedunculata* Roxb. were collected from Nagaon district of Assam (India). *Lactobacillus* strains were collected from the Department of Food Engineering and Technology, Tezpur University, Assam (India). These strains were isolated and identified in the laboratory from fermented bamboo shoot product (*khori*) of Assam, India.

2.2. Preparation of antioxidant extract

Antioxidant compounds were extracted from *G. pedunculata* Roxb. using microwave assisted extraction. In preliminary experiments the extraction process was optimized at 180W microwave power. The optimized values recorded were, solvent concentration of 70.79%, solvent to sample ratio of 20:1 and irradiation time of 4.73 min. Under these optimized conditions, the antioxidant activity of extract was found to reach up to 85.98% DPPH radical scavenging activity and 19.23% ABTS radical scavenging activity. The extract was filtered through cellulose acetate syringe filters and stored at 4 °C for further use.

2.3. Preparation of antimicrobial extract

Biometabolites were extracted and purified from a *Lactobacillus* strain isolated from fermented bamboo shoot of Assam and it was assessed for antimicrobial activity for three indicator strains of food contaminating microbes viz., *Escherichia coli*, *Streptococcus aureus* and *Bacillus cereus*. In preliminary study, submerged fermentation technique was used for the fermentation of biometabolite, and was purified by liquid-liquid extraction with chloroform. Minimum lethal dose concentration (LD_{min}) assay of the chloroform extract were reported as 27 mg/ml, 1.68 mg/ml and 1.68 mg/ml for *E. coli*, *S. aureus* and *B. cereus* respectively. The cell free purified supernatant was stored at 4 °C and used as antimicrobial agent during this study.

2.4. Preparation and standardization of film

The blend film composed of 6 different combinations (w/w) of alginate, starch and CMC varied at 15% to the amount of starch. The proportion of alginate and starch were taken in the ratio of 2:0(F₁), 2:1(F₂), 1:1(F₃), 1:1.5(F₄), 1:2(F₅), 0:2(F₆). The weight of total solid matter in all film forming solution was kept constant. Starch was mixed with distilled water (100 ml) and agitated by magnetic stirrer (500 rpm) for 30 min at 95 °C for complete gelatinization. In a separate vessel, alginate solution was prepared by dissolving alginate in distilled water (100 ml) and heated at 70 °C and stirred until the solution became clear (~30 min). CMC (15%, w/w starch) was solubilized in distilled water (50 ml) at 75 °C for 10 min (Ghanbarzadeh et al., 2010). The starch, alginate and CMC solutions were mixed together and stirred at 75 °C for 10 min for proper mixing, stability and keeping property of the suspension. Glycerol was added (40 ml/100 g of solid matter) to the suspension as a plasticizer for decreasing brittleness of the film.

Suspensions were then cooled to 40 °C and then kept under vacuum (600 mm Hg) for 24 h to release all air bubbles. Then, the suspension was poured into a Teflon casting tray resulting in thin films, and then dried at 60 °C in an oven to cast the films. Dry films were peeled intact from the casting surface. The films were then immersed in 2% (w/w) CaCl₂ solution for 10 min and dried at 40 °C for 6 h. CaCl₂ has been reported to increase strength of films, likely due to the development of crosslinks between the carboxyl groups in the film molecules and the calcium ions (Fazilah et al., 2011).

The prepared films were conditioned following ASTM D618-05 method (ASTM, 2005). For all tests the prepared films were conditioned at 55 ± 1% RH and 20 ± 1 °C in a desiccator containing a saturated solution of Mg(NO₃)₂·6H₂O for 72 h, or till further tests. All measurements were performed in three replicates. Based on mechanical and barrier properties of film, the best film was selected and the proportions of alginate, starch and CMC of best film were used for the formulation of coating solution.

2.5. Properties of film

2.5.1. Film thickness

Films were measured with hand-held micrometer (Alton M820-25, China) having a sensitivity of 0.01 mm. The ten pieces of films were stacked one above the other and average thickness was recorded. The average thickness of the coating formed around a shoot was also determined as the difference between the diameter of the shoot before and after coating at different points (Das, Dutta, & Mahanta, 2013).

