Synthesis, characterization and application of gelatin–carboxymethyl cellulose blend films for preservation of cherry tomatoes and grapes

Mimi Syakila Samsi¹ · Azlan Kamari¹ · Shahrulnizahana Mohammad Din² · Gabriel Lazar³

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Abstract In the present study, gelatin–carboxymethyl cellulose blend film was synthesized, characterized and applied for the first time to preserve cherry tomatoes (*Solanum lycopersicum* var. *cerasiforme*) and grapes (*Vitis vinifera*). Gelatin (Gel) film forming solution was incorporated with carboxymethyl cellulose (CMC) at three volume per volume (Gel:CMC) ratios, namely 75:25, 50:50 and 25:75. CMC treatment has improved the transparency, tensile strength (TS), elongation at break (EAB), water vapor permeability and oxygen permeability of gelatin films. A pronounced effect was obtained for 25Gel:75CMC film. The TS and EAB values were increased from 25.98 MPa and 2.34% (100Gel:0CMC) to 37.54 MPa and 4.41% (25Gel:75CMC), respectively. A significant improvement in antimicrobial property of gelatin films against two food pathogens, namely *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) was obtained in the presence of CMC. The effectiveness of gelatin–CMC blend films to extend the shelf life of agricultural products was evaluated in a 14-day preservation

study. The gelatin–CMC films were successfully controlled the weight loss and browning index of the fruits up to 50.41% and 31.34%, respectively. Overall, gelatin–CMC film is an environmental friendly film for food preservation.

Keywords Biopolymer films · Physicochemical properties · Antimicrobial activity · Food preservation

Introduction

Food preservation plays an important role in controlling physical and chemical changes of various food products such as vegetables, fruits and fresh meat. It extends the shelf life of perishable food products by preventing them from microorganism spoilage and oxidation deterioration reaction (Kumari et al. 2017; Hanani et al. 2014). Several traditional techniques such as drying, salting, freezing, smoking and addition of chemical preservatives are available to preserve food products. However, many of these techniques possess many limitations and therefore impractical for modern food industry. Due to their attractive properties such as cheap, abundantly available, good tensile and tear strength, synthetic petroleum-based films are widely used in food processing industry (Basu et al. 2017; Silva-Weiss et al. 2013). However, application of fossil derived polymers has caused serious environmental problems as they are non-biodegradable materials (Shanmuga et al. 2014; Dhumal and Sarkar 2018).

Based on a survey study conducted by Kaeb et al. (2016), the market for compostable and biodegradable plastic products in Europe was projected to grow beyond 300,000 tons in 2020. In fact, it is expected to continue to increase in the future as there is a great concern from the

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✉ Azlan Kamari
azlan.kamari@fsmt.upsi.edu.my

¹ Department of Chemistry, Faculty of Science and Mathematics, Universiti Pendidikan Sultan Idris, 35900 Tanjong Malim, Perak, Malaysia

² Department of Chemistry Malaysia, Johore State, Jalan Abdul Samad, 80100 Johor Bahru, Johor, Malaysia

³ Department of Environmental and Mechanical Engineering, “Vasile Alecsandri” University of Bacau, Calea Marasesti 157, 600115 Bacau, Romania

public in relation to food safety and human health. The increase in public demand for environmental friendly and safe packaging materials for food products has put biodegradable films production in very positive prospect. As a matter of fact, attention has been paid to development of biodegradable films from natural materials such as polysaccharides, proteins and lipids (Abdou and Sorour 2014; Xu et al. 2015). The aforementioned natural materials have good sensory qualities, biodegradable and offer microbial safety (Tulamandi et al. 2016).

Among these biopolymers, gelatin has received great attention from food scientists as an alternative eco-friendly packaging material mainly due to its biodegradability as well as excellent film-forming and mechanical properties (Hosseini et al. 2015). It is derived from partial hydrolysis of collagen which originated from skin, bone and connective tissue of animals or inner part of cattle hides (Li et al. 2016). Due to its polypeptide structure of protein, gelatin has been widely used as thickening and gelling agent in food industry. Carboxymethyl cellulose (CMC) is a water soluble cellulose derivative that possesses good film forming properties (Muppalla et al. 2014). It has been reported that CMC had an ability to form colorless, non-toxic, water soluble, stable and uniform film forming solution, but with weak mechanical properties (Arnon et al. 2014; Gregorova et al. 2015). CMC exhibits amphiphilic characteristics with hydrophobic polysaccharide backbone and hydrophilic carboxyl groups (Su et al. 2010). Blending of gelatin with several polymers such as starch and chitosan was reported able to form flexible composite films with improved mechanical properties as compared to single film forming solution (Fakhouri et al. 2015). However, these composite films exhibited poor antimicrobial property.

Based on the aforementioned issue, the ultimate objective of this study was to evaluate the potential of novel gelatin–CMC film to preserve agricultural products. To the best of our knowledge, the synthesis, characterization and application of gelatin–carboxymethyl cellulose blend films at different incorporation rates particularly for food preservation has never been studied and reported in literature. This study entailed three main studies, namely (1) characterization, (2) antimicrobial, and (3) preservation. The gelatin–CMC films were characterized using Fourier Transform Infrared (FTIR) Spectrometer, Ultraviolet–visible (UV–Vis) Spectrophotometer, Universal Testing Machine, Thermal Gravimetric Analysis (TGA) and Differential Scanning Calorimeter (DSC). The antimicrobial study was carried out using two microbial strains, namely *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*). The effectiveness of Gel–CMC films to preserve cherry tomatoes was evaluated in a 14-day preservation study.

