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Antimicrobial compound incorporated corn starch based self-supporting film and its properties

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Abstract

The issue of environmental pollution arising from disposable synthetic packaging films and the lack of petroleum resources demand development of alternatives from renewable natural biopolymers such as starch. In the present work, the effect of benzoic acid (BA), potassium sorbate (PS), and sodium propionate (SP) as antimicrobials on ultimate tensile strength (TS), elongation at break (%El), water vapour permeability (WVP) and optical properties of films developed from a mixture (100 mL) containing 7.50 g corn starch, 0.875 g polyvinyl alcohol and 0.125 g glutaraldehyde was evaluated. Antimicrobials varied within 0-1.791 g/100 g total polymers modified the properties of film containing no antimicrobial. At 1.791 g/100 g polymer, BA and PS decreased TS of control film by 24.01% and 26.84%, while SP indicated 12.35% increase. Simultaneously, BA decreased El by 82.82%, while PS and SP increased by 12.73% and 167.52%. All the three raised WVP, while SP decreased yellowness index, with minimum deterioration in opacity. SP at this level was, therefore, recommended as the best suitable antimicrobial, and the developed film coupled with high WVP could be useful to extend the storage life of fresh fruits and vegetables.

Introduction

Corn starch (CS), being widely available in high purity at low cost, renewable and easily biodegradable, is promising raw material for the development of environment-friendly packaging materials (Tharanathan, 2003). However, due to poor mechanical and water vapour barrier properties, attempts have been made to improve the properties of neat starch films by blending with synthetic polymers, most commonly linear polymer polyvinyl alcohol (PVA) (Sin *et al.*, 2010; Tang and Alavi, 2011) and crosslinking agent, usually glutaraldehyde (GA) (Parra *et al.*, 2004; Sreedhar, 2006; Al-Hassan *et al.*, 2012; Makishi *et al.*, 2013; Priya *et al.*, 2014). PVA increases the flexibility, while GA reinforces the matrix and helps to reduce the hydrophilicity that arises from the hydroxyl groups of PVA as well as starch (Liu *et al.*, 2012). However in most of the works, the PVA was kept higher than or equal to the amount of starch (Siddaramaiah, 2004; Imam *et al.*, 2005; Chai *et al.*, 2012), though considering the cost and limitation of petroleum resources, its minimal use is desirable. The authors in their previous work through rotatable central composite design in response surface methodology (Sen and Das, 2017) optimised the composition of a blend containing CS:PVA:GA in a ratio of 7.50:0.875:0.125 by weight (CS:PVA = 8.5:1); the developed film was flexible, self-supporting and had ultimate tensile strength (TS) of 11.66 MPa, elongation at break (%El) of 8.56% and water vapour permeability (WVP) of 0.132 $g.mm.m^2.kPa^{-1}h^{-1}$.

Recently, there is a growing interest for films embedded with antimicrobials to increase the shelf life of packaged foods. Additionally, starch being food materials, the films are also susceptible to airborne microorganisms, therefore requiring incorporation of antimicrobials for the protection of the film itself. Many reports are available on the use of plant-derived antimicrobials in biopolymer based films (Wu *et al.*, 2010; Sanla-Ead *et al.*, 2012; Kuorwel *et al.*, 2014; Cano *et al.*, 2015; Torrieri *et al.*, 2015). Wu *et al.* (2010) prepared starch-PVA films containing catechin of green tea and reported that the inclusion imparted green tinge in film and caused structural

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alterations thus degrading the mechanical properties. Torrieri *et al.* (2015) reported that rosemary and other essential oils caused phase separation in the film matrix thus affecting the structure and physical properties. According to Cano *et al.* (2015), though the incorporation of neem and oregano essential oils in PVA-starch film exhibited antimicrobial properties without a noticeable change in WVP, the mechanical properties, transparency, and gloss were affected, particularly after storage. Besides, the smell of the essential oil may also restrict its general use due to the alteration of packed food natural aroma. This problem can be solved using synthetic chemicals such as weak organic acids and their salts like benzoic acid (BA) (Manab *et al.*, 2011), potassium sorbate (PS) (Flores *et al.*, 2007; Silva *et al.*, 2013), and sodium propionate (SP) (Valencia-Chamorro *et al.*, 2008), which are among the 'generally recognized as safe (GRAS)' (Code of Federal Regulations Title 21) antimicrobials. Additionally, because of their availability in pure form, precise control over concentration is feasible. These antimicrobials have long been used in polyethylene and cellulose acetate based food packaging films (Soares *et al.*, 2002; Malhotra *et al.*, 2015). However, reports also say that such antimicrobials, depending on their concentration, chemical characteristics and the nature of polymer matrix, can form intermolecular complexes and modify the film properties (Ofman *et al.*, 2004; Chowdhury and Das, 2013; Han, 2013). Fama *et al.* (2005) and Flores *et al.* (2007) concluded that PS at a level of 6 g/100 g starch affected the solubility, colour, crystallinity and mechanical properties of tapioca starch films. Ofman *et al.* (2004) reported that even low levels of ionic compounds affected potato starch gel, while cassava starch remained insensitive. Shen *et al.* (2010) studied the effect of PS up to 15 g/100 g starch on the mechanical properties and WVP of sweet potato starch film. Jipa *et al.* (2012), while studying the effect of PS in bacterial cellulose-PVA film, mentioned that the antimicrobial decreased the transparency of pure PVA film. Chowdhury and Das (2013) observed that among BA, PS and SP, the last one appeared to be the best with minimum deterioration of film developed from CS mixed with hydroxypropylmethylcellulose (HPMC). da Rocha *et al.* (2014) prepared anchovy protein film in which they incorporated sorbic/benzoic acid up to 50 g/100 g protein. However, reports on the addition of PS to CS films, BA and SP to starch films in general, and BA, PS, and SP to films from CS_PVA_GA, in particular, are scanty.

