Journal of Materials and Environmental Sciences
ISSN : 2028-2508
CODEN : JMESCN

J. Mater. Environ. Sci., 2018, Volume 9, Issue 10, Page 2981-2993

http://www.jmaterenvironsci.com

Biopolymer films incorporated with ascorbic Acid: Characterisation, antimicrobial and fruit preservation studies

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Received 15 Jan 2018,
Revised 20 Dec 2018,
Accepted 22 Dec 2018

Keywords
- ✓ Biopolymers,
- ✓ Ascorbic acid,
- ✓ Physicochemical properties,
- ✓ Antimicrobial properties,
- ✓ Food preservation.

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Abstract

In the present study, chitosan (CS), gelatin (GL) and methyl cellulose (MC) film formulations were incorporated with ascorbic acid (AA) at a concentration of 15% (w/w). The addition of AA increased the transparency value of biopolymer films. A pronounced effect was obtained for CS, of which the transparency value was increased from 0.501 to 1.17 A/mm. A significant improvement in antimicrobial property against *Escherichia coli (E. coli)* and *Staphylococcus aureus (S. aureus)* was obtained in the presence of AA. CS-AA, GL-AA and MC-AA films were applied for the first time to preserve cherry tomatoes (*Solanum lycopersicum* var. *cerasiforme*) and grapes (*Vitis vinifera*). Based on a 14-day preservation study, the aforementioned biopolymer-AA films were successfully controlled the weight loss and browning index of both commodities. Overall, results from this study highlight the feasibility of biopolymer-AA films to extend the shelf-life of fruits.

1. Introduction

Petroleum-based materials have been widely used in food preservation. These materials are mechanically and chemically resistant, lightweight, heat-sealable and cheap [1]. However, these materials are not degradable and have caused serious environmental problems. In addition, pollutant from these materials could cause destruction in marine habitat of which it assists the transfusion of the persistent organic pollutants (POPs) that may travel into food chain [2].

Biopolymers are environmental friendly materials which can be degraded naturally. They can be produced from natural sources such as plant and animal. In recent years, scientists have focused on biopolymers as alternatives to petroleum-based materials to preserve food products [3]. Cellulose, chitin, chitosan, gums, gelatin, protein, lipids and starches have been tested for this purpose [4,5]. In spite of their attractive properties and prolific use, biopolymer materials however have several limitations such as high sensitivity to moisture, poor mechanical and antimicrobial properties [5]. Chitosan has low stability and poor water vapour barrier property [6]. On the other hand, gelatin has poor inhibition behaviour against food pathogens [7].

One of the most promising methods to improve the physical and chemical properties of biopolymer film is with addition of additives to film formulations. Synthetic additives are less favourable due their toxic effects which can pose adverse effects to consumer [8]. Natural additives have several active properties such as antioxidant, antimicrobial and antibrowning [8,9] Furthermore, sources of natural additives are abundant in nature.

Ascorbic acid is a natural additive with a weak acid property and soluble in water. It is widely used as an antioxidant agent in food and pharmaceutical products, nutrient supplement and therapy [10]. Pérez *et al*. [11] reported that the addition of ascorbic acid to high methoxyl pectin–methyl cellulose films improved the

performance of the films as antioxidant interfaces for protecting walnut oil. Meanwhile, a study by Robles-Sánchez *et al*. [12] has shown that the addition of ascorbic acid to alginate-based coatings had improved the colour retention and antioxidant property of fresh-cut mangoes.

To date, there are only a few research studies reported the effects of ascorbic acid (AA) on antimicrobial activity of biopolymer films. Furthermore, the application of biopolymer-AA films to preserve food is seldom studied. Therefore, the overall aim of this study was to evaluate the potential of biopolymer films (CS, GL and MC) incorporated with AA to prolong the shelf-life of fruits. This work entailed three main studies, namely (i) characterisation, (ii) antimicrobial, and (iii) preservation.

2. Material and Methods

2.1. Materials

CS with molecular weight of 600,000 Da and deacetylation degree of 85% was purchased from Acros Organics. GL with strength of 210 Bloom was supplied by Nur Halal Gelatine Resources. MC with viscosity of 4000 cP for a 2% solution in water at 20 ºC was obtained from Sigma-Aldrich. L-ascorbic acid was purchased from Fisher Scientific. Glycerol and acetic acid were supplied by Merck. Commercial cling (low-density polyethylene) was purchased from a local supermarket in Tanjong Malim, Perak, Malaysia. All chemicals used in this study were of analytical grade.

2.2. Preparation of film forming dispersions

To prepare CS film forming solution (FFS) (2% w/v), 2 g of CS was dissolved in 100 mL of acetic acid (1% v/v). The mixture was stirred using a magnetic stirrer for 1 h at 60 ºC. GL FFS (3.5% w/v) was prepared by dissolving 3 g of GL in 100 mL of deionised water and left for 1 h at 60 ºC under continuous stirring. For MC FFS (2% w/v), 2 g of MC was slowly added into 100 mL hot deionised water (80 ˚C). The solution was stirred vigorously for 1 h for total dispersion. Glycerol was added into each FFS (0.5 g glycerol per g biopolymer) as plasticiser. AA was then added (15% w/w) into each FFS under continuous stirring for 1 h at room temperature. In this study, control biopolymer films (without AA) were also prepared.

2.3. Preparation of films

Films were prepared by casting method. Each FFS (20 mL) was poured into a petri dish (90 mm diameter). The films were dried in an oven at 35 ºC for 24 h. Dried films were peeled off and stored in seal plastic packaging for protection against the light and humidity. A Senator 6"/150mm Digital Calliper was used to measure the thickness of the films. The measurement was performed at five random positions around the film.

2.4. Fourier transform infrared spectroscopy (FTIR)

The FTIR spectra of the films were recorded using a Thermo Nicolet 6700 FTIR Spectrometer at wavenumber range of 4000–400 cm^{-1} with over 10 cumulative scans and 2 cm^{-1} resolution. The analysis was carried out with the percent of transmittance (% T) mode and at room temperature. The film samples for FTIR analysis were thin enough to ensure that the absorption of IR was within the linearity range of detector and recorded by fixing films onto metallic slit in air medium.