2.5.2. Water vapor permeability (WVP)

WVP tests were carried out using the standard ASTM (1995) with some modifications (Mali, Grossmann, García, Martino, & Zaritzky, 2006). Glass beakers, with an average diameter of 2 cm and a depth of 4.5 cm, utilized to determine WVP of films. Films were cut into discs with a diameter slightly larger than the diameter of the cup. After placing 3 g of anhydrous CaSO₄ in each cup, they were covered with edible films of varying composition. Relative humidity (RH) of 0% was maintained using anhydrous CaSO₄ in the cup. Each cup was placed in a desiccators containing saturated K₂SO₄ solution to maintain the RH of 97% at 25 °C. The desiccators were kept in an incubator at 25 °C. Cups were weighed every 24 h and changes in the weight of the cup were recorded as a function of time. Slopes were calculated by linear regression (weight change vs. time) and the water vapor transmission rate (WVTR)

was defined as the slope (g/h) divided by the transfer area (m²). WVP (g m⁻¹ h⁻¹ Pa⁻¹) was calculated using Eq. (1).

$$\text{WVP} = \frac{\text{WVTR}}{P(R_1 - R_2)X} \quad (1)$$

where P is the saturation vapor pressure of water (Pa) at the test temperature (25 °C), R_1 is the RH in the desiccator, R_2 is the RH in the cup and X is the film thickness (m). Under these conditions, the driving force [$P(R_1 - R_2)$] is 3073.93 Pa.

2.5.3. Moisture absorption

The dried films of 20 mm × 20 mm were first conditioned at 0% RH (CaSO₄) for 24 h. The weight (W_0) of films were taken and they were conditioned in a desiccator containing CaNO₃ saturated solution at 20 °C to 25 °C to ensure a relative humidity of 55%. Each film was weighed at desired intervals (W_t) until the equilibrium state was reached (Ghanbarzadeh et al., 2010). The moisture absorption of the samples was calculated using Eq. (2).

$$\text{Moisture absorption (\%)} = \left[\frac{W_t - W_0}{W_0} \right] \times 100 \quad (2)$$

where W_t and W_0 are the weights of the sample after t time at 55% RH and the initial weight of the sample, respectively.

2.5.4. Solubility in water

Solubility in water was defined as the percentage of the dry matter of film which is solubilized after 24 h immersion in water (Gontard, Duchez, Cuq, & Guilbert, 1994). Film specimens were kept in a desiccator containing dry calcium sulphate (CaSO₄) till they reached constant weight. Afterwards, about 500 mg of each film were immersed in beakers containing 50 ml of distilled water at 23 °C for 24 h with periodical gentle manual agitation. The films were removed from the water and were placed back in the desiccator until they reached a constant weight to obtain the final dry weight of the film. The percentage of the total soluble matter (%TSM) of the films was calculated using Eq. (3).

$$\%TSM = \left[\frac{(\text{Initial dry weight} - \text{Final dry weight})}{\text{Initial dry weight}} \right] \times 100 \quad (3)$$

2.5.5. Tensile properties

Length of elongation and strain to break (SB) of the films were measured by Kieffer Dough and Gluten Extensibility Rig (A/KIE) with the help of Texture Analyzer (TA-HDPlus, Stable Microsystems, UK). The thin strips (80 mm × 3 mm) were cut from each film and were used to analyze textural properties. The test had a tension mode with following settings. Pre-test speed of 2 mm/s, test speed of 3 mm/s, post test speed of 10 mm/s, distance of 75 mm, trigger force of 10 g was used and the probe was attached to a 5 kg load cell.

2.5.6. Film color

CMC and starch based films were highly transparent, but the addition of alginate in the film forming solution imparted an amber color to the developed films. The color of the developed films was measured using a Hunter Lab colorimeter (Ultrascan VIS, Hunter Lab, Inc., USA) with reflectance mode, CIELab scale (L , a and b), D65 as illuminant and a 10° observer angle as a reference system. The color measurements were expressed in terms of lightness L ($L=0$ for black and $L=100$ for white), and the chromaticity parameters a (green [−] to red [+]) and b (blue [−] to yellow [+]). In addition, the total color change (dE) values were calculated (Eq. (4)) from the Hunter L , a and b scale measured with absorption mode and used to describe the color change during coating of bamboo shoot.

$$dE = \sqrt{(L_0 - L_t)^2 + (a_0 - a_t)^2 + (b_0 - b_t)^2} \quad (4)$$

where L_0 , a_0 , b_0 are the initial color measurements of fresh bamboo shoot cubes and L_t , a_t , b_t are the color measurements of coated shoot with reflectance mode (RSIN), CIELab scale (L^* , a^* and b^*), D65 as illuminant and a 10° observer angle as a reference system.