The objective of the present work was therefore to study the effect of BA, PS, and SP on TS, %El, WVP and optical properties of the film developed from the aforementioned optimised CS_PVA_GA blend (Sen and Das, 2017).

Materials and methods

Materials

Commercial grade white free flowing CS (21.7% amylose) was procured from Angel Starch and Chemicals Pvt. Ltd., Tamil Nadu, India. PVA (molecular weight 14,000) was obtained from S.D Fine-Chem. Ltd., Mumbai, India. GA (25%), glycerol (GLY) (87% GR), BA and PS were supplied by Merck Specialities Pvt. Ltd., Mumbai, India. SP was supplied by Loba Chemie Pvt. Ltd., Mumbai, India. All chemicals were of Analar/Extra pure grade. Glass distilled water was used for dispersion of ingredients.

Preparation of film

Blend (100 mL) containing the previously optimised amount of CS, PVA and GA at 7.50 g, 0.875 g and 0.125 g, respectively (Sen and Das, 2017), was used for making film in the present work. The PVA was soaked overnight at room temperature in 30 mL water, followed by heating in the water bath to obtain a clear solution. Glycerol (plasticiser, 2.46 g/100 mL blend) and prescribed amount of CS and GA were added to this solution. Antimicrobial agents (BA, PS, and SP at the levels of 0.02, 0.04, 0.08, 0.10 and 0.15 g/100 mL blend) were separately added to this solution followed by making the volume up to 100 mL with water. The levels of antimicrobials corresponded to 0.238, 0.477, 0.955, 1.194 and 1.791 g/100 g total polymer comprising CS and PVA. The ingredients were mixed by swirling and kept undisturbed for 3 h to initiate interaction between the ingredients. The mixture was then gelatinised by heating in boiling water bath for about 10-12 min, and hot gelatinised mass was cast (2 mm thickness) on a polypropylene plate using thin layer chromatography applicator. The cast film was dried in an incubator maintained at 40±1°C for 24 h. The dried film was peeled off, kept over activated charcoal overnight to remove any residual GA content, and stored at ambient condition over a period of 90 d. The variable composition of the films was coded as CS_PVA_GA_BA(0.238-1.791), CS_PVA_GA_PS(0.238-1.791) and CS_PVA_GA_SP(0.238-1.791) for the whole range of antimicrobial agent incorporation, respectively, for BA, PS and SP; for individual cases, it was represented similarly mentioning the particular concentration in parenthesis. Control film, CS_PVA_GA(Control), referred to the film prepared similarly but without the addition of any antimicrobial agent.

For each composition, 25-30 films were prepared.

Measurement of properties

Following 90 d of storage, films were randomly picked up from a lot and cut manually into pieces of specific shape. For tensile strength and elongation, pieces were rectangular (100 mm × 10 mm); dimension was ensured by cutting the film both vertically (along the direction of casting) and horizontally (perpendicular to the direction of casting), at five replications each. For WVP and optical properties, the film was cut in the form of circular disc. For evaluation of mechanical and barrier properties, thickness of the cut samples was a necessary input, and the same was measured at least at 5 random positions by a hand dial thickness gauge (model 7301, Mitutoyo Co., Tokio, Japan; with an accuracy of 0.01 mm) to find the average thickness. Irrespective of properties, prior to measurement, the cut samples were conditioned at 50% relative humidity (RH) at 25 ± 1ºC for at least 48 h (ASTM D618).

Tensile strength and elongation at break

The ultimate tensile strength (TS) and elongation at break (%El) were measured according to ASTM methodology (ASTM D882-97) using Universal Testing Machine (INSTRON 5965 U 2597, USA). The sample was stretched at a constant rate of 10 mm/min and the stress-strain data recorded for the whole profile of pulling. The TS was noted as the peak stress that material could sustain before breakage. Strain, i.e., elongation at break (%El) represented the percentage change in length, due to pulling apart, experienced by the film just before breakage.

Water vapour permeability

The water vapour permeability (WVP) was determined by gravimetric method (ASTM E96) using a perspex cup. About 50% volume of the cup was filled with distilled water. The test sample was placed at the mouth of the cup covering its aperture. The cup was then placed over the saturated solution of magnesium nitrate placed in a desiccator to maintain at 50% RH (Wexler and Hasegawa, 1954; Chowdhury and Das, 2010). The desiccator was kept at 25ºC in an incubator. Thus, a fixed RH gradient 100%/50% was maintained across the film surface. Initial weight of the cup (W_1, g) and the final weight (W_2, g) after 24 h, were recorded, and the WVP was calculated using Equation 1.

$$\mathrm{WVP}\ (\mathrm{g.mm/kPa.m^2.h}) = \frac{(W_1-W_2)h}{(P_{A1}-P_{A2})A_1T_1} \quad (1)$$

where h = film thickness (mm), P_{A1} and P_{A2} = partial pressure (kPa) of water vapour at 100% RH and 50% RH, respectively, A_1 = area of the exposed film (m^2). and T_1 = time (h) during which the change in weight had taken place. The measurement was done in three replications for each film composition.