2.5. Light transmission and transparency

Film samples were cut into rectangles (1 cm x 3 cm) and placed in a spectrophotometer test cell. An empty test cell was used as the reference. The percent transmittance (% T) at selected wavelengths between 200 and 800 nm was measured using an Agilent Cary 60 UV-Visible Spectrophotometer. The measurements were performed in three replicates. The following Equation 1 was used to determine the transparency of the films [7]:

$$Transparency = \frac{A_{600}}{x} \quad (1)$$

where, A_{600} is the absorbance at 600 nm, and x is the film thickness (mm).

2.6. Thermogravimetric analysis (TGA)

The thermal stability and decomposition temperature of film samples were assessed using a Perkin-Elmer Pyris 1 Thermal Gravimetric Analyser. The analysis was conducted within temperature range of 30-600 ºC at a

heating rate of 10 ºC per min under a nitrogen atmosphere. The mass of the sample pan was continuously recorded as a function of temperature.

2.7. Scanning electron microscopy (SEM)

Microstructural analysis of the films was carried out by using a Hitachi SU 8020 UHR Scanning Electron Microscope. Film samples were coated with a conductive layer of sputtered platinum. Samples were then viewed at several magnifications at an accelerating voltage of 15 kV to ensure a suitable image resolution.

2.8. Mechanical properties

Tensile strength (TS) and elongation at break (EAB) were determined according to ASTM D822 Standard test (ASTM) using an Instron 5967 Electromechanical Testing System. Film specimens (100 mm × 10 mm) were cut and analysed, taking an average of ten measurements for each film. The initial grip separation and crosshead speed were set at 50 mm and 50 mm/min, respectively. Tensile strength (TS, MPa) was expressed as a ratio of maximum load for breaking the film to initial cross-sectional area of the sample [13]. Meanwhile, percentage of elongation (EAB, %) was expressed as a ratio of the elongation at the break point to the initial length of the sample multiplied by 100 [13].

2.9. Water vapour permeability (WVP)

The water vapour permeability (WVP) of biopolymer films was performed according to ASTM E96-95 (ASTM 1995) procedure using a PERME W3/060 Water Vapour Transmission Rate Test System, which was based on the cup method. The film sample (50 cm^2) was placed on the top of the aluminium cup filled with fused calcium chloride and sealed with a mix of microcrystalline and paraffin waxes, as outlined by Joseph *et al*. [14] The temperature and relative humidity of chamber were set as 38 ºC and 90%, where the cups were placed and weighed every hour for 6 h. The water vapour transmission rate (WVTR) was determined by a slope of linear portion of the weight gained versus time (Equation 2). This corresponds to the diffusion of water vapour through the film, which was measured at a steady state. The WVP were calculated using the following Equation 3 [14].

$$WVTR = \frac{slope}{film\ area} \tag{2}$$

$$WVP = \frac{WVTR \times L}{(P_2 - P_1)} \tag{3}$$

where, P_1 is partial pressure inside the cup (kPa), P_2 is water vapour partial pressure at the film outer surface (kPa), and L is average film thickness (mm). The values of WVP were expressed in g m^{-1} day^{-1} atm^{-1}.

2.10. Oxygen permeability (OP)

The effectiveness oxygen permeability of films was determined volumetrically using permeability cell according to the ASTM D1434 procedure (ASTM 1983). The change in volume of the oxygen permeated (inferred from the displacement of mercury) was plotted as a function of time. The slope of the obtained straight line was derived by using simple linear equation. The oxygen transmission rate (OTR) under standard experimental condition was calculated as follow (Equation 4) [14]:

$$OTR = 34,029 \times \frac{slope}{pressure} \tag{4}$$

where, 34,029 is capillary constant. Oxygen permeability was calculated by multiplying the oxygen transmission rate and thickness of the film and reported in cc m^{-1} day^{-1} atm^{-1}.

2.11. Colour differences

Measurement of colour was determined using a ColorFlex EZ Spectrophotometer. Total difference of colour (ΔE*) was calculated as follow (Equation 5) [15]:

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (5)$$

where, ΔL^*, Δa^* and Δb^* are the differences between the colour parameter of the film samples and the colour parameter of the white standard (L*= 93.45, a*= -0.81 and b*= 0.33). In addition, the L* parameter represents the lightness index scale ranges from 0 (black) to 100 (white). The parameter a* measures the degree of red (+a*) or green (-a*), while the degree of yellow (+b*) or blue (-b*) colour is represented by parameter b* [16].

2.12. Antimicrobial study

The antimicrobial activity of the biopolymer solutions was performed using agar diffusion method outlined by Hosseini *et al*. [17], with some modifications. Two microbial strains, namely *E. coli* and *S. aureus* were used in this study. Sterilised filter paper discs (5 mm diameter) were dipped into each biopolymer solution and applied to the surface of agar plates containing bacteria. The plates were incubated at 37 ºC for 24 h in an incubation chamber. After incubation, the diameter of the inhibition zone (considered as the antimicrobial activity) around the disc was measured with a ruler. Results were expressed as mm of inhibition growth and each determination was performed in quadruplicate**.**

2.13. Preservation study

Preservation study was conducted according to a method suggested by Priya *et al*. [18], with some modifications. Fresh cherry tomatoes and grapes with almost equal size were randomly selected and used in this study. Fruits were washed with deionised water and wiped with a clean towel. The fruits were weighed, wrapped with each biopolymer film (in triplicate), and stored at 21-23 ºC and 75% RH for 14 days. Fruit samples were wrapped with biopolymer films as shown in Figure 1. The weight loss and browning index of the fruits were calculated using the following Equations 6 and 7 [18]. The browning level was classified as: 1 = no browning, 2 = less than 0.25%, 3 = around 0.25 to 0.5%, and 4 = more than 0.5%.