2.5.7. Film surface characteristics

Surface morphology of the films was observed under a Scanning Electron Microscope (JEOL JSM 6390 LV, Singapore). Dried film samples were sputter coated with platinum and the images were taken at an accelerating voltage of 5 kV and magnification of 1000×.

2.5.8. Infrared spectroscopy

The infra-red spectra for all the films were obtained with a FTIR spectrometer (PerkinElmer, USA). The equipment was operated with scanning range of 4000–450 cm⁻¹ and spectrum of 100. The films were ground to a fine powder and then sample (clear glassy disk) for FTIR analysis were prepared by mixing powdered sample with IR grade KBr using FTIR hand operated press at around 12,000 psi pressure.

2.5.9. Thermal properties

The thermal degradation properties of the films were determined by Thermo Gravimetric Analysis (TGA). Thermo gravimetric measurement was carried out on a Shimadzu TGA-50 thermogravimetric analyzer. Non-isothermal experiments were performed in the temperature range 25–900 °C at heating rates of 10, 20 and 40 °C min⁻¹ on each sample. The average sample size was 10 mg and the nitrogen flow-rate was 30 ml min⁻¹.

2.6. Effect of coating on bamboo shoot quality

2.6.1. Incorporation of antioxidant and antibacterial extracts in film forming solution

The proportions of alginate, starch and CMC of films having best mechanical and barrier properties were chosen for preparing the final basic coating formulation. Antioxidant extract (4 ml/100 ml) were incorporated (Das et al., 2013) and antibacterial extracts was adjusted to 27 mg/ml in the film forming solution during the last 5 min of mixing. Film forming solution was coated on bamboo shoots for prevention of enzymatic browning and control of microbial degeneration.

2.6.2. Coating of bamboo shoot samples

Bamboo shoots were dipped in the coating solutions for 1 min and left to dry at room temperature for 30 min by hanging with constant air flow. Coated shoots were again dipped in the coating solution for another 10 s and dried again to a uniform coating layer. The uniform coating is achieved by turning the side of shoot during second dipping in film forming solution. All the samples were stored at room temperature (30 ± 2 °C) and relative humidity of 64 ± 3% for 5 days.

2.6.3. Effects of coating on weight loss and surface color

The effect of coating on weight loss and surface color of bamboo shoot was tested at 12 h intervals for 5 days. Weight was recorded after removing the coating from shoots and weight loss was measured as the percentage weight loss from the original weight. The surface color of bamboo shoot cubes was measured using a Hunter Lab colorimeter as discussed for film color. The measurements were made in triplicate (three cubes per treatment taken from different trays) and each sample was scanned at four different regions of the shoot.

Table 1
Properties of film prepared from different compositions of alginate, starch and CMC.

Film	Water vapor permeability (g/Pa h m)	Moisture absorption (%)	Solubility in water (%)	Breakage strength (g)	Elongation capacity (mm)	Color value		
						<i>L</i>	<i>a</i>	<i>b</i>
F ₁ (2:0)	4.01 × 10 ⁻⁹ ^a	18.29 ± 0.82 ^a	89.38 ± 2.65 ^a	273.2 ± 11.23 ^a	49.43 ± 2.45 ^a	63.16 ± 1.34 ^a	6.66 ± 0.31 ^a	11.59 ± 0.37 ^a
F ₂ (2:1)	3.13 × 10 ⁻⁹ ^b	15.67 ± 1.05 ^b	55.21 ± 1.57 ^b	411.5 ± 15.68 ^b	31.55 ± 1.73 ^b	67.19 ± 2.36 ^b	6.29 ± 0.30 ^{ab}	10.02 ± 0.25 ^b
F ₃ (1:1)	2.90 × 10 ⁻⁹ ^c	13.97 ± 1.23 ^c	53.56 ± 1.84 ^b	488.9 ± 9.42 ^c	22.23 ± 1.02 ^c	74.61 ± 1.39 ^c	6.22 ± 0.26 ^b	9.85 ± 0.13 ^{bc}
F ₄ (1:1.5)	1.26 × 10 ⁻⁹ ^d	11.64 ± 0.68 ^d	52.16 ± 1.65 ^{bd}	856.5 ± 10.59 ^d	21.02 ± 1.32 ^c	81.67 ± 2.67 ^d	5.74 ± 0.18 ^c	9.40 ± 0.26 ^c
F ₅ (1:2)	1.21 × 10 ⁻⁹ ^d	9.37 ± 1.12 ^e	40.00 ± 2.45 ^c	977.3 ± 17.42 ^e	20.76 ± 1.14 ^c	85.47 ± 1.73 ^e	4.39 ± 0.38 ^d	7.60 ± 0.63 ^d
F ₆ (0:2)	4.06 × 10 ⁻⁹ ^a	17.43 ± 0.45 ^a	50.91 ± 1.23 ^d	208.4 ± 12.42 ^f	14.62 ± 1.08 ^d	90.36 ± 2.69 ^f	1.66 ± 0.10 ^e	2.59 ± 0.12 ^e