Optical properties

The colour parameters L, a and b and other optical properties namely whiteness index (WI), an estimate of the degree of whiteness; yellowness index (YI), an estimate of yellowness usually arising from product degradation by light, chemical changes etc.; and brightness index (Z%), an estimate of brightness of a colour, that is the amount of light reflection from the film surface were measured, all in three replications, in a Hunter Lab Colour Flex^{R}EZ (Reston, Virginia, United States) using D65 illuminant and 10° observer angle. The film sample was rested on a white background such that the exposed area was sufficiently greater relative to the illuminated area to avoid any light trapping effect.

The total colour difference (ΔE) of the film with respect to the control film (reference) was calculated using Equation 2.

$$\Delta E = \sqrt{(L^c-L)^2+(a^c-a)^2+(b^c-b)^2} \quad (2)$$

where L^c, a^c, and b^c = mean values of the control film, and L, a, and b = mean values of the tested film.

To measure the opacity of film, an indication of cloudiness or transparency, the sample was placed backed by, consecutively, white plate and black plate as per the manual of the instrument (Chowdhury and Das, 2013).

To compare with a commercially available synthetic plastic film, all the aforementioned optical properties were also measured for transparent film used in making zip lock pouch.

Statistical analysis

The number of replications has been mentioned in respective cases. For each replication, separate film was used by randomly taking from a lot. The mean value of different replications and corresponding standard deviation (SD) were evaluated. The treatment effect of the concentration of antimicrobials on film properties was determined by analysis of variance (ANOVA) for single factor experiment with completely randomised design

Table 1. Effect of antimicrobials on mechanical and water vapour barrier properties of CS_ PVA_GA film.

Antimicrobial, (g/100 g polymer)	Properties		
BA	TS (MPa)^{1}	El (%)^{1}	WVP (g.mm/kPa. m^{2}.h)^{2}
0.00	11.66 ± 1.58^{a}	8.56 ± 0.69^{a}	0.132 ± 0.016^{a}
0.238	9.12 ± 0.24^{b}	2.24 ± 0.18^{b}	0.135 ± 0.002^{a}
0.477	8.95 ± 0.15^{b}	2.15 ± 0.20^{b}	0.140 ± 0.002^{ab}
0.955	8.78 ± 0.29^{b}	1.96 ± 0.02^{b}	0.148 ± 0.003^{bc}
1.194	8.71 ± 0.32^{b}	1.91 ± 0.10^{bc}	0.155 ± 0.004^{cd}
1.791	8.74 ± 0.17^{b}	1.47 ± 0.32^{c}	0.158 ± 0.003^{d}
LSD$_{0.01}$	1.335	0.649	0.016
LSD$_{0.05}$	0.932	0.454	0.010
PS	TS (MPa)^{1}	El (%)^{1}	WVP (g.mm/kPa. m^{2}.h)^{2}
0.00	11.66 ± 1.58^{a}	8.56 ± 0.69^{a}	0.132 ± 0.016^{ab}
0.238	10.90 ± 0.55^{a}	8.92 ± 0.23^{a}	0.125 ± 0.002^{a}
0.477	8.74 ± 0.92^{b}	9.45 ± 0.13^{b}	0.129 ± 0.002^{ab}
0.955	8.94 ± 0.71^{b}	9.71 ± 0.46^{b}	0.137 ± 0.002^{b}
1.194	8.43 ± 0.67^{b}	9.75 ± 0.16^{b}	0.138 ± 0.002^{bc}
1.791	8.02 ± 0.62^{b}	9.65 ± 0.18^{b}	0.147 ± 0.001^{c}
LSD$_{0.01}$	1.785	0.722	0.015
LSD$_{0.05}$	1.246	0.504	0.010
SP	TS (MPa)^{1}	El (%)^{1}	WVP (g.mm/kPa. m^{2}.h)^{2}
0.00	11.66 ± 1.58^{a}	8.56 ± 0.69^{a}	0.132 ± 0.016^{a}
0.238	11.91 ± 0.16^{ab}	5.50 ± 1.33^{b}	0.130 ± 0.011^{a}
0.477	12.51 ± 0.82^{ab}	5.99 ± 0.91^{b}	0.137 ± 0.003^{a}
0.955	12.72 ± 0.35^{b}	10.43 ± 0.17^{c}	0.140 ± 0.001^{a}
1.194	13.21 ± 0.36^{b}	15.37 ± 0.69^{d}	0.142 ± 0.004^{a}
1.791	13.38 ± 0.33^{b}	22.90 ± 0.77^{e}	0.160 ± 0.003^{b}
LSD$_{0.01}$	1.052	1.631	0.020
LSD$_{0.05}$	1.506	1.139	0.013

Values are mean ± SD of ^{1}10 replications, ^{2}3 replications. Means with common superscripts denote no significant ($p > 0.05$) difference.

with equal replications. Least significant differences (LSD; confidence level $p < 0.05/0.01$) were also evaluated (Gomez and Gomez, 1984). All the statistical calculations were done using Microsoft Excel (Microsoft Corp., 2010).

Results and discussion

The developed self-supported antimicrobial films (as per Table 1) were flexible, light in weight (density≈ 970-1253 kg/m^{3}) and thin (thickness ≈ 0.1 mm). The following section describes the effect of each independent variable on relevant responses.

Mechanical properties

Irrespective of the vertical or horizontal direction of cutting, TS and %El of the films were almost similar. Hence, the average of all ten replicated values was considered for analysis.