Materials and methods

Materials

Gelatin (Gel) with the strength of 210 Bloom was purchased from Nur Halal Gelatine Resources, Malaysia. Meanwhile, carboxymethyl cellulose (CMC) sodium salt was supplied by Acros Organics, Malaysia.

Preparation of Gel–CMC films

All films were prepared by using solvent casting method. The single film forming solutions of 2% (w/v) of Gel and CMC were prepared by weighing about 2.0 g of each material in 100 mL of deionized water. The solution was continuously stirred at 55 °C for 30 min. Meanwhile, blend films with different Gel:CMC volume ratios were prepared by mixing Gel and CMC solutions (75Gel:25CMC, 50Gel:50CMC, 25Gel:75CMC). All mixtures were stirred continuously for 30 min until homogeneous. The solution was left to rest for about 30 min to remove all bubbles formed during stirring. The solution was then poured onto the centre of a polystyrene petri dish and spread uniformly. The resulting films were dried in an oven for about 12 h at 35–40 °C before being peeled off. Film thickness was measured using a Senator Digital Caliper DCS150 with an accuracy of ± 0.01 mm.

Characterization studies

Fourier transform infrared (FTIR) spectroscopy

FTIR analysis of Gel–CMC films was conducted on a Thermo Nicolet 6700 Fourier Transform Infrared Spectrometer. FTIR spectra were recorded at the wave number range of 500–4000 cm^{-1} with a resolution of 2 cm^{-1} and an average of 25 cumulative scans.

Light transmission and transparency

The transparency of the films was studied using an Agilent Cary 60 UV–Visible Spectrophotometer. The films were analyzed at a wavelength ranged from 200 to 800 nm. The opacity values were calculated based on a formula proposed by Hosseini et al. (2015):

$$\text{Transparency} = \frac{A_{600}}{x}$$

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

Scanning electron microscopy (SEM)

The surface morphology of gelatin, CMC and Gel–CMC blend films were observed by using a Hitachi FESEM SU 8020UHR Field Emission Scanning Electron Microscope. Films were coated with a conductive layer of platinum using a Quorum Q150P S cooled Sputter Coater. The acceleration of electron was set at 15 kV and film samples were then viewed at several magnifications.

Mechanical properties

Elongation at break (EAB) and tensile strength (TS) of the films were determined according to ASTM D822 Standard test (ASTM) using an Instron 5967 Universal Testing Machine. The sample was cut at 50×10 mm and mechanical crosshead speed was fixed at 30 mm/min. Each film was examined for five times.

Water vapor permeability (WVP) and oxygen permeability (OP) analyses

WVP was measured gravimetrically using ASTM E-96 method (2005) adapted to hydrophilic films (McHugh et al. 1993). Briefly, the Gel–CMC film was sealed using a carbon tape around a cup, which was filled with 10 mL deionized water. Then, the cup was placed in a desiccators containing dry silica gel at 50% relative humidity (RH) and temperature of 23 °C. The cup was left for 2 h until the initial equilibrium was achieved and continuously weighed at 8 h intervals. The permeation of water vapors onto the silica gel through the films was continuously observed for 48 h. Weight loss was recorded using an analytical scale (± 0.0001 g). Water vapor transmission rate (WVTR) was obtained by plotting the weight loss versus time in a linear regression ($r^2 \geq 0.99$) and dividing the slope by the exposed film area (m^2). The water vapor permeability was calculated according to the following equation:

$$WVP = \frac{WVTR \times L}{\Delta P}$$

where WVTR is water vapor transmission rate, L is the mean film thickness (mm) and ΔP is the partial water vapor pressure difference (kPa) across the two sides of the film.

Meanwhile, OP of each film was determined using ASTM D3985 method on a 8001 Oxygen Permeation Analyzer (Bang and Kim 2012). The OP analysis was conducted at temperature of 23 ± 1 °C and $50 \pm 5\%$ RH. The OP values were calculated based on following formula (Yap et al. 2016):

$$OP = \frac{OTR \times \ell}{\Delta P}$$

where the OTR represents oxygen transmission rate, ℓ was the films thickness and ΔP is the partial pressure of oxygen (kPa) of the film.

Thermogravimetric (TGA) analysis

Thermal stability of each film was determined by using a Pyris 1 Perkin-Elmer Thermal Gravimetric Analyzer. The initial temperature of TGA analysis was set at 25 until 1000 °C and at a heating rate of 40 °C/min. The TGA analysis was carried out in nitrogen atmosphere.

Antimicrobial analysis

In this study, *S. aureus* (Gram-positive bacteria) and *E. coli* (Gram-negative bacteria) were used to test the antimicrobial activity of each film. Disc shaped filter paper was soaked in film forming solutions of each sample before being placed in an agar which was seeded with *S. aureus* and *E. coli* bacteria. The tested sample was incubated in an incubator at 37 °C for 24 h. The inhibition zone appeared around the disc shaped filter paper was referred as the inhibitory effects of the films against the bacteria.