$$Weight\ loss\ (\%) = \frac{(W_i - W_f)}{W_i} \times 100 \quad (6)$$

where, W_i is the initial weight of fruit, and W_f is the final weight of fruit after preservation study.

$$Browning\ index = \sum (Browning\ scale \times percentage\ of\ corresponding\ fruit\ within\ each\ class) \quad (7)$$

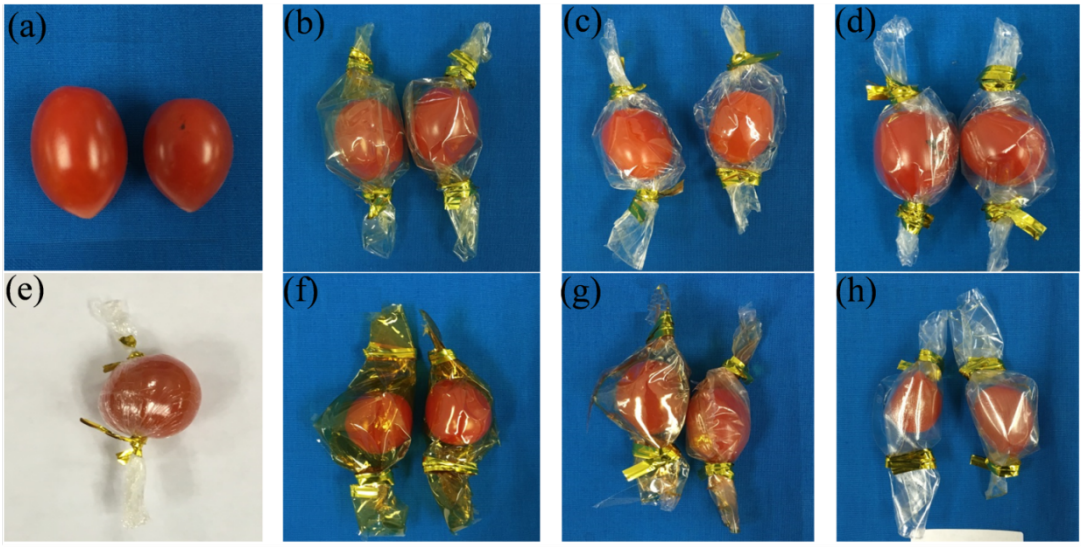

Figure 1: Fruits preservation using (a) control, (b) chitosan film, (c) gelatin film, (d) methylcellulose film, (e) cling film, (f) chitosan-ascorbic acid film, (g) gelatin-ascorbic acid film, and (h) methylcellulose-ascorbic acid film.

2.14. Statistical analysis

Results were expressed as the mean ± standard deviation and were analysed by analysis of variance (one way ANOVA) using Minitab 17 (Minitab Inc., State College, PA). The probability value of $p < 0.05$ was used as the criterion for significant differences.

3. Results and discussion

3.1. FTIR analysis

Based on Figure 2a, absorption bands of AA observed at 3519, 3399 and 3299 cm^{-1} can be assigned to −OH stretching, 1752 cm^{-1} represents C=O stretching and 1649 cm^{-1} corresponds to C=C stretching [19]. FTIR spectrum of CS (Figure 2b) exhibits a broad band at 3159 cm^{-1} which can be related to $–NH_2$ and –OH groups, and a discernible absorption band at 1536 cm^{-1} can be assigned to NH_2 [18]. As shown in Figure 2c, the band of GL at 1622 cm^{-1} corresponds to C=O stretching and the band observed at 1528 cm^{-1} can be related to N−H bending [20]. In the case of MC (Figure 2d), the O−H stretching band was observed at 3381 cm^{-1}, while the C−O−C stretch was appeared at 1048 cm^{-1} [3].

Following reaction with AA, there were significant changes in the wavenumber and absorption intensity of functional groups of biopolymer films (Figure 3 and Table 1). It is interesting to note that all biopolymer films exhibited new absorption bands at 1749, 1754 and 1760 cm^{-1} which correspond to C=O stretching of AA. As shown in Table 1, the absorption band of amide-II for CS at wavenumber of 1536 cm^{-1} shifted to 1575 cm^{-1} indicating a possible formation of hydrogen bonding between AA and biopolymer matrix. A similar explanation was discussed by Hosseini *et al*. [17], who synthesised gelatin-chitosan nanoparticles. Moreover, amide-A of GL shifted to a lower wavenumber suggesting the involvement of free –OH group of GL in the formation of hydrogen bonding with AA [17]. Generally, the functional groups ($–NH_2$ and –OH) exist in CS, GL and MC are capable of forming hydrogen bonds with –OH of AA.