The effect of BA, PS and SP on mechanical properties of films is shown in Table 1. For TS, it was observed that BA at a level of 0.238 g/100 g significantly reduced TS ($p < 0.01$) of the CS_PVA_GA(Control) from 11.66 MPa to 9.12 MPa. Further addition within the limit, however, could not make any significant reduction ($p > 0.05$) and maintained an average value of 8.86 ± 0.17 MPa. Thus, for CS-PVA-GA-BA(0.238-1.791), the decrease in TS was ≈ 24.01% as compared to CS_PVA_GA(Control). In the case of PS also, decreasing tendency in TS was observed. First decrease ($p < 0.01$) from that of CS_PVA_GA(Control) occurred with the addition of 0.477 g/100 g. Following this, with increasing concentration, no significant change was noticed; on average, the value was 8.53 ± 0.40 MPa for CS_ PVA_GA_PS(0.477-1.791), ≈26.84% decrease from CS_PVA_GA(Control). On the contrary, the addition of SP increased TS; the first significant difference from CS_PVA_GA(Control) was noticed only at 0.955 g/100 g. The following higher concentrations did insignificant ($p > 0.05$) contribution. Thus for CS_PVA_GA_SP(0.955-1.791), the average value was 13.10 ± 0.34 MPa, ≈12.35% increase from that of CS_PVA_GA(Control).

In the case of CS-HPMC film where HPMC shared only 4.16 g/100 g of total polymer, BA and PS at the level of 2.66 and 1.40 g/100 g polymer indicated a comparable extent of lowering in TS at 24.18% and 22.13%, respectively (Chowdhury and Das, 2013). Shen *et al.* (2010) also reported a progressive decrease in TS of sweet potato starch film when the addition of PS was over 5 g/100 g of the polymer. While incorporating 6 g PS/100 g starch in tapioca starch film, Flores *et al.* (2007) opined that movement of polymer chains was facilitated due to the plasticising action of sorbate, and as a consequence, TS reduced. Mechanical behaviour of starch films is affected by the presence of crystalline phases, and Fama *et al.* (2005) verified the fact that the tapioca starch films without PS showed a higher degree of crystallinity than that with potassium sorbate. Ofman *et al.* (2004) observed that PS and sodium benzoate (SB) at 2.85 g/100 g tapioca starch interact with starch at water activity 0.5 and above. The decrease in TS by BA and PS in the present work, therefore, may be due to the interaction of these antimicrobials with CS_PVA_GA matrix, which reduced the cohesive force of the film. Probably, the film in the present work was much more sensitive to BA and PS than the CS-HPMC film where the reduction in TS required a higher concentration of these antimicrobials (Chowdhury and Das, 2013). However, commensurate to the behaviour shown by CS_PVA_GA film for addition of higher concentration of SP, TS of CS-HPMC as well as HPMC-lipid films also documented the lack of effect for this antimicrobial agent (Valencia-Chamorro *et al.*, 2008; Chowdhury and Das, 2013).

The effect of incorporation of different antimicrobials on %El is shown in Table 1. Control film indicated elongation of 8.56%. With the incorporation of 0.238 g BA/100 g polymer, El significantly dropped ($p < 0.01$) to 2.24%. With further increase of BA to 1.791 g, El diminished to 1.47%. In the case of PS, El was found to increase, first significant ($p < 0.01$) change occurring at 0.477 g/100 g. For ≥ 0.477 g, i.e., CS_PVA_GA_PS(0.477-1.791), an average value of 9.64 ± 0.13% was maintained. In the case of SP, a different mode was observed. El first significantly decreased ($p < 0.01$) to 5.50% at 0.238 g/100 g polymer. Then for CS_PVA_GA_SP(0.238 - 0.477), insignificant ($p > 0.05$) increase was noted. After this, El started increasing ($p < 0.01$) significantly for each successive increase in concentration, finally reaching to a value of 22.90% for maximum addition. Thus, the change in elongation appeared to be 82.82% decrease, 12.73% increase and 167.52% increase for CS_PVA_GA_BA(1.791), CS_PVA_GA_PS(1.791) and CS_PVA_GA_SP(1.791), respectively.

Structurally, PS and SP being low molecular weight straight chain compounds may easily penetrate and accommodate within PVA and starch chains, compared to BA which has a benzene ring. Therefore, PS and SP confer more flexibility to the chains thereby recording higher elongation of the films as compared to that of the control film (Cagri *et al.*, 2001). The inclusion of benzene ring, on the other hand, disrupts the continuity of the matrix to withstand sliding of the chains during pulling, ultimately decreasing the %El. However, several publications report observations similar and opposite to this interpretation. No change in elongation due to the addition of BA in CS-HPMC film, while 17.57% and 55.48% increase from control were observed at 2.66 g SP and ≥ 1.40 g PS per 100 g polymer, respectively (Chowdhury and Das, 2013). Valencia-Chamorro *et al.* (2008) reported brittle character of HPMC-lipid film due to the inclusion of sodium benzoate. Fama *et al.* (2005) reported significantly higher elongation of PS-containing tapioca starch film than that with no PS film. Cagri *et al.* (2001) reported that sorbic acid added at 15 g/100 g protein increased %El of wheat protein film from 6.37 (no sorbic acid film) to 73.01, ≈1,046% increase. Conversely, continuous decrease in El of sweet potato starch film up to incorporation of 15 g PS/100 g starch was also documented (Shen *et al.*, 2010).