All data are the mean ± SD of three replicates. Mean followed by different letters in the same column differs significantly ($P \leq 0.05$). Different letters in superscripts along the column represent the significance difference between the values.

2.6.4. Effects of coating on surface microbial count

Surface microbial count of the uncoated (control) and coated (coating was removed at the time of analysis) bamboo shoots were observed for a period of 5 days after every 24 h. Both coated and uncoated bamboo shoot pieces (1 cm × 1 cm × 1 cm) were immersed in 90 ml sterile peptone water and vortexed for 2 min in a vortex shaker. Precisely 100 ml of this peptone water was taken and spread on plate count agar media and incubated for 36 h. Visible colonies were counted and cfu/cm² was calculated.

2.7. Statistical analysis

All the analyses were performed taking three replicates and data were reported as mean ± SD. Single factor ANOVA was used to determine the critical difference of means, and variance among the different samples were checked at significance level $P \leq 0.05$ (Steel & Torrie, 1980).

3. Results and discussion

3.1. Effect of different combinations of alginate, starch and CMC on film properties

3.1.1. Film thickness and water vapor permeability

The thickness of the prepared films and the coatings formed around the bamboo shoots were 0.43 ± 0.08 mm and 0.15 ± 0.02 mm respectively. The water vapor permeability (WVP) values of the obtained films are shown in Table 1. The WVP value of pure alginate film (F₁) was 4.01 × 10⁻⁹ g/Pa h m, which reduced to 1.21 × 10⁻⁹ g/Pa h m for F₆ film. The addition of starch in alginate caused the significant decrease in WVP values ($P \leq 0.05$), resulting in better film resistance to water vapor transmission. However, pure starch-CMC (F₆) film had WVP of 4.06 × 10⁻⁹ g/Pa h m, which is slightly higher than the alginate film (F₁). It might be attributed to gelatinized starch with α-(1 → 6) glycosidic linkages, which resulted in much tightened structures, resulting in limited mobility even after plasticizing. The tightened structures could possibly have offered greater resistance to mass transfer.

3.1.2. Moisture absorption and water solubility

The moisture absorption and water solubility patterns of film are shown in Table 1. The films F₁ and F₆ evinced the highest moisture absorption of 18.29% and 17.43% respectively and there is no significant difference in water absorption of both films. However, moisture absorption of alginate films decreased significantly with an increase in starch-CMC content ($P \leq 0.05$). The F₁ film had moisture absorption of 18.29% which reduced to 9.37% for F₅ film. The results are in line with the finding of Ghanbarzadeh et al. (2010) for starch/CMC blend film.

The water solubility of alginate film (F₁) was 89.38%, which was more compared to the starch-CMC blend film (F₆). It was also observed that, as the concentration of starch in the blend increased

there was a marked decreased in the solubility of the films in water (Table 1). This is contrary to the findings of Rachtanapun and Tongdeesoontorn (2009), who reported that starch based films are highly hydrophilic in nature. In the present study the decrease in solubility could be due to a more closed matrix, owing to addition of alginate and CMC, making the blend film less accessible to water. This result is comparable with the decrease in WVP with concomitant increase in the concentration of starch in the blend.

3.1.3. Tensile properties

Breakage strength and elongation of the film under stress conditions could be used to describe how the tensile properties of the film are related to the chemical structure. In this study, the films capacity for stretching could be described by elongation capacity, and breakage strength. It represents the film resistance to elongation and the amount of load it can handle. The end use handling properties and mechanical performance of the films are governed by these parameters. The breakage strength of film increased significantly ($P \leq 0.05$) with an increase in the amount of starch in the film composition and recorded the highest for F₅ film and the lowest in F₁ and F₆ films.