Preservation study

The effectiveness of Gel–CMC films to extend the shelf life of food products was further evaluated in a preservation study. In this study, cherry tomatoes (*Solanum lycopersicum* var. *cerasiforme*) and grapes (*Vitis vinifera*) were obtained from an agricultural farm in Cameron Highlands, Malaysia. The total number of fruit samples used was 42, of which 7 films $\times$ 2 types of fruit $\times$ 3 replicates. Equal size of fruits were chosen at random and washed with deionized water to remove the dirt around the fruits. The selected fruits were weighed and wrapped by using the films. The fruits were then kept at room temperature (23–25 °C) and monitored daily. The percentage of weight loss for each fruits was calculated by using the following formula (Priya et al. 2014):

$$\text{Weight loss (\%)} = \frac{(W_o - W)}{W_o} \times 100$$

where W_o is the initial weight of the fruits and W is the final weight of the fruits after 14 days of preservation study. The browning index was calculated by using the following equation (Tian et al. 2005):

$$\begin{aligned} \text{Browning index} &= \frac{\sum (\text{Browning level}) \times \text{number of fruit at the browning level}}{\text{Total number of fruit in the treatment}} \\ &\times 100 \end{aligned}$$

The browning level was classified as (Priya et al. 2014): 1 = no browning, 2 = less than 20%, 3 = around 20–40%, 4 = around 40–60%, and 5 = more than 60%.

Statistical analyses

Each experiment was performed in triplicates. Results were presented as the mean $\pm$ standard deviation and were analyzed by analysis of variance (one way ANOVA) using Minitab 17 Statistical Software. The probability value of $p < 0.05$ was used as the criterion for significant differences. It is known that the correlation coefficient can reflect the intensity of the linear correlation between two variables (Kuan 2018; Kim et al. 2019). In this study, Pearson correlation analysis was used to analyze the linear correlation between two variables at confidence level of 0.01. Treatment means were compared by Dunnett's test at $p < 0.05$ and $p < 0.05$, where the control group was fruits wrapped with commercial cling film.

Results and discussions

FTIR analysis

Figure 1 presents FTIR spectra of gelatin, CMC and gelatin–CMC films. FTIR spectrum of gelatin

(100Gel:0CMC) film exhibits a broad absorption band at the wavenumber of 3308 cm^{-1} which corresponds to the overlapped characteristics of hydroxyl (OH) and amine (NH) stretches (Arnon et al. 2014). The two discernible absorption bands observed at 1639 and 1544 cm^{-1} can be assigned to amide-I (C=O stretching) and amide-II (NH_2), respectively (Arfat et al. 2014). These findings are in accordance with previous studies by Singh et al. (2009) and Singh et al. (2010), which have described the amide-I and amide-II regions comprehensively. These results support the discussion made by Ahmed and Ikram (2016), which reported that gelatin exhibits its characteristic bands at the wavenumbers of 1632 – 1539 cm^{-1} .

Based on FTIR spectrum of CMC (0Gel:100CMC) film, the strong absorption band observed at 3368 cm^{-1} indicates the presence of hydroxyl group (O–H) while the adjacent absorption band at 2927 cm^{-1} confirmed the existence of C–H stretch (Su et al. 2010). Meanwhile, the peak appeared at 1592 and 1057 cm^{-1} represent the absorption bands of cellulose structure which correspond to the antisymmetric vibrations of C=O and C–O–CH₂, respectively (Zhang et al. 2013). The absorption bands at 1417 cm^{-1} and 1324 cm^{-1} are related to amide-III (C–N stretching) and hydroxyl (OH) bending (Singh et al. 2010).

As depicted in FTIR spectra of 75Gel:25CMC and 25Gel:75CMC films, it is apparent that Gel–CMC blend films exhibit both gelatin and carboxymethyl cellulose

Fig. 1 FTIR spectra of gelatin (Gel), carboxymethyl cellulose (CMC) and gelatin–carboxymethyl cellulose (Gel–CMC) films

characteristics. The addition of CMC to gelatin film forming solution has led to the formation of intermolecular and intramolecular hydrogen bond between the polymer structures which shifted the absorption band of hydroxyl group (O–H) from 3308 to 3297 cm^{-1} . The increase in CMC volume added to gelatin film forming solution has significantly reduced the absorption intensity of N–H stretching vibration at 1544 cm^{-1} and shifted the wavenumber from 1544 to 1594 cm^{-1} . Conversely, the absorption intensity of C–O stretching for ether group (CH–O–CH₂) at the wavenumbers of 1065, 1061 and 1060 cm^{-1} increased markedly upon addition of CMC. A pronounce effect was observed for 25Gel:75CMC blend film.

Light transmission and transparency

Film with excellent UV barrier properties determines its potential to protect the wrapped or coated food against oxidation reaction which contributes to deterioration rate, shorten the shelf life and loss of flavor. The light transmission and transparency values of gelatin, CMC and Gel–CMC blends films are given in Table 1. The light transmission was measured as percentage of transmission (%T) within the range of 200–800 nm. The transmission of UV light was determined at the wavelength range of 200–350 nm. When comparing to gelatin and gelatin–CMC blends films, CMC had higher transmission value of 86.85% at 350 nm suggesting a low barrier property of the film towards UV light.