Interestingly, it is clear that (Table 1) BA simultaneously decreased TS and El, whereas SP simultaneously increased the same. This may be attributed to the interpenetration and entanglement of the polymer chains formed by the strong hydrogen bond and network (Cao *et al.*, 2006). This type of simultaneous change in different polymer based films has also been reported by several other researchers (Siddaramaiah *et al.*, 2004; Larotonda *et al.*, 2004; Shen *et al.*, 2010; Malkappa and Jana, 2013).

Water vapour permeability

The effect of incorporation of different antimicrobials on WVP is shown in Table 1. All the three antimicrobials accelerated water vapour permeation. The concentration (g/100 g polymer) responsible for the first significant increase was found to be 0.955 for BA, and 1.791 for both PS and SP. Thus, the film CS_PVA_GA_BA(0.955), CS_PVA_GA_PS(1.791) and CS_PVA_GA_SP(1.791), respectively, exhibited WVP (g.mm/kPa.m^2.h) of

Table 2: Effect of antimicrobials on optical properties of CS_ PVA_GA film

Antimicrobial, (g/100 g polymer)	Optical properties							
BA	L	a	b	ΔE	WI	YI	Z%	Opacity%
0.00	88.03 ± 0.01^{a}	-1.11 ± 0.01^{ab}	6.45 ± 0.03^{a}	0.00	50.77 ± 0.01^{a}	9.35 ± 0.01^{a}	74.63 ± 0.02^{a}	3.27 ± 0.01^{a}
0.238	84.33 ± 0.03^{b}	-1.13 ± 0.02^{a}	5.34 ± 0.04^{b}	3.86	50.66 ± 0.04^{b}	9.14 ± 0.02^{b}	74.33 ± 0.02^{b}	3.88 ± 0.02^{b}
0.477	83.83 ± 0.03^{c}	-1.08 ± 0.02^{bc}	5.24 ± 0.02^{c}	4.37	50.94 ± 0.02^{c}	8.93 ± 0.03^{c}	73.65 ± 0.04^{c}	4.15 ± 0.03^{c}
0.955	83.95 ± 0.03^{d}	-1.06 ± 0.02^{dc}	5.63 ± 0.03^{d}	4.16	51.26 ± 0.03^{d}	8.86 ± 0.02^{d}	73.42 ± 0.03^{d}	5.03 ± 0.02^{d}
1.194	84.06 ± 0.02^{e}	-0.96 ± 0.03^{e}	5.54 ± 0.02^{e}	4.07	51.84 ± 0.03^{e}	8.86 ± 0.03^{d}	73.25 ± 0.04^{e}	6.04 ± 0.02^{e}
1.791	83.93 ± 0.03^{d}	-1.04 ± 0.03^{d}	4.95 ± 0.04^{f}	4.37	52.24 ± 0.03^{f}	8.64 ± 0.04^{e}	72.92 ± 0.02^{f}	6.28 ± 0.03^{f}
$LSD_{0.01}$	0.054	0.048	0.060		0.060	0.054	0.060	0.047
$LSD_{0.05}$	0.036	0.032	0.040		0.040	0.036	0.040	0.031
PS	L	a	b	ΔE	WI	YI	Z%	Opacity%
0.00	88.03 ± 0.01^{a}	-1.11 ± 0.01^{a}	6.45 ± 0.03^{a}	0.00	50.77 ± 0.01^{a}	9.35 ± 0.01^{a}	74.63 ± 0.02^{a}	3.27 ± 0.01^{a}
0.238	83.96 ± 0.02^{b}	-1.01 ± 0.01^{b}	5.45 ± 0.04^{b}	4.19	51.04 ± 0.02^{b}	9.56 ± 0.04^{b}	73.63 ± 0.02^{b}	4.73 ± 0.02^{b}
0.477	84.86 ± 0.02^{c}	-1.12 ± 0.01^{a}	5.74 ± 0.01^{c}	3.25	51.42 ± 0.02^{c}	9.76 ± 0.01^{c}	73.46 ± 0.03^{c}	5.29 ± 0.01^{c}
0.955	83.22 ± 0.02^{d}	-1.08 ± 0.03^{c}	5.46 ± 0.03^{b}	4.90	50.65 ± 0.02^{d}	10.04 ± 0.03^{d}	73.20 ± 0.03^{d}	6.94 ± 0.02^{d}
1.194	83.57 ± 0.03^{e}	-1.13 ± 0.01^{a}	5.56 ± 0.02^{d}	4.55	49.74 ± 0.02^{e}	10.36 ± 0.02^{e}	72.86 ± 0.02^{e}	7.12 ± 0.01^{e}
1.791	83.96 ± 0.02^{b}	-1.18 ± 0.02^{d}	5.04 ± 0.03^{e}	4.30	49.56 ± 0.03^{f}	10.65 ± 0.02^{f}	72.16 ± 0.02^{f}	7.73 ± 0.04^{f}
LSD0.01	0.039	0.036	0.056		0.039	0.047	0.046	0.044
LSD0.05	0.026	0.024	0.037		0.026	0.031	0.030	0.029
SP	L	a	b	ΔE	WI	YI	Z%	Opacity%
0.00	88.03 ± 0.01^{a}	-1.11 ± 0.01^{a}	6.45 ± 0.03^{a}	0.00	50.77 ± 0.01^{a}	9.35 ± 0.01^{a}	74.63 ± 0.02^{a}	3.27 ± 0.01^{a}
0.238	84.76 ± 0.01^{b}	-1.12 ± 0.01^{ab}	4.92 ± 0.01^{b}	3.61	50.94 ± 0.04^{b}	9.22 ± 0.02^{b}	74.62 ± 0.03^{a}	4.36 ± 0.01^{b}
0.477	84.16 ± 0.02^{c}	-1.14 ± 0.02^{bc}	4.94 ± 0.01^{b}	4.15	51.33 ± 0.02^{c}	9.25 ± 0.02^{b}	74.56 ± 0.02^{b}	4.47 ± 0.02^{c}
0.955	83.33 ± 0.02^{d}	-1.22 ± 0.02^{d}	5.05 ± 0.03^{c}	4.91	51.53 ± 0.02^{d}	9.14 ± 0.04^{c}	74.16 ± 0.02^{c}	4.92 ± 0.02^{d}
1.194	83.92 ± 0.02^{e}	-1.26 ± 0.01^{e}	6.26 ± 0.04^{d}	4.12	51.82 ± 0.02^{e}	9.07 ± 0.03^{d}	73.75 ± 0.02^{d}	5.02 ± 0.04^{e}
1.791	84.82 ± 0.02^{f}	-1.29 ± 0.02^{f}	5.82 ± 0.01^{e}	3.27	52.05 ± 0.02^{f}	8.94 ± 0.01^{e}	73.60 ± 0.00^{e}	5.35 ± 0.03^{f}
$LSD_{0.01}$	0.032	0.033	0.054		0.044	0.052	0.037	0.050
$LSD_{0.05}$	0.021	0.022	0.036		0.030	0.034	0.025	0.033