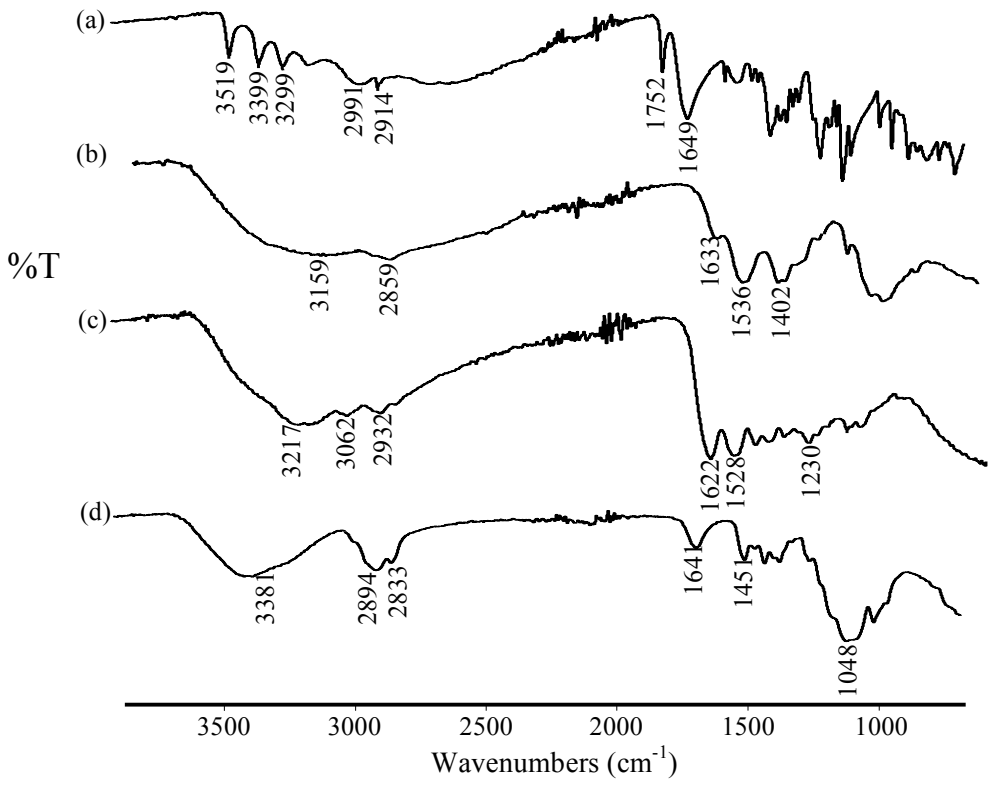

Figure 2: FTIR spectra of (a) ascorbic acid powder, (b) chitosan film, (c) gelatin film, and (d) methylcellulose film.

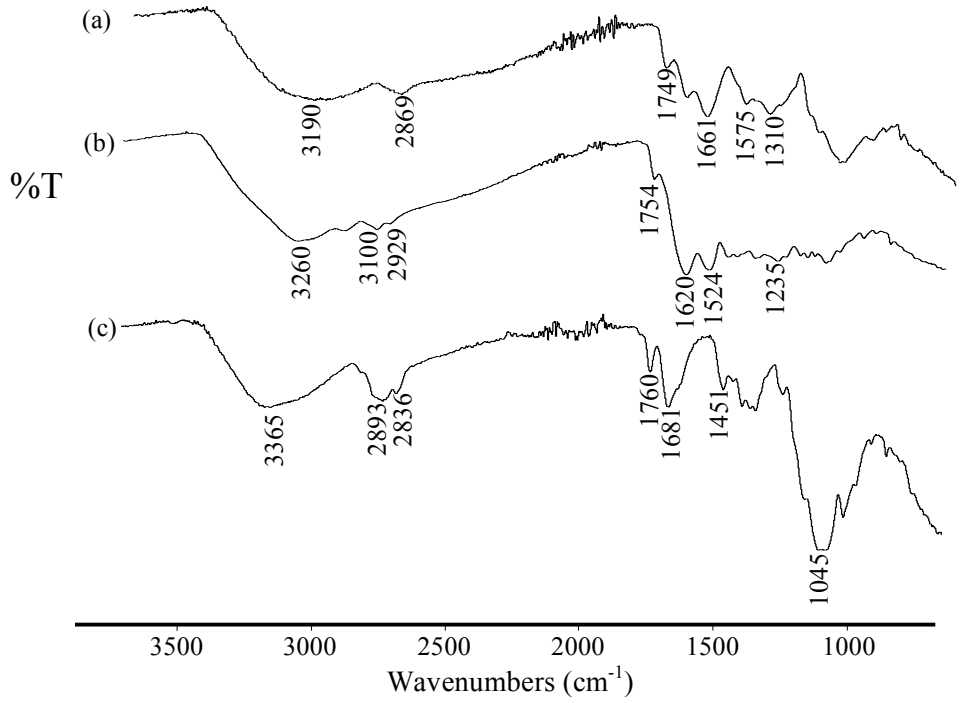

Figure 3: FTIR spectra of (a) chitosan-ascorbic acid film, (b) gelatin-ascorbic acid film, and (c) methylcellulose-ascorbic acid film.

Table 1: Wavenumber and assignment of biopolymer films.

Biopolymer film	Wavenumber (cm^{-1})		Assignment
	Without ascorbic acid	With ascorbic acid	
Chitosan	3159	3190	Amide-A, $–NH_2$ and –OH stretching
	2859	2869	Amide-B, C-H stretching
	1633	1661	Amide-I, C=O stretching
	1536	1575	Amide-II, NH_2 bond stretching
	1402	1310	Amide-III, C–N bond stretching
Gelatin	3217	3260	Amide-A, N−H and C−H stretching
	2932	2929	Amide-B, C−H stretching
	1622	1620	Amide-I, C=O stretching
	1528	1524	Amide-II, N−H bending
	1230	1235	Amide-III, C−N bond stretching
Methylcellulose	3381	3365	O−H stretching
	2894	2893	C−H stretching
	2833	2836	C−H stretching
	1641	1681	C−O stretching
	1451	1451	C−OH stretching
	1048	1045	C−O−C stretch

3.2. Light transmission and transparency

Light transmission and transparency values of biopolymer films at selected wavelength are given in Table 2. For UV region at 280 nm, the transmission values for CS, GL, and MC films were 34.9%, 29.5% and 68.6%, respectively. The transmission values were decreased significantly after addition of AA. CS-AA had the lowest

light transmission value with 0.002% followed by GL-AA (0.003%) and MC-AA (2.12%). The values at 280 nm for AA derivative films were negligible. This suggests that the biopolymer-AA films have excellent UV barrier property as compared to control films, which may reduce oxidation in the food system. This will prevent nutrient loss, discolouration and off-flavours of food products [21].

As shown in Table 2, the transmission values of visible light for all films were in the range of 0.007-89.5% for 350-500 nm, and 74.4-90.8% for 600-800 nm. The values for biopolymer-AA films at 600 nm were between 74.4% and 84.9% which lower than control films (89.1-90.1%). From Table 2, the addition of AA slightly increased the transparency value of films at 600 nm, CS from 0.501 to 1.17 A/mm, GL from 0.412 to 0.559 A/mm and MC from 0.572 to 0.889 A/mm. As discussed by Tongnuanchan *et al*. [23], the higher the transparency value, the lower the transparency of films. The transparency values obtained for biopolymer-AA films were for found to be lower than those reported for commonly used synthetic films such as low-density polyethylene (LDPE) (3.05 A/mm) and oriented polypropylene (PP) (1.67 A/mm) [7]. These findings suggest that the light barrier property of the biopolymer films is comparable with LDPE and PP films.