As expected, gelatin formed film with good UV light barrier and transparency values. The UV light transmission of gelatin (100Gel:0CMC) film showed the lowest %T values (0.03%, 16.43% and 39.09%). The lower values of UV light transmittance for gelatin film can be attributed to the presence of aromatic amino acid along the gelatin polypeptide structure, which consequently absorb the penetration of UV light through the film (Ahmed and Ikram 2016).

Meanwhile, the transmission values measured within visible light region (400 nm to 800 nm) were greater than 60% for all composite films and above 80% at wavelength 800 nm. From Table 1, the transparency value of gelatin (0.87) was slightly higher than CMC (0.66). The transparency value of the films increased from 0.87 (100Gel:0CMC) to 1.92 (75Gel:25CMC), 1.13 (50Gel:50CMC) and 0.73 (25Gel:75CMC) following the addition of CMC. As discussed by Hosseini et al. (2013), the higher the transparency value, the lower the transparency of films. Therefore of Gel–CMC blend films fabricated, 25Gel:75CMC film was the most transparent with a transparency value of 0.73.

The correlation between UV light transmission (%T) and transparency at 600 nm was analyzed using Pearson correlation analysis. From Table 1, it is clear that the %T had a strong relationship with transparency whereby the Pearson coefficient values were between the range of 0.750 and 0.985. The most significant correlation is for 25Gel:75CMC film with Pearson coefficient of 0.985.

SEM analysis

SEM analysis has been conducted in order to observe the microstructure and compatibility between the polymer blends. Figure 2 shows SEM images of gelatin, CMC and gelatin–CMC films at 10,000 $\times$ magnification. Gelatin and CMC films exhibited a homogeneous and smooth surface texture (Fig. 2a, b). However, several dark spots were observed on the surface of gelatin's film (Fig. 2a). Gelatin inherited protein's chemical structure, therefore the formation of dark spots might be due to the reorganization of the polymer chains during drying process forming a fibrous structure (Rawdkuen et al. 2012).

The existence of micro-cracks present on certain parts of the films might be from the effects of electron beam during the analysis. Gelatin–CMC formed films with a smooth texture and without any aggregations. Overall, the smooth and dense surface texture represents a good distribution of

Table 1 Light transmission, transparency and Pearson coefficient of gelatin, carboxymethyl cellulose and gelatin–carboxymethyl cellulose films

Film	Light transmission (%T)							Transparency	Pearson coefficient
	200 nm	280 nm	350 nm	400 nm	500 nm	600 nm	800 nm		
100Gel:0CMC	0.03	16.43	39.09	65.40	80.71	85.12	88.17	0.87 ^c	0.929
0Gel:100CMC	0.28	67.09	86.85	89.67	90.90	91.33	91.66	0.66 ^d	0.814
75Gel:25CMC	0.05	31.36	69.55	79.17	85.59	87.55	89.33	1.92 ^a	0.750
50Gel:50CMC	0.04	28.62	72.80	83.15	88.95	90.47	91.31	1.13 ^b	0.783
25Gel:75CMC	0.04	31.12	68.87	80.02	87.76	90.11	91.42	0.73 ^c	0.985**

Values represent mean $\pm$ standard deviation. Different letters indicate significant statistical differences ($p < 0.05$)

**The correlation is significant when the confidence level (double test) is 0.01

Fig. 2 SEM images of **a** gelatin film, **b** carboxymethyl cellulose film, **c** 75Gel:25CMC film, **d** 50Gel:50CMC film and **e** 25Gel:75CMC film at $\times 25,000$ magnification

particles thus confirmed the homogeneity between gelatin and carboxymethyl cellulose.

Mechanical properties

Tensile strength (TS) and elongation at break (EAB) play an important role in determining the flexibility and strength of a film especially to overcome the external stress that occurs within packaging or manufacturing process. Table 2 presents the TS and EAB values for gelatin, CMC and gelatin–CMC blend films at different v/v ratios. From Table 2, gelatin film had a slightly higher TS value of 35.98 ± 0.91 MPa than 33.28 ± 1.89 MPa which was measured for CMC film. In terms of flexibility behavior, gelatin film exhibited a lower flexibility with an EAB value of 2.34% as compared to CMC film which possessed an EAB value of 14.18%.

The addition of CMC into gelatin film forming solution has greatly influenced the strength and flexibility of the film. Initially, gelatin (100Gel:0CMC) film had a TS and EAB value of 35.98 MPa and 2.34%, respectively. The incorporation of CMC with gelatin has caused an increment in the TS and EAB values for gelatin. A significant increase was obtained for 25Gel:75CMC film formulation, of which the TS and EAB values were increased to 37.54 MPa and 4.41%, respectively.