Values are mean ± standard deviation of three replications. Means with common superscripts denote no significant ($p > 0.05$) difference.

0.148, 0.147, and 0.160. The increase of BA to still higher level, i.e., 1.791 g further accentuated the WVP to 0.158 g.mm/kPa.m^2.h. Thus, at the same maximum concentration, the increase WVP was 19.69%, 11.36%, and 21.21% respectively for CS_PVA_GA_BA(1.791), CS_PVA_GA_PS(1.791), and CS_PVA_GA_SP(1.791). Such effect could be due to the disruption of the CS_PVA_GA matrix structure by the incorporation of small molecules of antimicrobials (Chowdhury and Das, 2013) in between the polymer chains, thereby loosening the compactness, as well as the polarity of the antimicrobials, thus helping to attract the water molecules (Cagri *et al.*, 2001).

The increase in WVP due to the addition of varying amount of PS in starch based film has been reported by several researchers. Shen *et al.* (2010) reported that in the case of sweet potato starch film, PS concentration lower than 5 g/100 g starch did not cause any significant change on film's WVP. However, when PS was increased to 15 g/100 g, the WVP increased by five times as compared to no sorbate film. The insensitiveness towards PS in the concentration range within 4-6 g/100 g starch was reported for tapioca starch film (Flores *et al.*, 2007). Chowdhury and Das (2013) observed that PS added at 2.66 g/100 g polymer to CS-HPMC film increased the WVP by 15% as compared to that of no PS film; below this level, WVP was not affected. Vásconez *et al.* (2009), while working with chitosan-tapioca starch (1:2 w/w) film, demonstrated about 2.39 times increment in WVP owing to the addition of 1.66 g PS/100 g polymer. Besides starch, the enhancement of WVP due to the incorporation of PS has also been reported for other biopolymers based films. In the case of whey protein based film at up to 15 g sorbic acid/100 g protein, there was no significant change in WVP; for 20% addition, WVP increased significantly (Cagri *et al.*, 2001). Similar fact has been reported for chitosan film at 15 g PS/100 g chitosan (Pranoto *et al.*, 2005) and for HPMC film at 30 g PS/100 g HPMC (Park *et al.*, 2005).

As mentioned earlier, the information on addition of BA and SP to starch based film is rather limited. Chowdhury and Das (2013) observed that at ≤ 2.66 g/100 g polymer, BA and SP did not affect WVP of CS-HPMC film. While adding SP supplemented with either SB or PS in HPMC-lipid composite coating, Valencia-Chamorro *et al.* (2008) observed that SP+PS exhibited higher WVP than that of SP+SB. However, in this context, it may be noted that starch film with high water absorbing capacity coupled with high WVP may be helpful to maintain the proper condition to extend the storage life and fresh fruits and vegetables, over commercial low WVP packages which allow in-package moisture accumulation which could accelerate microbial and other qualities deterioration (Mahajan *et al.*, 2014).

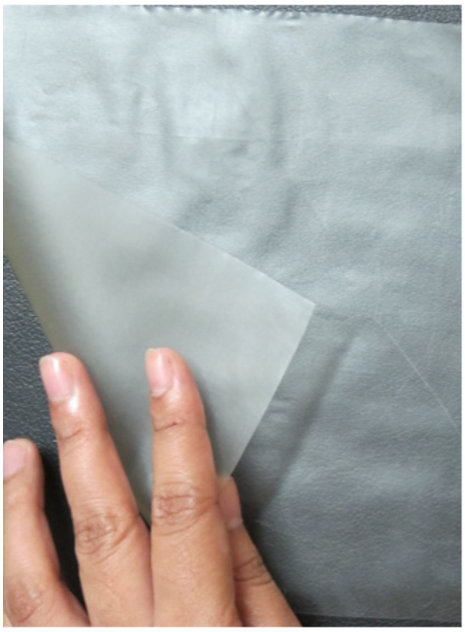

Figure 1. A visual image of starch based self-supporting CS_PVA_GA film with added antimicrobial agent SP at 1.791 g/100 g polymer.