Table 2: Light transmission and transparency of biopolymer films.

Biopolymer film	Light transmission at different wavelength (%)							Transparency (A/mm)
	200	*280*	*350*	*400*	*500*	*600*	*800*	
Cling film	8.10	83.92	90.24	91.52	91.72	92.33	91.86	0.495
CS	0.07[a]	34.9[a]	54.6[a]	76.4[a]	87.0[a]	89.1[a]	90.3[a]	0.501[a]
GL	0.046[a]	29.5[a]	76.4[a]	85.2[a]	89.2[a]	90.1[a]	90.8[a]	0.412[a]
MC	6.00[a]	68.6[a]	86.3[a]	87.9[a]	89.5[a]	90.0[a]	90.5[a]	0.572[a]
CS-AA	0.012[b]	0.002[b]	0.007[b]	3.04[b]	46.7[b]	74.4[b]	87.8[b]	1.17[b]
GL-AA	0.015[b]	0.003[b]	9.01[b]	36.0[b]	73.7[b]	86.8[b]	89.8[b]	0.559[b]
MC-AA	0.057[b]	2.12[b]	72.5[b]	79.6[b]	83.5[b]	84.9[b]	86.3[b]	0.889[b]

3.3. SEM analysis

The surface morphology of biopolymer films are shown in Figure 4. CS-AA film (Figure 4b) exhibits a non-porous and smoother surface texture than CS film (Figure 4a). In contrast, there was no significant change in the surface morphology of GL film following addition of AA (Figure 4c and Figure 4d). Both films display a smooth and homogenous surface texture. AA treatment has reduced the freckles-like surface texture of MC films (Figure 4e and Figure 4f). Overall, the addition of AA into FFS may alter the surface morphology of biopolymer films. A study by Ahmed *et al*. [22] has shown that the incorporation of gelatin and chitosan with addition of boric acid produced a uniform, compact and homogenous appearance of composite film.

3.4. TGA analysis

The TGA thermograms of biopolymer films are illustrated in Figure 5. All films exhibit two stages of decomposition with a slight weight loss (9.21-14.9%) at the first decomposition stage between the temperature of 30 and 214 ˚C. The second decomposition stage occurred at temperature of 198 to 487 ˚C with a more significant weight loss (37.9-75.6%). The first weight loss may be due to evaporation of water and glycerol, while the second decomposition stage may be attributed to degradation of side chain and the loss of CO_2 [23, 24]. Table 3 represents the decomposition stage and percentage weight loss for biopolymer films. The peak temperature (T_{peak}) of the main degradation step was shifted to a higher temperature following application of AA. For example, the second T_{peak} of MC was shifted from 300 ˚C to 370 ˚C, which indicates that AA treatment improved the heat stability of MC film. Obviously, the chemical structures of biopolymer-AA films play a crucial role in the thermal decomposition process. As discussed in FTIR section, it was speculated that the hydrogen bond was formed due to interaction between AA and biopolymer matrix. Therefore, higher temperature is required by biopolymer-AA film to overcome strong and stable bond.