In the case of 75Gel:25CMC and 50Gel:50CMC films, the incorporation of CMC has reduced the TS value of gelatin (100Gel:0CMC) film from 35.98 to 21.98 and 25.81 MPa, respectively. Based on Table 2, it is apparent that TS and EAB had a negative relationship. The Pearson coefficient values for correlation between TS and EAB for 75Gel:25CMC and 50Gel:50CMC films were -0.212 and -0.347 , respectively. In contrast, a significant positive relationship between TS and EAB was obtained for

Table 2 Physical properties and Pearson coefficient of gelatin, carboxymethyl cellulose and gelatin–carboxymethylcellulose films

Film	TS (MPa)	EAB (%)	Pearson coefficient	WVP (g m ⁻¹ day ⁻¹ atm ⁻¹)	OP × 10 ⁻⁴ (cc m ⁻¹ day ⁻¹ atm ⁻¹)	Pearson coefficient
100Gel:0CMC	35.98 ± 0.91 ^a	2.34 ± 0.06 ^c	0.835	1.76 ± 0.06 ^a	1.54 ± 0.30 ^a	0.794
0Gel:100CMC	33.28 ± 1.89 ^a	14.18 ± 0.06 ^a	0.719	1.25 ± 0.15 ^c	1.14 ± 0.22 ^c	0.835**
75Gel:25CMC	21.98 ± 3.36 ^b	2.47 ± 0.21 ^c	− 0.212	1.65 ± 0.12 ^a	1.50 ± 0.08 ^a	0.761
50Gel:50CMC	25.81 ± 1.13 ^b	3.18 ± 0.08 ^{ab}	− 0.347	1.50 ± 0.07 ^b	1.43 ± 0.21 ^{ab}	0.809
25Gel:75CMC	37.54 ± 0.43 ^a	4.41 ± 0.23 ^b	0.962**	1.33 ± 0.04 ^c	1.32 ± 0.26 ^b	0.948**

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

**The correlation is significant when the confidence level (double test) is 0.01

25Gel:75CMC film with a Pearson coefficient value of 0.962.

The addition of CMC into the film forming solution of gelatin creates an active site within the polypeptides chains for hydrogen bonding and electrostatic interaction between the polymers. An increase in the volume of CMC ratio may have increased the potential active sites for anion-cation interactions between the polymers. This interaction has results in a more compact film between the polymer structures which exhibits a stronger film with improving flexibility.

WVP and OP analyses

The migrations of gases from the surroundings determine the senescence and respiration rate of perishable food products. Low level of O₂ (below 3%) may contribute to anaerobic respiration (Rahman 2007; Dhumal and Sarkar 2018). The anaerobic respiration promotes formation of lactate, acetaldehyde and ethanol which eventually caused off flavors and reduce in quality of food products (Olivas and Barbosa 2009; Rahman 2007). However, high concentration of O₂ gas may lead to oxidative deterioration within packaged products, one of main causes of nutrient loss and increasing senescence rate (Yap et al. 2016). Therefore, controlling oxygen permeability is the crucial step to maintain the quality and freshness of agricultural products. On the other hand, water content is essential for preservation of cell turgor within the products. Minimizing water losses is one of important properties of a good functional film in order to delay the senescence process of perishable food (Olivas and Barbosa 2009). The WVP and OP values for each film studied are given in Table 2.

As shown in Table 2, gelatin film had the highest WVP (1.76 g m⁻¹ day⁻¹ atm⁻¹) and OP (1.54 × 10⁻⁴ cc m⁻¹ day⁻¹ atm⁻¹) values, suggesting its poor barrier properties against moisture and O₂ gas. Gelatin film possesses a poor water vapor barrier property which can be linked to its hydrophilic properties (Hanani et al. 2014). In contrast,

CMC had an excellent WVP and OP barrier properties with the values of 1.25 g m⁻¹ day⁻¹ atm⁻¹ and 1.14 × 10⁻⁴ cc m⁻¹ day⁻¹ atm⁻¹, respectively. A significant improvement was obtained for 25Gel:75CMC film, of which the WVP and OP values were decreased to 1.33 g m⁻¹ day⁻¹ atm⁻¹ and 1.32 × 10⁻⁴ cc m⁻¹ day⁻¹ atm⁻¹, respectively.

Based on Pearson correlation analysis, it was found that WVP and OP had a strong positive relationship with Pearson coefficient values ranged from 0.761 to 0.948. A significant correlation between WVP and OP was obtained for CMC (0Gel:100CMC) and 25Gel:75CMC films, whereby the Pearson coefficient values were 0.835 and 0.948, respectively.

The presence of cellulose fibers within the CMC structure may have provided a tortuous path for the water vapor to penetrate the films (Su et al. 2010; Gregorova et al. 2015). As discussed earlier in FTIR analysis, the inter-molecular interaction of both polymers is connected by the formation of hydrogen bonding throughout the polymer chain. Good interaction between the polymers has resulted in more compact structure of film matrix that able to restrict and control the penetration of O₂ gas.

TGA analysis

The characterization of biopolymer films was further studied by using TGA analysis. TGA analysis monitored the thermal degradation behavior of gelatin films incorporated with CMC, as a function of temperature in controlled surroundings. The TGA thermograms of gelatin, CMC and gelatin–CMC films are shown in S1. The first degradation of all films started from 48 to 116 °C with percentage weight loss ranged from 5.95 to 19.94%. The second stage of weight loss for gelatin was observed at 352 °C with percentage weight loss of 77.89%. This observation was might be due to the decomposition of highly connected amino acids group in the protein films (Akelah 2013; Arfat et al. 2014). Meanwhile, CMC film exhibited four thermal degradation stages where the second stage began at 289 °C

with a percentage weight loss of 37.16%. The third and fourth decomposition stages of CMC stage started at 437 and 717 °C respectively.