Optical properties

Hunter L, a, and b colour parameters and other optical properties of the films are shown in Table 2. It is clear from the Table that CS_PVA_GA(Control) indicated L, a, and b of 88.03, -1.11 and 6.45, respectively. It may be worth mentioning here that the L, a, and b for commercial zip lock film were 91.09, -0.87 and 1.99, respectively. Thus, both lie in the same green-yellow quadrant of the colour sphere but in the different location. Apparently, the developed films had slight yellowish tinge, probably due to the presence of GA. With the addition of BA, PS, and SP, the L value further decreased from that of the CS_PVA_GA(Control), i.e., some darkness developed, and the first significant change occurring at 0.238 g/100 g polymer in each case. However, for CS_PVA_GA_BA(0.238-1.791), CS_PVA_GA_PS(0.238-1.791) and CS_PVA_GA_SP(0.238-1.791), the L maintained an average value of 84.02 ± 0.19, 83.91 ± 0.61, and 84.19 ± 0.61, respectively, which are comparable to each other. With BA, the first significant change ($p < 0.01$) from control in a value occurred at 0.955 g; for CS_PVA_GA_BA(0.0.955-1.791), an average of 1.02 ± 0.05 was indicated. In the case of PS, up to 1.194 g/100 g, average a value of -1.09 ± 0.05 was maintained; at 1.791 g/100 g, the value decreased to -1.18 ± 0.02. For SP, a value significantly decreased first ($p < 0.05$) at 0.477 g/100 g, following which continuous lowering ($p < 0.01/0.05$)

was observed for each successive addition. Regarding b, the value first significantly ($p < 0.01$) decreased for the addition of 0.238 g of all the antimicrobials. The films CS_PVA_GA_BA(0.238-1.194) and CS_PVA_GA_PS(0.238-1.194) corresponded to average values of 5.43 ± 0.18 and 5.55 ± 0.13, respectively, while an increasing trend was seen for CS_PVA_GA_SP(0.238-1.194). At 1.791 g/100 g, b decreased again for all. Compared to the effect of BA, PS and SP on CS-HPMC film (Chowdhury and Das, 2013), in the present work the values of L were found to be lower whereas, a and b were higher.

Values of ΔE (Equation 2) (Table 2) for CS_PVA_GA_BA(0.238-1.791), CS_PVA_GA_PS(0.238-1.791) and CS_PVA_GA_SP(0.238-1.791) were found within the range of 3.86-4.37, 3.25-4.90, and 3.27-4.91, respectively. In case of CS-HPMC film, ΔE between control and the film containing a variable concentration of antimicrobial (maximum 2.66 g/100 g) has been reported as ≈0.5-1.0 for BA, ≈1.5-3.5 for PS, and ≈1.0-1.5 for SP (Chowdhury and Das, 2013). The higher value of ΔE in the present work, particularly for BA and SP, may indicate that the propensity of colour development was more in CS_PVA_GA matrix, as compared to that of CS-HPMC matrix.

The WI of the CS_PVA_GA(Control) (50.77) (Table 2) usually increased significantly ($p < 0.01$) for each successive addition of BA and SP, ultimately raising the value by 2.89% and 2.52% over the incorporation of 1.791 g/100 g. In the case of PS, up to 0.477 g/100 g, WI increased, however, following that, it consecutively decreased for up to 1.791 g at which the value reduced by 2.38%. It may be worth mentioning that, WI of CS-HPMC film (72.76) and zip lock film (72.85) were much higher. WI of CS-HPMC film, irrespective of the concentration of antimicrobials, decreased by 4.52% for BA and 5.60% for SP; PS exhibited a concentration-dependent lowering to 48.57 at 2.66 g/100 g polymer (Chowdhury and Das, 2013).

The YI of zip lock film was only 0.49. Compared to this, YI in the present work (Table 2) varied within 8.64 to 10.65, indicating much higher yellowish tinge of the films in general. Except for the range CS_PVA_GA_BA(0.955–1.194), and CS_PVA_GA_SP(0.238-0.477), the YI significantly changed ($p < 0.01$) for each successive increment of the antimicrobials. Over the entire range of concentration of BA and SP, YI decreased from CS_PVA_GA(Control) by 7.59% and 4.38%; in the case of PS, on the contrary, the YI increased by 13.90%. About three times increase (from 2.36 to 7.49) has been reported in YI of CS-HPMC control film due to inclusion of 2.66 g PS/100 g polymer (Chowdhury and Das, 2013). However, according to them, YI increased for the addition of BA and SP as well. Interaction of antimicrobials with film ingredients possibly changed the YI, as recommended by Ofman *et al.* (2004) and Valencia-Chamorro *et al.* (2008), Additionally, PS can undergo auto-oxidation forming carbonyl moieties to cause browning in film matrix (Ferrand *et al.*, 2000).

The brightness index (Z%) of CS_PVA_GA(Control) was 74.63 ± 0.02 (Table 2). The value was comparable to that of zip lock film (74.25) and CS-HPMC film (78.07) (Chowdhury and Das, 2013). Z% significantly decreased ($p < 0.01$) for each successive interval of all the three antimicrobials concentration (except for 0 - 0.238 g for SP). Over the entire range, the reduction was 2.29% for BA, 3.30% for PS and 1.38% for SP. Thus, among the three antimicrobials, SP rendered the least reduction. Moreover, except for PS where the reduction over entire concentration was around 5%, the reduction in Z% due to BA and SP were comparable to that exhibited by CS-HPMC film (Chowdhury and Das, 2013).