However, increase in starch in the film composition produced an inverse effect on the film elongation capacity. This effect suggested that an increase in starch content resulted in films with more load capacity and stiffness and leading to a decrease in elongation capacity (Table 1). This could be attributed to the presence of α-(1 → 6) glycosidic linkages. Films produced solely from starch-CMC had significantly lower tensile properties compared to the other blend films and alginate stand-alone film. Therefore, additions of CMC in the blend films did not have profound effect on the mechanical properties as a whole. The breakage strength and elongation capacity for pure starch-CMC (F₆), pure alginate (F₁) and alginate-starch-CMC (F₅) film were reported as 208.4 g and 14.62 mm, 273.2 g and 49.43 mm and 977.3 g and 20.76 mm respectively.

3.1.4. Film color

The six different films produced varied in their color, mostly due to the addition of alginate in the blend. Alginate left an amber color in the films. Hunter color data revealed that starch-CMC film (F₆) had the highest *L* value (90.36) and lowest for the F₁ film (Table 1). The *L* value of F₁ film was 63.16, which increased significantly to 85.47 for F₅ film after addition of starch in alginate. The concentration of alginate in the final films was responsible for the difference in the color of the films. Redness (*a* value) and yellowness (*b* value) value of films decreased with concomitant increase in the starch content and it was least for the pure starch-CMC film.

3.1.5. Film surface characteristics

The scanning electron micrographs of outer surface for different film specimens are shown in Fig. 1. Overall, the surface

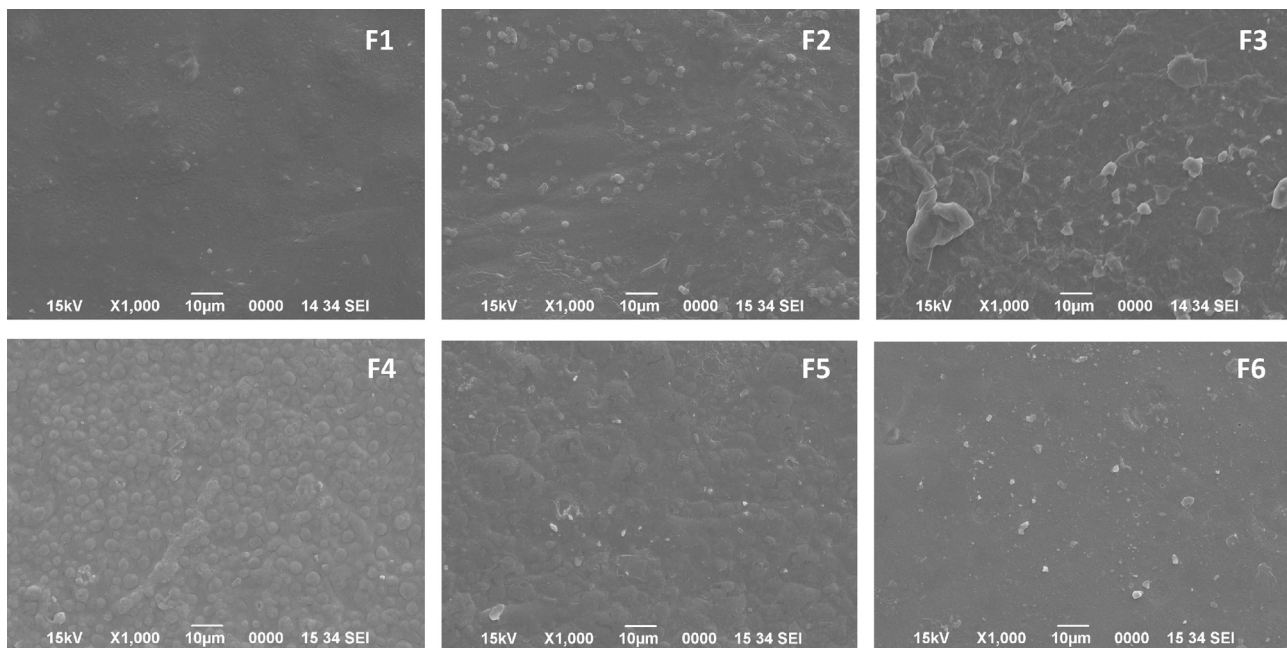


Fig. 1. Scanning electron micrographs of blend films.

demographics for all films showed uniform structures without cracks, pores or major disturbances. The micrograph for the six films was homogeneous and there were no signs of phase separation between the components. This indicated that the three polymers are physically compatible with each other. The film F_3 showed irregularity with large crystal like particles on it; however some granules particles did appear in the F_2 and F_3 films. The crystal like particles in F_3 film might be due to gelatinized starch granules remaining on film after majority of internal starch polymers have been released (Das et al., 2013; Han & Hamaker, 2002).