The degradation stage of CMC was associated to the decomposition of highly interacted side chain and carbonyl groups (Bella et al. 2016). Due to its crystalline structure, the incorporation of CMC into the gelatin film forming solution is able to improve the thermal stability the films. The thermal decomposition of gelatin–CMC blend films is shifted towards higher temperature as compared to gelatin films. The enhancement in thermal stability of gelatin–CMC films can be attributed to the intermolecular and intramolecular interaction between the polymers. Overall, the results obtained from TGA analysis corroborate the earlier findings for FTIR analysis, particularly the interaction behavior between the polymers.

Antibacterial analysis

In this study, the antimicrobial properties of the films against two different types of food pathogenic bacteria, namely *S. aureus* (Gram-positive bacteria) and *E. coli* (Gram-negative bacteria) were also studied. The antibacterial activity of each film was measured based on area of the inhibitory zone around the film after 24 h of incubation (S2). The results clearly show that the incorporation of CMC into the gelatin film has improved the antibacterial properties of the film as the inhibition area changed significantly (Table 3).

From Table 3, gelatin and CMC films did not possess any antimicrobial properties against both Gram-positive and Gram-negative food pathogens. However, the blends films of gelatin–CMC exhibited antimicrobial properties. A clear trend of inhibition was obtained particularly when the volume of CMC was increased. A significant correlation

between inhibition of *S. aureus* and *E. coli* was obtained following CMC treatment. The Pearson coefficient values were determined as 0.822, 0.890 and 0.977 for 75Gel:25CMC, 50Gel:50CMC and 25Gel:75CMC, respectively.

Of blend films studied, 25Gel:75CMC film was superior in inhibiting *S. aureus* and *E. coli* with inhibition zone area of 1.30 and 1.50 cm², respectively. This observation can be explained by the fact that CMC consists of linear β -(1 → 4)-glycosidic bonds which possess weakly acidic effects due to its polyelectrolyte property. This characteristic may favor the inhibition of microorganism activities (Muppalla et al. 2014).

Preservation study

Another important characteristic of a good film is being able to prolong shelf life of perishable products. The feasibility of gelatin–CMC blend films to preserve agricultural products was assessed in a preservation study using two types of fruits, namely climacteric (cherry tomatoes) and non-climacteric (grapes). Table 4 presents the percentage of weight loss and browning index of cherry tomatoes and grapes after 14 days of preservation. The total period of 14 days was chosen due to the fact that both fruits become fully ripe and start to deteriorate after 2 weeks of harvest. The decaying processes of fruits were monitored, as the outer membrane of the fruits started to shrink and the browning spots was seen (S2). The unwrapped fruits developed bad smell and completely damaged at day 7. The shrinkage and soft texture on the outer membrane might be due to polyphenols oxidation activities within the fruits (Ioannou 2013).

As shown in Table 4, gelatin film had the least effect in preserving the fruits. In fact, fruits preserved in gelatin film exhibited a significant moldy spots with strong senescence odor. As expected, 25Gel:75CMC blend film did not show any mildew or browning signs on the outer layer of the fruits. Fruits preserved in 25Gel:75CMC blend film has resulted in low values of weight loss (%) and browning index, suggesting the best treatment for fruits preservation.

The correlation between weight loss and browning index was further analyzed using Pearson correlation analysis. From Table 4, it is clear that the weight loss had a strong positive relationship with browning index whereby the Pearson coefficient values were ranged from 0.748 to 0.952 for cherry tomatoes and from 0.761 to 0.969 for grapes, respectively. The most significant correlations are for 25Gel:75CMC and 50Gel:50CMC films with Pearson coefficient values of 0.952 and 0.874 for cherry tomatoes, respectively. Meanwhile, in the case of grapes the most significant Pearson coefficient values were 0.969 and 0.908 for 25Gel:75CMC and 50Gel:50CMC films, respectively.

Table 3 Inhibition zone and Pearson coefficient of gelatin, carboxymethyl cellulose and gelatin–carboxymethyl cellulose films against *S. aureus* and *E. coli*

Film	Inhibition zone area (cm ²)		Pearson coefficient
	<i>S. aureus</i>	<i>E. coli</i>	
100Gel:0CMC	No inhibition	No inhibition	–
0Gel:100CMC	No inhibition	No inhibition	–
75Gel:25CMC	0.50 ± 0.11 ^c	1.0 ± 0.79 ^c	0.822
50Gel:50CMC	0.75 ± 0.85 ^b	1.20 ± 0.61 ^b	0.890
25Gel:75CMC	1.30 ± 1.03 ^a	1.50 ± 1.24 ^a	0.977**

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

**The correlation is significant when the confidence level (double test) is 0.01

Table 4 Percentage of weight loss, browning index and Pearson coefficient of cherry tomatoes and grapes after 14 days of preservation