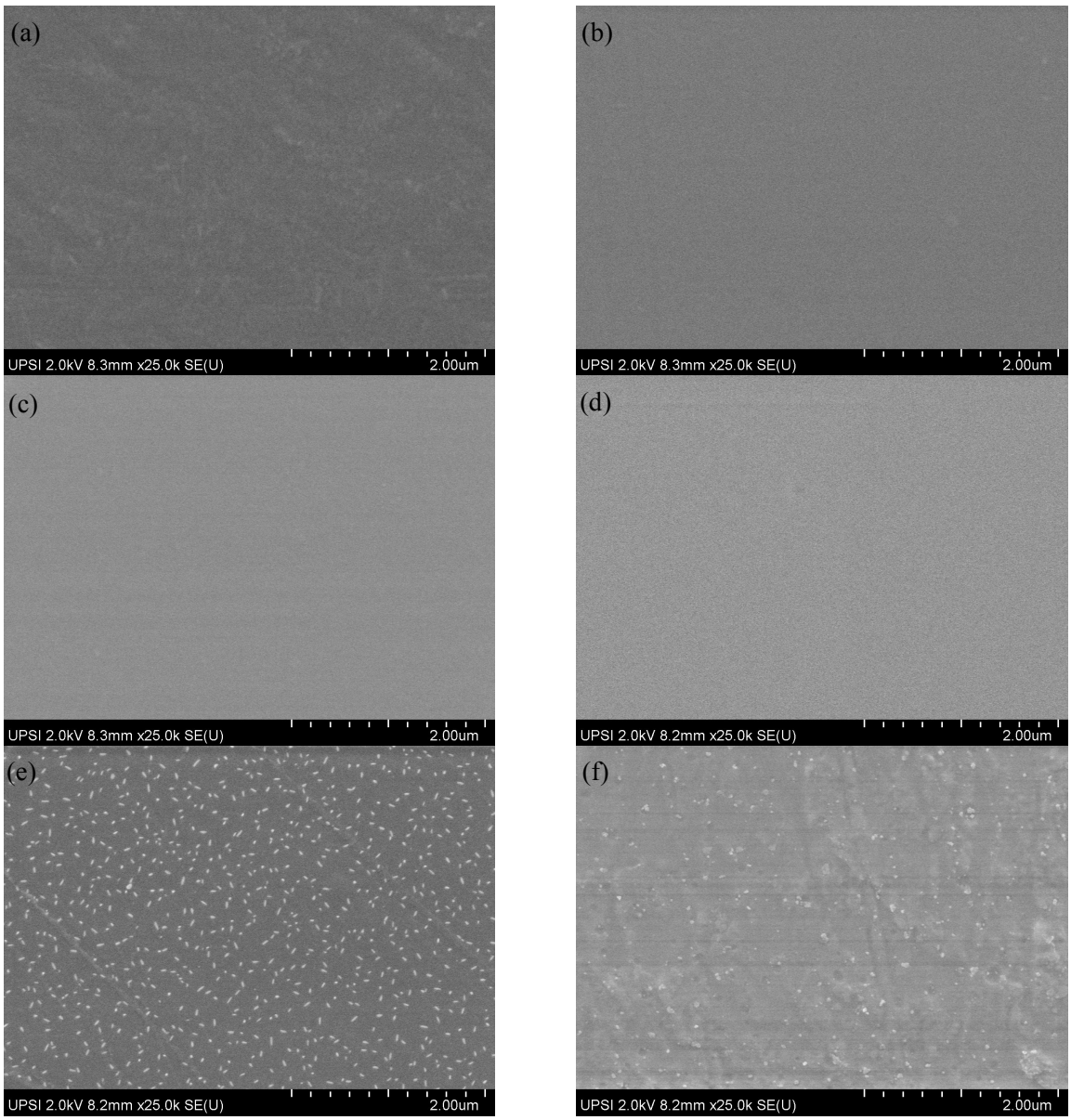

Figure 4: SEM images of (a) chitosan, (b) chitosan-ascorbic acid, (c) gelatin, (d) gelatin-ascorbic acid, (e) methylcellulose, and (f) methylcellulose-ascorbic acid film at a 25,000× magnification.

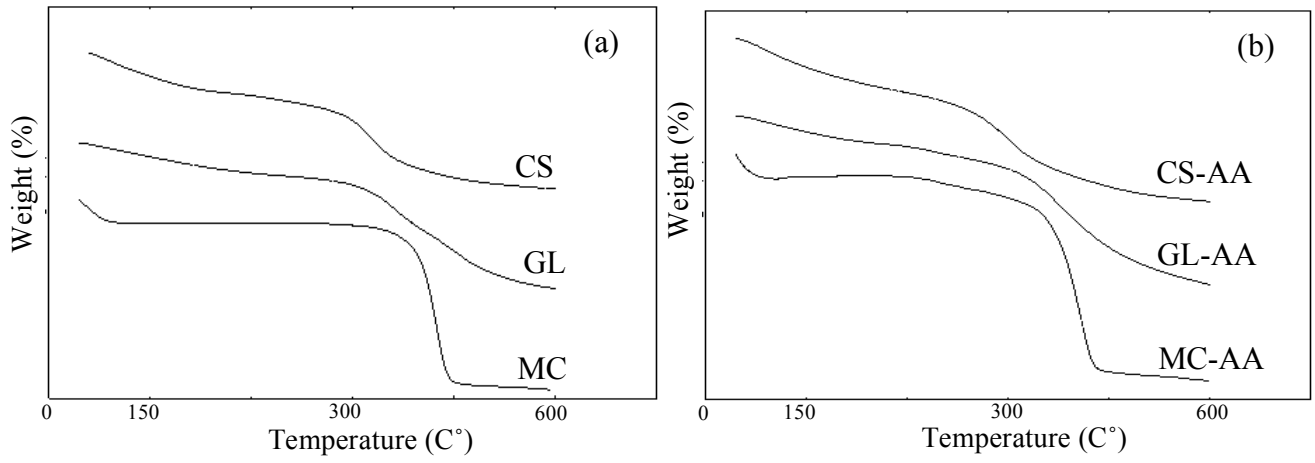

Figure 5: TGA thermograms of (a) biopolymer-control films, and (b) biopolymer-ascorbic acid films.

Table 3: TGA data for biopolymer films.

Biopolymer film	Decomposition stage	Temperature (˚C)			Weight loss (%)	
		Start	End	T_{peak}	Partial	Total
CS	1st	39	95	58	9.91	47.8
	2nd	252	470	300	37.9	
GL	1st	35	214	56	14.9	66.4
	2nd	241	480	341	51.4	
MC	1st	33	84	60	10.7	86.3
	2nd	198	410	300	75.6	
CS-AA	1st	34	87	62	9.21	50.2
	2nd	262	473	317	41.0	
GL-AA	1st	31	87	65	10.7	57.7
	2st	265	487	348	47.0	
MC-AA	1st	33	86	61	9.33	76.4
	2nd	201	438	370	67.1	

3.5. Mechanical properties

The ability of film to resist stress during its application and to protect the inner packaging reflects by TS and EAB, respectively [25]. Table 4 shows the TS and EAB of biopolymer films and commercial cling film. After addition of AA, the TS values of biopolymer films were decreased while the EAB values were increased. The addition of 15 % (w/w) of AA reduced the TS value from 27.3 to 23.6 MPa for CS film, 10.5 to 6.53 MPa for GL film, but a marginal reduction was obtained for MC (18.8 to 16.3 MPa). In contrast, the corresponding EAB values for CS, GL, and MC were increased from 30.1 to 58.6%, 57.0 to 85.6% and 25.4 to 36.2%, respectively with the addition of AA.

Results from this study suggest that the addition of AA could provide plasticising effect to biopolymer films. Cao *et al*. [26] observed an increment in the EAB value and a reduction in the TS and Young's Modulus values for gelatin film after addition of malic acid, another common natural additive. According to conventional standards, the TS of packaging film must be more than 3.50 [17, 27]. It is important to note that the TS values measured for biopolymer-AA films were comparable with typical packaging plastics such as high-densitypolyethylene (HDPE) (17.9-33.1 MPa) and LDPE (15.2-78.6 MPa) [17].

Table 4: Properties of biopolymer films.

