Gelatin film incorporated with banana leaf essential oil for food preservation

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Abstract. The potential of gelatin (GL) film incorporated with banana leaf essential oil (BA) as an alternative to non-biodegradable cling films was investigated. The environmental friendly GL-BA film was prepared by casting method, with incorporating 1% (v/v) of BA to GL film. Their physicochemical properties were determined by using FTIR Spectrometer, Scanning SEM, TGA, UV-Vis Spectrophotometer, Water Vapour and Oxygen Permeability Analyser, and Universal Testing Machine. The FTIR analysis revealed a new formation of absorption band in the FTIR spectrum of GL at wavenumber 1732 cm\(^{-1}\) after addition of BA. The roughness of GL film was increased following incorporation of BA. Application of BA increased the opacity of GL film from 0.501 to 1.32 A/mm. Moreover, the incorporation of BA significantly increased the elongation at break (with increment of 26.6%) and slightly decreased the tensile strength (with decrement of 1.8 MPa) of GL film. Results from antimicrobial study showed an improvement of antimicrobial property of GL-BA film when tested against both \textit{Escherichia coli} (Gram-negative) and \textit{Staphylococcus aureus} (Gram-positive) as compared to GL film. Based on a 14-day preservation study, GL-BA film was successfully able to preserve cherry tomatoes (\textit{Solanum lycopersicum}) by lowering the weight loss and browning index values. Overall, results from this study highlight the feasibility of BA as a natural additive to improve the applicability of GL film for food preservation.

1. Introduction
The food preservation is essential to human being in order to ensure a better food quality and prevent food from contaminate. According to United Nations [1], the consumption of contaminated food had caused more than one-third of the total population in developing countries experienced food poisoning. Plastic materials are widely used in food packaging however they are not degradable and had caused serious environmental problems [2]. Biopolymer materials are commonly derived from plant or animal which are able to degrade naturally [3]. The incorporation of biopolymer films such as gelatin, chitosan and methylcellulose with natural additives has attracted great interest of food scientists [4]. This is mainly because biopolymer films can act as vehicles that carry active agent such as antimicrobial and/or antioxidant which can improve the biopolymer films properties to preserve food [3]. Gelatin is abundance in nature as a major constituent of bones, connective tissues and skin such as beef, chicken, pork and fish [5]. However, fish as a source of gelatin is important nowadays particularly for Muslim consumers. Bovine gelatin may cause diseases and pork is banned for religious reason [6]. Gelatin has good film forming and oxygen barrier properties but has several disadvantages such as poor water barrier, weak mechanical properties and non-antibacterial properties [6]. This research aimed to synthesis an environmental friendly bioactive film by incorporation of gelatin and banana leaf essential oil that possesses key features for food preservation.
2. Materials and methods

2.1. Chemicals
Gelatin (GL) with a bloom strength of 210 was supplied by Nur Halal Gelatine Resources, Malaysia. Banana leaf essential oil (BA) was purchased from Best Formula Industries, Malaysia. Tween 20 and glycerol were provided by Merck. Commercial cling wrap (low-density polyethylene) was purchased from a local supermarket in Tanjong Malim, Malaysia. All chemicals used in this study were of analytical grade.

2.2. Preparation of GL and GL-BA Films
GL solution (3.5% w/v) was prepared by dissolving 3.5 g of GL in 100 mL of deionised water. The mixture was stirred until homogenous using a magnetic stirrer at 60 °C. The solution was then added with glycerol at a concentration of 0.5 mL/g gelatin and stirred for 30 minutes. Tween 20 was mixed with 1 mL of BA at a concentration of 0.2% (v/v) for 30 minutes. The mixture was added into GL solution and continues stirred for 1 hour at room temperature (25 °C). GL solution (20 mL) was poured onto a petri dish (9 mm diameter) and dried in an oven at 35 °C for 24 hours. Dried film was peeled off and stored in a seal packaging to avoid moisture effect. GL films without addition of BA were also prepared as controls.

2.3. Characterisation Study
The physicochemical properties of GL and GL-BA films were characterised by using Fourier Transform Infrared (FTIR) Spectrometer, Thermogravimetry Analyser (TGA), UV-Visible (UV-Vis) Spectrophotometer, Scanning Electron Microscope (SEM) and Universal Testing Machine (UTM). The FTIR spectra were obtained in the wavenumber range between 4000 and 400 cm⁻¹ with over 25 cumulative scans using a Thermo Nicolet 6700 FTIR Spectrometer. TGA characterisation was performed using a Perkin-Elmer Pyris 1 Thermal Gravimetric Analyser to determine the thermal stability and decomposition stage of film samples. The film samples were heated from 30 until 600 °C at a heating rate of 10 °C per min under a nitrogen flow and the mass of the sample pan was recorded. The UV-Visible analysis was conducted on an Agilent Cary 60 UV-Vis Spectrophotometer in the wavelength range of 200 to 800 nm. The transparency value of the film was calculated using the following Equation (1) [7]:

\[
\text{Transparency} (A/mm) = - \log T / x
\]

where \( A \) is the absorbance at wavelength 600 nm, \( T \) is the transmittance (%) at wavelength 600 nm and \( x \) is the film thickness (mm). The SEM images of the film samples were captured by using a Hitachi SU 8020 UHR SEM to observe the surface morphology of the film. The mechanical property (tensile strength and elongation at break) of the film was determined using an Instron 5067 UTM. The water vapour permeability (WVP) of the films was determined according to ASTM E96-95 (ASTM 1995) procedure by using a PERME W3/060 Water Vapour Transmission Rate Test System. The method was outlined by Joseph et al. [8], where the film sample (50 cm²) was placed on the top of aluminium cup filled with fused calcium chloride. The aluminium cup was sealed with a mix of microcrystalline and paraffin waxes. The temperature was set at 38 °C and 90% of relative humidity, where the cups were placed and weighed every hour for 6 hours. Based on Equation (2), the water vapour transmission rate (WVTR) was determined by a slope of linear portion of the weight gained versus time. The Equation (3) was used to calculate the WVP value of film samples.

\[
WVTR = \text{slope} / \text{film area}
\]

\[
WVP = (\text{WVTR} \times L) / (P_2 - P_1)
\]

where \( P_1 \) is the partial pressure inside the cup (kPa), \( P_2 \) is the water vapour partial pressure at the film outer surface (kPa) and \( L \) is the average film thickness (mm). The values of WVP were expressed
in g m\(^{-1}\) day\(^{-1}\) atm\(^{-1}\). The oxygen permeability (OP) of the films was determined according to ASTM D1434 procedure (ASTM 1983) by using permeability cell. 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. Equation (4) was used to calculate the oxygen transmission rate (OTR) under standard experimental condition.

\[
OTR = 34,029 \times \text{slope/pressure}
\]  

(4)

The 34,029 value represents capillary constant. The OP values of the film samples were calculated by multiplying the OTR with thickness of the film and reported in cc m\(^{-1}\) day\(^{-1}\) atm\(^{-1}\).

2.4. Antimicrobial Study

The antimicrobial study of GL film was conducted by the diffusion method in agar, using sterile filter paper discs (5 mm diameter) which were first dipped in biopolymer film solution [9]. The antimicrobial activity of GL and GL-BA were tested against \textit{Staphylococcus aureus} (Gram-positive bacteria) and \textit{Escherichia coli} (Gram-negative bacteria). The dipped filter papers were then placed into each bacterium and were incubated at 37 ºC for 24 hours. The inhibition zone which considered as a measure of the antimicrobial activity was measured after incubation.

2.5. Preservation Study

Cherry tomatoes (\textit{Solanum lycopersicum}) were obtained from an agricultural farm in Cameron Highlands, Malaysia and used as food samples. The fruits were washed and air-dried before the initial weight was recorded. The fruits were wrapped with synthesised biopolymer films and commercial cling wrap (CC, low-density polyethylene) as shown in figure 1. The unwrapped fruits serve as controls. The fruits were kept in the laboratory at room temperature for 14 days. The final weight of each cherry tomato was measured at the end of preservation study. The weight loss (%) and browning index were calculated using Equations (5) and (6), respectively [10]:

\[
\text{Weight loss} \%(\%) = \left( \frac{W_i - W_f}{W_i} \right) \times 100
\]  

(5)

where \(W_i\) and \(W_f\) are the initial and final weights of the fruits, respectively. The browning level was classified as: 1 = no browning, 2 = less than 20%, 3 = around 20 to 40%, 4 = around 40 to 60% and 5 = more than 60%.

\[
\text{Browning index} = \frac{\sum (\text{Browning level}) \times \text{number of fruit at the browning level}}{\text{Total number of fruit in the treatment}} \times 100
\]

(6)

\(\text{Figure 1.}\) Unwrapped cherry tomatoes (a), cherry tomatoes wrapped with CC film (b), GL film (c) and GL-BA film (d).
3. Results and discussion

3.1. FTIR
From figure 2(a), the absorption bands of GL film at 3217 cm\(^{-1}\) can be assigned to N–H and –OH stretching (Amide-A), at 2932 cm\(^{-1}\) can be attributed to C−H stretching (Amide-B), at 1622 cm\(^{-1}\) corresponds to C=O stretching (Amide-I), at 1528 cm\(^{-1}\) can be related to N–H bending (Amide-II) and the peak appeared at 1230 cm\(^{-1}\) represents C–N stretching (Amide-III) [11]. Moreover, the presence of –OH group of glycerol was confirmed by the appearance of an absorption band at the wavenumber of 1031 cm\(^{-1}\) [12, 13]. The BA spectrum (figure 2(b)) shows two sharp absorption peaks at 2919 cm\(^{-1}\) and 2851 cm\(^{-1}\) which can be assigned to the stretching of methyl and methylene groups, a prominent band at 1731 cm\(^{-1}\) can be attributed to C=O stretching of ketone and the band observed at 1466 cm\(^{-1}\) may be due to C-C stretching in the phenyl ring [14]. After incorporation of GL and BA, the spectrum of GL-BA film (figure 2(c)) exhibits a shoulder at 1732 cm\(^{-1}\) which might be due to C=O stretching. This can be related to polyphenol group of essential oil which is able to form hydrogen and covalent bonds with functional groups such as C-O, N-H and O-H [15].