Film	Weight loss (%)		Browning index		Pearson coefficient	
	Cherry tomatoes	Grapes	Cherry tomatoes	Grapes	Cherry tomatoes	Grapes
Commercial cling film	21.88 ± 15.08	18.62 ± 1.57	300 ± 12	367 ± 19	0.864	0.885
Unwrapped	46.82 ± 11.65 ^{##}	36.48 ± 2.77 ^{##}	485 ± 17 ^{##}	500 ± 26 ^{##}	0.755	0.790
100Gel:0CMC	35.80 ± 6.54 [#]	30.23 ± 5.62 [#]	460 ± 23 [#]	475 ± 10 [#]	0.802	0.761
0Gel:100CMC	29.13 ± 2.04	28.57 ± 2.65	450 ± 15	460 ± 16	0.748	0.798
75Gel:25CMC	26.62 ± 6.15	28.54 ± 3.03	435 ± 14	440 ± 18	0.763	0.815
50Gel:50CMC	25.50 ± 7.90	27.49 ± 6.80	392 ± 19	418 ± 27	0.874 ^{**}	0.908 ^{**}
25Gel:75CMC	23.22 ± 2.82	20.97 ± 8.32	333 ± 24	385 ± 13	0.952 ^{**}	0.969 ^{**}

Values represent mean ± standard deviation. ^{##}Treatments are significantly different from the control group at $p < 0.05$ and $p < 0.01$, respectively (Dunnett's test)

^{**}The correlation is significant when the confidence level (double test) is 0.01

Based on Dunnett's test, it is obvious that unwrapped fruits and fruits wrapped with gelatin (100Gel:0CMC) film are significantly different from the control group (fruits wrapped with commercial cling film) at $p < 0.01$ and $p < 0.05$, respectively. It is interesting to note that the ability of 25Gel:75CMC blend film to preserve fruits was comparable with a commercial cling film. The ability of the films to preserve fruits was greatly influenced by their WVP and OP characteristics as well as with their antibacterial activity as discussed earlier in WVP and OP analyses together with antibacterial studies. In both sections, 25Gel:75CMC blend film exhibited excellent WVP and OP barrier properties and antimicrobial property against *S. aureus* and *E. coli*.

Conclusion

Carboxymethyl cellulose treatment increased the tensile strength and flexibility of gelatin film. The incorporation of carboxymethyl cellulose into gelatin film forming solution significantly improved the barrier properties on UV light and permeability of water vapor and O₂ gas. An enhancement in antimicrobial activity of the film was obtained following addition of carboxymethyl cellulose, particularly at 25Gel:75CMC v/v ratio. Gelatin–carboxymethyl cellulose films were successfully preserved cherry tomatoes and grapes at almost similar degree to that of a commercial cling film. Of blend films studied, 25Gel:75CMC film was the best film to extend the shelf life of agricultural products. In conclusion, gelatin–carboxymethyl cellulose film possesses key unique features that favor its application as an alternative to non-environmental friendly films for food preservation.

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References

- Abdou ES, Sorour MA (2014) Preparation and characterization of starch/carrageenan edible films. *Int Food Res* 21:189–193
- Ahmed S, Ikram S (2016) Chitosan and gelatin based biodegradable packaging films with UV-light protection. *J Photochem Photobiol B Biol* 163:115–124
- Akelah A (ed) (2013) Polymers in food packaging and protection. In: *Functionalized polymeric materials in agriculture and the food industry*. Springer, New York, USA, pp 293–347
- Arfat YA, Benjakul S, Prodpran T, Osako K (2014) Development and characterisation of blend films based on fish protein isolate and fish skin gelatin. *Food Hydrocolloid* 39:58–67
- Arnon H, Zaitsev Y, Porat R, Poverenov E (2014) Effects of carboxymethyl cellulose and chitosan bilayer edible coating on postharvest quality of citrus fruit. *Postharvest Biol Technol* 87:21–26
- Bang G, Kim SW (2012) Biodegradable poly (lactic acid)-based hybrid coating materials for food packaging films with gas barrier properties. *J Ind Eng Chem* 18:1063–1068
- Basu A, Kundu S, Sana S, Halder A, Abdullah MF, Datta S, Mukerjee A (2017) Edible nano-bio-composite film cargo device for food packaging applications. *Food Packag Shelf Life* 11:98–105
- Bella GR, Jeba Jeevitha RS, Avila Thanga Booshan S (2016) Polyvinyl alcohol/starch/carboxymethyl cellulose ternary polymer blends: synthesis, characterization and thermal properties. *Int J Curr Res Chem Pharm Sci* 3:43–50
- Dhumal CV, Sarkar P (2018) Composite edible films and coatings from food-grade biopolymers. *J Food Sci Technol* 55:4369–4383
- Fakhouri FM, Martelli SM, Caon T, Velasco JI, Mei LHI (2015) Edible films and coatings based on starch/gelatin: film properties and effect of coatings on quality of refrigerated Red Crimson grapes. *Postharvest Biol Technol* 109:57–64
- Gregorova A, Saha N, Kitano T, Saha P (2015) Hydrothermal effect and mechanical stress properties of carboxymethylcellulose based hydrogel food packaging. *Carbohydr Polym* 117:559–568
- Hanani ZN, Roos YH, Kerry JP (2014) Use and application of gelatin as potential biodegradable packaging materials for food products. *Int J Biol Macromol* 71:94–102