Biopolymer film	Thickness (mm)	Tensile strength (MPa)	Elongation at break (%)	Water vapour permeability (g m^{-1} day^{-1} atm^{-1})	Oxygen permeability × 10^{-4} (cc m^{-1} day^{-1} atm^{-1})
Cling film	0.07	39.7 ± 1.33	238 ± 2.06	0.86 ± 0.73	0.72 ± 0.43
CS	0.100^{a}	27.3 ± 1.92^{a}	30.1 ± 2.11^{a}	1.44 ± 0.18^{a}	1.63 ± 0.05^{a}
GL	0.110^{a}	10.5 ± 2.22^{a}	57.0 ± 2.74^{a}	1.73 ± 0.26^{a}	1.51 ± 0.30^{a}
MC	0.080^{a}	18.8 ± 2.32^{a}	25.4 ± 1.05^{a}	1.27 ± 0.12^{a}	1.10 ± 0.08^{a}
CS-AA	0.110^{a}	23.6 ± 1.66^{b}	58.6 ± 1.48^{b}	1.46 ± 0.36^{a}	1.69 ± 0.27^{a}
GL-AA	0.110^{a}	6.53 ± 1.99^{b}	85.6 ± 2.17^{b}	1.76 ± 0.08^{a}	1.56 ± 0.14^{a}
MC-AA	0.080^{a}	16.3 ± 2.41^{b}	36.2 ± 2.88^{b}	1.36 ± 0.26^{b}	1.22 ± 0.37^{b}

Values represent mean of 3 replicates ± standard deviation. Different letters indicate significant statistical differences ($p < 0.05$).

3.6. WVP and OP analyses

The WVP and OP values of commercial cling and biopolymer films with and without addition of AA at concentration of 15% (w/w) are shown in Table 4. Of biopolymers studied, MC exhibited the best water vapour

barrier property with WVP value of 1.27 g m^{-1} day^{-1} atm^{-1}, followed by CS (1.44 g m^{-1} day^{-1} atm^{-1}) and GL (1.73 g m^{-1} day^{-1} atm^{-1}). GL had the highest WVP value due to the presence of hydrophilic amino acids in its structure [28]. On the other hand, MC consists of hydrophobic methoxide groups. As shown in Table 4, the OP values of CS, GL and MC were determined as 1.63, 1.51 and 1.10 cc m^{-1} day^{-1} atm^{-1}, respectively. The differences of OP values depend mainly on the chemical composition and molecular structure of biopolymer [8]. As discussed by Ariaii *et al*. [29], in many cases MC has a better molecular structure as oxygen barrier than other biopolymers.

However, the addition of AA caused a slight increase in WVP and OP values for all films. This scenario can be explained by the fact AA possess a number of hydroxyl groups, which favoured the formation of hydrogen bonding with water molecule. Furthermore, the interactions of AA could alter the arrangement of polymeric network that could change the rigidity of biopolymers. In the preceding section, it was apparent that the TS values of biopolymer films decreased following AA treatment. Presumably, both aspects (the formation of hydrogen bonding and the change in polymeric network) will induce water vapour and oxygen permeation.

3.7. Colour Measurement

The incorporation of AA with biopolymers significantly increased the b* value of the biopolymer films as shown in Table 5. From Table 5, it is clear that the ΔE* value was greatly influenced by both a* and b* values. The lower the a* value and the higher the b* value will results in the higher the ΔE* value of the biopolymer-AA films [30]. The increase of b* value indicated an increase in the yellowness of the biopolymer-AA films. Kowalczyk [31] reported that the AA increased the yellowing index of carboxymethyl cellulose, oxidised potato starch, soy protein isolate and gelatin film. As discussed by Kowalczyk [31] and Song & Cheng [32], the colour change of biopolymer films following incorporation of AA might be due to oxidation of ascorbic acid to dehydroascorbic acid which increased the yellowing index of the biopolymer films. These results suggest that the incorporation of AA influenced the colour of biopolymer films. This phenomenon can also be related to the increase in the opacity of the biopolymer-AA films, as discussed earlier in light transmission and transparency section, which favour the protection against light.

Table 5: Colour parameters (L*, a*, b*) and total colour difference (ΔE*) of biopolymer films.

Biopolymer film	L*	a*	b*	ΔE*
CS	89.82^{a}	-0.64^{a}	0.71^{a}	-
GL	96.78^{a}	-0.39^{a}	0.50^{a}	-
MC	98.51^{a}	-0.94^{a}	0.28^{a}	-
CS-AA	75.66^{b}	-0.89^{b}	47.35^{b}	48.74
GL-AA	90.85^{a}	-1.14^{b}	22.67^{b}	22.96
MC-AA	96.03^{a}	-2.72^{b}	14.59^{b}	14.63

Values represent mean of 3 replicates. Different letters indicate significant statistical differences ($p < 0.05$).

3.8. Antimicrobial properties

The effects of AA addition on antimicrobial activities of biopolymer films are shown in Table 6. In this study, two common food-borne pathogens namely *E. coli* (gram-negative) and *S. aureus* (gram-positive) were used as test bacteria. The control films (GL and MC) showed no inhibition zone against both bacteria. For CS film, the inhibition zone values against *E. coli* and *S. aureus* were 7 and 10 mm, respectively. The antimicrobial property of CS can be related to its cationic nature. It is expected that the cation of CS ($(R{-}N(CH_3)_3^+$ sites) and anion of bacteria cell membranes will interact electrostatically. This interaction will cause cellular lysis to occur and provide antimicrobial property to CS [33].

After reaction with AA, there was a significant increase in inhibition zone for both bacteria. The inhibition zone values against *E. coli* for CS-AA, GL-AA and MC-AA were 18, 10 and 10 mm, respectively. Meanwhile for *S. aureus*, the inhibition zone was recorded as 20, 15 and 16 mm for CS-AA, GL-AA and MC-AA, respectively. Furthermore, biopolymer-AA films are more effective against gram-positive bacteria because gram-negative bacteria are surrounded with impermeable outer membrane [34]. Presumably, AA enhanced the

acidity of the solution which hindered the oxygen source for aerobic bacteria [35]. The inhibition of microbial growth can also be related to electrostatic interaction between positively charged both biopolymers and AA with negatively charged surface of bacterial membrane. This interaction, which normally occurred at low pH medium, will disrupt cell membrane of *S. aureus* and *E. coli*. This lysis phenomenon will improve antimicrobial property of the biopolymers. This electrostatic interaction could be the main antimicrobial mechanism of ascorbic acid for chitosan, gelatin and methylcellulose studied. Therefore, the incorporation of ascorbic acid to biopolymer films improved their antimicrobial property against *S. aureus* and *E. coli*.