3.1.6. Infrared spectroscopy of films

The C–H stretching resulted in peaks for all films at around 2900 cm^{-1} as seen in the IR spectral graph (Fig. 2). Wideband was visible around $3400\text{--}3200\text{ cm}^{-1}$ and found in all the films and was caused by N–H stretching, to some extent O–H stretching and intra-intermolecular hydrogen bonds. The blend films exhibited

peaks at around 1600 and 1400 cm^{-1} may be due to symmetric and asymmetric vibrations for COO^- group (Biswal & Singh, 2004; Günzler & Gremlich, 2002; Xiao, Li, Song, Peng, & Hu, 2000). Change in concentration of starch content in the films however, resulted in a tiny gradual shift in the peaks toward a higher IR range. This suggests that symmetric and asymmetric vibrations of the C–O and C=O increased. When starch was added to the blend, the disarray in the intermolecular hydrogen bonds that were present between the carboxylic groups might have caused the above shift. In F_3 film prominent peaks were observed in the range of 1150 cm^{-1} , this peak might be originated from glycosidic linkages in polysaccharides and these bands were assigned to antisymmetric α -(1 $\rightarrow$ 4) stretching mode of glycosidic linkages (Nikonenko, Buslov, Sushko, & Zhabankov, 2000; Kacurakova, Capek, Sasinkova, Wellner, & Ebringerova, 2000; Sekkal, Dincq, Legrand, & Huvenne, 1995). However, these peaks were found in other film but they were less prominent. Overall there are

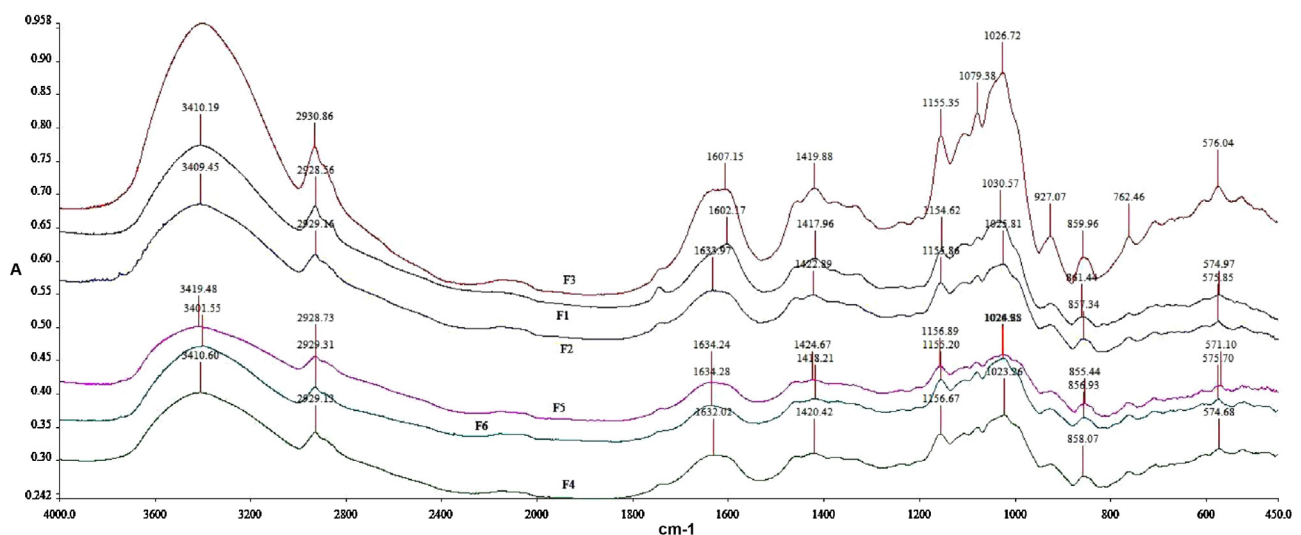


Fig. 2. FTIR spectra of films and individual polymers.