- Hosseini SF, Rezaei M, Zandi M, Ghavi FF (2013) Preparation and functional properties of fish gelatin–chitosan blend edible films. *Food Chem* 136:1490–1495
- Hosseini SF, Rezaei M, Zandi M, Farahmandghavi F (2015) Fabrication of bio-nanocomposite films based on fish gelatin reinforced with chitosan nanoparticles. *Food Hydrocolloids* 44:172–182
- Ioannou I (2013) Prevention of enzymatic browning in fruit and vegetables. *Eur Sci J* 9:310–341
- Kaeb H, Aeschelmann F, Dammer L, Carus M (2016) Market study on the consumption of biodegradable and compostable plastic products in Europe 2015 and 2020. nova-Institut GmbH, Hürth, pp 2–3
- Kim S-J, Lee J-Y, Yoon S-R, Lee H-W, Ha J-H (2019) Regression analysis for predicting the fermentation state of packaged Kimchi using a colorimetric indicator. *J Food Eng* 240:65–72
- Kuan J (2018) Regression analysis estimation of stature from foot length. *Cogn Syst Res* 52:251–260
- Kumari M, Mahajan H, Joshi R, Gupta M (2017) Development and structural characterization of edible films for improving fruit quality. *Food Packag Shelf Life* 12:42–50
- Li D, Ye Y, Li D, Li X, Mu C (2016) Biological properties of dialdehyde carboxymethyl cellulose crosslinked gelatin–PEG composite hydrogel fibers for wound dressings. *Carbohydr Polym* 137:508–514
- McHugh TH, Avena BR, Krochta JM (1993) Hydrophilic edible films: modified procedure for water vapor permeability and explanation of thickness effects. *J Food Sci* 58:899–903
- Muppalla SR, Kanatt SR, Chawla SP, Sharma A (2014) Carboxymethyl cellulose–polyvinyl alcohol films with clove oil for active packaging of ground chicken meat. *Food Packag Shelf Life* 2:51–58
- Olivas GI, Barbosa GV (2009) Edible films and coatings for fruits and vegetables. In: Embuscado ME, Huber KC (eds) *Edible films and coatings for food applications*. Springer, New York, pp 211–244
- Priya DS, Suriyaprabha R, Yuvakkumar R, Rajendran V (2014) Chitosan-incorporated different nanocomposite HPMC films for food preservation. *J Nanopart Res* 16:1–16
- Rahman MS (2007) *Handbook of food preservation*. CRC Press, London
- Rawdkuen S, Suthiluk P, Kamhangwong D, Benjakul S (2012) Mechanical, physico-chemical, and antimicrobial properties of gelatin-based film incorporated with catechin–lysozyme. *Chem Cent J* 6:131
- Shanmuga PD, Suriyaprabha R, Yuvakkumar R, Rajendran V (2014) Chitosan-incorporated different nanocomposite HPMC films for food preservation. *J Nanopart Res* 16:2248
- Silva-Weiss A, Ihl M, Sobral PJA, Gómez-Guillén MC, Bifani V (2013) Natural additives in bioactive edible films and coatings: functionality and applications in foods. *Food Eng Rev* 5:200–216
- Singh N, Georget DMR, Belton PS, Barker SA (2009) Zein-Iodine complex studied by FTIR spectroscopy, dielectric and dynamic rheometry in films and precipitates. *J Agric Food Chem* 57:4334–4341
- Singh N, Georget DMR, Belton PS, Barker SA (2010) Physical properties of zein films containing salicylic acid and acetyl salicylic acid. *J Cereal Sci* 52:282–287
- Su JF, Huang Z, Yuan XY, Wang XY, Li M (2010) Structure and properties of carboxymethyl cellulose/soy protein isolate blend edible films crosslinked by Maillard reactions. *Carbohydr Polym* 79:145–153
- Tian SP, Li BQ, Xu Y (2005) Effects of O₂ and CO₂ concentrations on physiology and quality of litchi fruit in storage. *Food Chem* 91:659–663
- Tulamandi S, Rangarajan V, Rizvi SSH, Singhal RS, Chattopadhyay SK, Saha NC (2016) A biodegradable and edible packaging film based on papaya puree, gelatin and defatted soy protein. *Food Packag Shelf Life* 10:60–71
- Xu H, Shen L, Xu L, Yang Y (2015) Low-temperature crosslinking of proteins using non-toxic citric acid in neutral aqueous medium: mechanism and kinetic study. *Ind Crops Prod* 74:234–240
- Yap YM, Rahman AR, Chin NL (2016) Mechanical and oxygen barrier properties of sodium caseinate edible film. In: *Third international conference on agricultural and food engineering (Cafei2016)*. Kuala Lumpur Malaysia, UPM
- Zhang L, Zhang G, Lu J, Liang H (2013) Preparation and characterization of carboxymethyl cellulose/polyvinyl alcohol blend film as a potential coating material. *Polym Plast Technol Eng* 52:163–167

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