Table 6: Inhibition zone of biopolymer films against *E. coli* and *S. aureus*.

Biopolymer film	Inhibition zone [mean ± SD (mm)]	
	E. coli	*S. aureus*
CS	7 ± 0.57^{a}	10 ± 0.33^{a}
GL	0^{a}	0^{a}
MC	0^{a}	0^{a}
CS-AA	18 ± 0.64^{b}	20 ± 0.53^{b}
GL-AA	10 ± 1.29^{b}	15 ± 0.34^{b}
MC-AA	10 ± 1.00^{b}	16 ± 0.72^{b}

Values represent mean of 4 replicates ± standard deviation. Different letters indicate significant statistical differences ($p < 0.05$).

3.9. Preservation study

The effectiveness of biopolymer films to preserve food was further evaluated in a preservation study using two local commodities, namely cherry tomatoes and grapes. To assess the potential global applicability of the biopolymer films, the preservation study was performed using two types of fruits, namely climacteric (cherry tomatoes) and non-climacteric (grapes). The fruits' appearance and freshness were monitored for 14 days. In this study, unwrapped fruits and fruits wrapped with commercial cling (CC) film act as controls. The percentage of weight loss and browning index of fruits are presented in Table 6.

From Table 7, it is clear that unwrapped fruits had the highest percentage of weight loss and browning index as compared to wrapped fruits. At day 14 of preservation study, a major shrinkage effect was observed on unwrapped grapes suggesting their low ability to sustain freshness. Based on observation, the effectiveness of film treatment on fruit freshness was in the order of CC film > biopolymer-AA film > biopolymer > unwrapped fruits. It is interesting to note that biopolymer-AA films had almost similar preservation effect with CC film, particularly in reducing the weight loss and browning index of fruits. For instance, the weight loss for cherry tomatoes wrapped with CC and CS-AA films were determined as 14.3 and 15.5%, respectively. Grapes received CC and GL-AA films treatment showed browning index value of 44 and 46, respectively. The ability of biopolymer-AA films to sustain the freshness of the fruits can be related to their ability to protect fruits against light and food pathogens.

It is known that light, oxygen, water and microorganism activity can promote the spoilage of foods [36, 37]. An effective biopolymer film for food preservation should possess several protective characteristics against aforementioned factors. As previously discussed from light transmission and transparency findings, AA treatment increased opacity of the biopolymer films. This increment will provide a better protection for biopolymer films against light. Furthermore, from antimicrobial study it was found that the addition of AA has improved the antibacterial properties of the biopolymer films. In this context, the biopolymer-AA films may act as vehicle carrying antimicrobial agents which protect the fruits against microorganism. According to Avila-Sosa *et al*. [38], the migration of antimicrobial agents from the biopolymer film occurred due to: (1) electrostatic interactions between the antimicrobial agent and the polymer chains, (2) osmosis, (3) structural changes induced by the presence of antimicrobial and (4) environmental conditions. Overall, results from preservation study corroborate the earlier findings obtained for light transmission and transparency and antimicrobial activity studies.

Table 7: Percentage of weight loss and browning index of preserved cherry tomatoes and grapes.

Samples	Weight loss (%) [mean ± SD (mm)]		Browning index [mean ± SD (mm)]	
	Tomato	Grape	Tomato	Grape
Unwrap	32.3 ± 0.06^{a}	49.1 ± 0.85^{a}	306 ± 18^{a}	360 ± 33^{a}
Cling	14.3 ± 0.74^{c}	18.4 ± 1.04^{c}	41 ± 14^{f}	44 ± 15^{d}
CS	20.4 ± 1.04^{b}	22.1 ± 0.46^{b}	90 ± 27^{d}	114 ± 23^{c}
GL	21.2 ± 1.10^{b}	23.1 ± 0.82^{b}	102 ± 18^{c}	138 ± 13^{b}
MC	25.9 ± 1.02^{b}	24.8 ± 0.95^{b}	154 ± 24^{b}	137 ± 19^{b}
CS-AA	15.5 ± 1.07^{c}	20.2 ± 0.39^{c}	46 ± 14^{f}	47 ± 17^{d}
GL-AA	17.9 ± 0.68^{c}	19.4 ± 0.79^{c}	52 ± 17^{e}	46 ± 24^{d}
MC-AA	18.7 ± 1.48^{c}	19.9 ± 0.99^{c}	57 ± 15^{e}	47 ± 26^{d}

Values represent mean of 3 replicates ± standard deviation. Different letters indicate significant statistical differences ($p < 0.05$).

Conclusion

The incorporation of ascorbic acid into biopolymer (chitosan, gelatin and methyl cellulose) films significantly improved the light barrier property, surface morphology and heat stability of the films. Ascorbic acid induced plasticising effect, which has caused a reduction in tensile strength and an increment in elongation at break of biopolymer films. Water vapour permeability was affected by this scenario. Ascorbic acid inhibited *E. coli* and *S. aureus* activities. The antimicrobial properties of biopolymer films against *E. coli* and *S. aureus* were improved following ascorbic acid treatment. Biopolymer-AA films were successfully preserved cherry tomatoes and grapes at almost similar degree to that of commercial cling films. In conclusion, results from this study suggest that biopolymer-AA films possess key features as environmental friendly agents for food preservation.

Acknowledgements - This work was supported by Majlis Amanah Rakyat (MARA) Malaysia under Skim Geran Penyelidikan dan Inovasi MARA (SGPIM 2016-0005-102-20).

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(2018) ; http://www.jmaterenvironsci.com