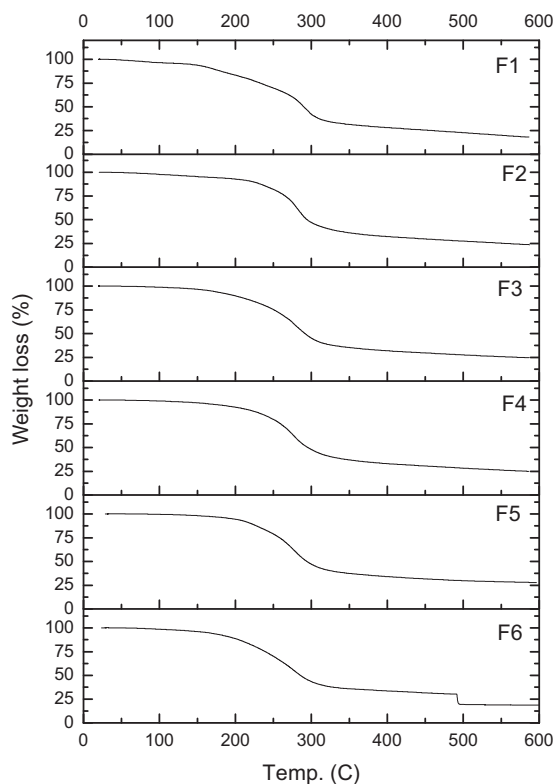


Fig. 3. Thermo gravimetric analysis curves of blended films.

less changes were observed in FTIR spectra of all the prepared film.

3.1.7. Thermal stability assessment by TGA

The curves of thermo gravimetric analysis for the films with the varying concentrations are shown in Fig. 3. A gradual loss of weight was observed in all films till about 200 °C which could be attributed to loss of free and bound moisture from the films. A relatively low decomposition temperature of about 180 °C was found for F₁ and F₆ films. The films F₂, F₃, F₄ and F₅ evinced similar thermal decomposition behavior above 200 °C, and recorded sharp weight loss in the 250–350 °C region. As the starch content in the films increased, the thermal stability also enhanced. However, CMC content in the blends helped to enhance for thermal stability of film. In case of pure CMC, mostly weight loss was observed in the temperature range of 300–500 °C (Su, Huang, Yuan, Wang, & Li, 2010). It corroborates that the three polymers were greatly compatible with one another.

3.1.8. Standardization of film

The film F₅ with a polymer blend of alginate and starch (1:2) and 15% CMC equivalent to starch (w/w) was finally selected on the basis of better performance than the other blended films. WVP, moisture absorption and water solubility of F₅ film were reported as 1.21×10^{-9} g/Pa h m, 9.37% and 40% respectively and it was ahead of the other films. Tensile properties of F₅ film were acceptable and gave the highest breakage strength of 977.30 g. Colors' value revealed the lightness of film, which is acceptable for coating purposes. Surface of F₅ film was smooth and uniform, without cracks, pores or major disturbances. Thermal stability of this film was more and decomposition behavior started above 200 °C. Considering the suitability of film with respect to different film properties, same film forming solution of F₅ film was selected for coating of bamboo shoot.

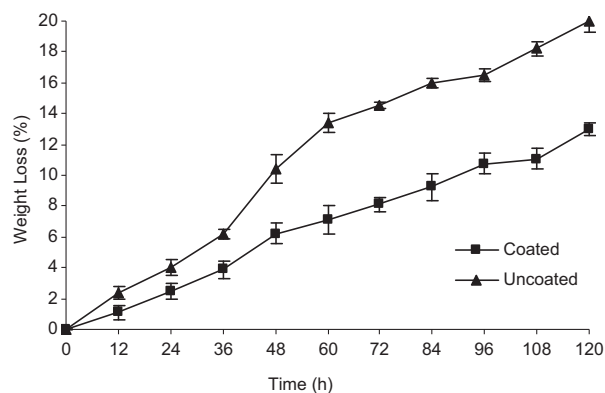


Fig. 4. Weight loss in coated and uncoated bamboo shoot during storage.

3.2. Effect of coating on bamboo shoot quality

Film forming solution of selected film (F₅) was incorporated with the antioxidant and antimicrobial extract and same was used for coating of bamboo shoots. The effect of coating on bamboo shoot was studied as discussed below.

3.2.1. Weight loss

The percentage of weight loss in bamboo shoots as a function of time, with and without the coating is shown in Fig. 4. Weight loss of around 20% was reported in uncoated sample and around 13% in case of coated sample at the end of 5th day. Coating of shoot reduces the weight loss by 7%; this may be attributed to low WVP of film, which restricted the surface moisture loss from the bamboo shoot. Reduction of weight loss during storage by application of starch coating on tomatoes was reported by Das et al. (2013) which correlates the present findings.