Transparency increases the aesthetic sense of polymer film for food packaging. From the opacity values in Table 2, the developed films appeared to be more opaque than the zip lock film with opacity of only 1.05. At each level of the antimicrobials, the opacity significantly increased ($p < 0.01$) from the previous one. Over the full range of addition, the opacity of the control film, i.e., 3.27 increased to 6.28, 7.73 and 5.35 for BA, PS, and SP, accounting about 92%, 136% and 64% increase, in that order. The increase in opacity may be due to the development of structural inhomogeneity in the film caused by added antimicrobials (Pritchard, 1964). Chowdhury and Das (2013) also documented change in the opacity of CS-HPMC film, which increased in case of BA and SP incorporation, and decreased for PS. Even for low density polyethylene (LDPE) film, the decrease in transparency due to the incorporation of PS was reported (Han and Floros, 1997). The interaction of antimicrobials thus produces a modification in the polymer network.

Based on the above discussion, it is seen that among the three antimicrobials, SP at 1.791 g/100 g polymer comprising CS and PVA, increased both tensile strength and elongation, decreased YI, increased WI, and rendered minimum deterioration in opacity, as compared to film with no antimicrobial. SP at this level was, therefore, recommended as the best suitable antimicrobial for the incorporation in CS_PVA_GA blend in prescribed composition.

The recommended film, i.e., CS_PVA_GA_SP(1.791) exhibited WVP of 0.160 g.mm/kPa.

Table 3. Mechanical properties of commercially used polymers and CS_PVA_GA_SP(1.791) film formulated in the present work (*Clarinval and Halleux, 2005; Jamshidian et al., 2010; @Matweb, 2015)

Packaging materials	Tensile Strength (MPa)	Young's Modulus (GPa)	Elongation at Break (%)
LDPE (film grade)@	7.85-34.5	0.16-0.442	50-1000
HDPE (film grade)@	29-40	0.02-1.10	400-1700
EVOH@	37-74	1.08-3.65	130-330
PET*	48-72	0.20-4.10	30-300
PS*	34-50	2.30-3.30	1.2-2.5
PCL*	4-28	0.39-0.47	700-1000
PLA*	48-53	3.5	30-240
PHB*	25-40	3.5	5-8
CS_PVA_GA_SP(1.791)	13.38 ± 0.33	0.53 ± 0.21	22.90 ± 0.77

LDPE: low density polyethylene; HDPE: high density polyethylene; EVOH: ethylene vinyl alcohol; PET: polyethylene terephthalate; PS: polystyrene; PCL: polycaprolactone; PLA: polylactic acid; PHB: polyhydroxybutyrate

m^2.h (Table 1). Such high value of WVP could be a positive help for extending the shelf life of fresh fruits/vegetables, and in a separate empirical investigation, the authors established this capability with noteworthy prevention of microbial spoilage over commercially used synthetic packaging film (Sen and Das, 2017). Practically, the film, because of its water absorbing capacity and relatively high WVP as compared to LDPE, minimised the possibility of in-package water accumulation arising from respiration and transpiration. Perhaps, the case specific permeation characteristics in joint action with the embedded antimicrobial extended the shelf life. It may be mentioned that in a separate experiment, the antimicrobial activity of the recommended film was confirmed by a clear zone of inhibition in disc diffusion method against *Staphylococcus aureus*. However, in spite of the presence of SP, the film being mainly made of starch showed over 90% biodegradation within 28 days of soil burial (Sen and Das, 2018), which ensures environmental protection after disposal. Besides these specific advantages, to assess the practical usage, the mechanical properties of the CS_PVA_GA_SP(1.791) have been compared (Table 3) with that of commercially available synthetic and biodegradable packaging material (Song *et al.*, 2011). It was observed that TS of the CS_PVA_GA_SP(1.791) was in the same range with that of LDPE and PCL; LDPE being the most widely used synthetic polymer for making flexible packaging film (Krochta, 2006). Young's modulus (tensile strength/elongation, a measure of flexibility) was also observed to be in the range of HDPE and comparable to the higher limit of LDPE and PCL. The %El, however, was in close proximity to lower limit of PLA and PET, and higher than PS and PHB. The Young's modulus of the CS_PVA_GA_SP(1.791) also exhibited similarity with other biodegradable starch films (Dias *et al.*, 2010; Tapia-Blácido *et al.*, 2011; Andrade-Mahecha *et al.*, 2012). The modulus value and appearance of the film (Figure 1) supported the fact that it possessed sufficient flexibility, and may be targeted for making small pouches where the exhibited elongation suffices the purpose.

Conclusion

Depending on concentration, benzoic acid (BA), potassium sorbate (PS) and sodium propionate (SP) added as antimicrobials influenced TS, El, WVP and optical properties of the developed flexible film from CS_PVA_GA blend. Among the three antimicrobials (BA, PS and SP) at a concentration of 1.791 g/100 g polymer comprising CS and PVA, SP increased both tensile strength and elongation, decreased YI, increased WI, and rendered minimum deterioration in opacity, as compared to control film with no antimicrobial compound. Coupled with WVP on the higher side (0.160 g.mm/kPa.m^2.h), such SP (1.791 g/100 g polymer) incorporated film appears promising to be used for extending shelf life of fresh fruits and vegetables.

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