3.2.2. Surface color

The change in color and *L* values of coated and uncoated bamboo shoot as a function of time, are given in Fig. 5. The lightness values decreased significantly for all coated and uncoated samples during storage. Control samples presented the lowest *L* values at the end of storage. Despite the addition of antioxidant extract on the film, the coated samples showed a gradual decrease in the *L* values after the 36th hour. However, it recorded better results than the uncoated samples. The *dE* value of uncoated shoot increased exponentially with time; however, in case of coated shoot very less change were observed up to 48th day and afterwards the value increased slowly. It has been reported that polysaccharides have good gas barrier properties, and thereby help in decreasing the respiration rate and eventually limiting metabolic activities.

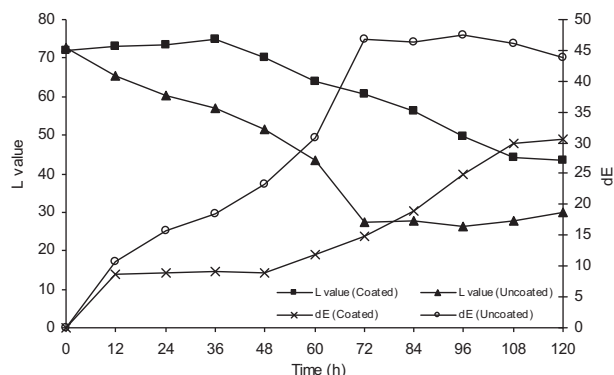


Fig. 5. Effects of coating on surface color of bamboo shoot.

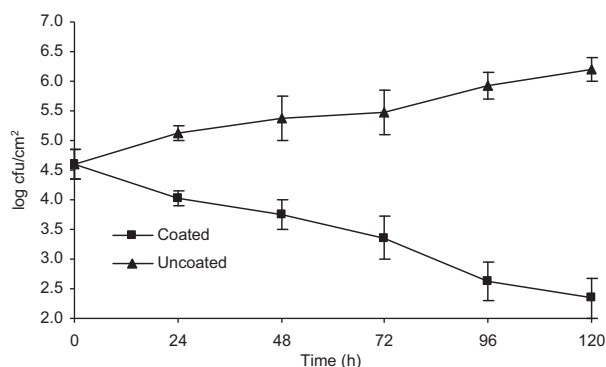


Fig. 6. Effects of coating on surface microbial count of bamboo shoots.

This helps to delay enzymatic browning. The use of alginate, other polysaccharides and antioxidants help to limit or delay browning of fruits; however, sometime the amber color of the alginate solution imparts lower *L* value of fruits. Results of present study are in line with finding of several research works (Fontes, Sarmento, Spoto, & Dias, 2008; Lee et al., 2003; Olivas et al., 2007).

3.2.3. Surface microbial count

The microbial count increased in the uncoated samples during storage from 4.60 to 6.20 log cfu/cm² (Fig. 6). However, in the coated samples the surface microbial load dropped significantly ($P \leq 0.05$) from 4.64 to 2.34 log cfu/cm². This might be due to addition of the antimicrobial agent in film forming solution, as this antimicrobial agent earlier tested proved effective against *E. coli*, *S. aureus* and *B. cereus*. An antimicrobial agent incorporated film declined the microbial growth and kept the surface microflora in control. Polysaccharide films also provide good barrier against entry of microbes through the film (Das et al., 2013). The microbial barrier properties of the F₅ film contributed to decline in the surface microbial load of coated bamboo shoots might be due to use of polysaccharides and addition of antimicrobial metabolites.

4. Conclusions

Alginate, starch, and CMC were successfully employed to fabricate the edible film. The F₅ film prepared from alginate and starch (1:2) blend with added CMC (15% of starch) evinced lower water vapor permeability, moisture absorption, water solubility and elongation capacity; however, breakage strength were reported high with less yellowness and lighter film. Surface characteristics showed the uniformity of film as well as more thermal stability. Addition of starch and CMC imparted an important influence on the barrier and mechanical properties of the resulted composite films. Coating of bamboo shoot resulted in less water loss, lowered the color change (dE) value and reduction in surface microbial count, which could be attributed to incorporation of antioxidant and antibacterial extracts in film forming solution.

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