Figure 2. FTIR spectra of GL (a), BA (b) and GL-BA (c).

3.2. SEM
Figures 3(a) and 3(b) show surface morphology of GL and GL-BA films, respectively. GL film (figure 3(a)) exhibits a smooth, dense and non-porous morphology, which a similar observation was reported by Ahmed and Ikram [16]. After addition of BA, the surface morphology of GL-BA film (figure 3(b)) became rough with grooves and network patterns texture. The roughness of film surface could be due to the incorporation of different phase of film formulations, namely hydrophobic (essential oil) and hydrophilic (biopolymer materials) [17]. As discussed by Tongmuanchan et al. [12], the addition of essential oil results in an increase in the roughness of film surface which might be due to the disruption of protein-protein interaction in film matrix. The addition of essential oil may also interrupt the hydrogen bond which could lead to the roughness of the film [18]. From figure 3(b), it is clear that
there was no formation of pores on the surface of GL film following addition of BA. Results from SEM analysis suggest that incorporation of BA to GL film affects the surface morphology of GL film with no porosity texture was observed.

![SEM images of GL (a) and GL-BA films (b) at 25,000× magnification.](image)

3.3. TGA

Figure 4 illustrates the TGA thermograms of GL and GL-BA films which were heated from 30 until 600 °C. Both films present two decomposition stages. The first decomposition stage of GL (figure 4(a)) occurred at temperature range of 67-79 °C, which may be due to evaporation of water and glycerol with 15.4-16.2% of weight loss [19]. Meanwhile, the second decomposition stage of GL was observed at 342-420 °C with a higher weight loss of 51.3-58.7%, as compared to the first decomposition stage. The second decomposition stage can be attributed to degradation of biopolymer materials [12]. From figure 4(b), it is apparent that the temperature for second decomposition stage of GL-BA was higher than GL film. This result suggests that the formation of bond between GL and BA was stable since more heat is required to decompose the compound.

![TGA thermograms of GL (a) and GL-AA films (b).](image)

3.4. UV-Vis

The films for food preservation should be able to block or minimise the light diffusion, which responsible for food deterioration [20]. Hence, films with opaque properties are particularly important in order to prevent the effects of light. Therefore, it is imperative to study the light barrier property of films. In this study, the transparency and opacity of the films were determined by UV-Vis spectroscopy technique. Table 1 lists the light transmission (%) values at different wavelength and transparency values of films including commercial cling (CC) film as a synthetic material. At UV region of 280 nm, the highest light transmission (%) value was recorded for CC film with 83.9% followed by GL film (29.5%) and GL-BA film (16.4%). The obtained result suggests that the GL film had a better barrier to prevent UV light as compared to CC film. This phenomenon could be due to the presence of chromophore groups such as tyrosine and phenylalanine amino acid in GL that are able to absorb UV light [16]. After addition of BA, the light transmission (%) decreased significantly from 83.9% to 16.4%, which could be related to light scattering at the interface of essential oil droplets.
imbedded in the film matrix [21]. As shown in table 1, the lowest transparency value was CC film (0.495 A/mm) followed by GL (0.501 A/mm) and GL-BA (1.32 A/mm) films. According to Hosseini et al. [6], a high transparency value indicates a high opaque property of film. Furthermore, the increase in opacity of film can be positively related to the improvement of light barrier [22]. The results obtained suggest that the GL-BA film had a better light barrier property as compared to CC and GL films.

**Table 1. Light transmission and transparency value of CC, GL and GL-BA films.**

| Film      | Light transmission (%) at different wavelength | Transparency value (A/mm) |
|-----------|-----------------------------------------------|--------------------------|
|           | 200   | 280   | 350   | 400   | 500   | 600   | 800   |                  |
| CC        | 8.10  | 83.9  | 90.2  | 91.5  | 91.7  | 92.3  | 91.8  | 0.495            |
| GL        | 0.0460| 29.5  | 76.4  | 85.2  | 89.2  | 90.1  | 90.8  | 0.501            |
| GL-BA     | 0.0200| 16.4  | 48.4  | 57.3  | 64.4  | 67.4  | 70.7  | 1.32             |

3.5. Mechanical properties

Table 2 lists the tensile strength (TS) and elongation at break (EAB) values of films. The evaluation of TS and EAB are important in order to determine the ability of films to protect the food products throughout distribution [9]. The results showed a slight decrement of TS value following addition of BA from 10.5 to 8.70 MPa. However, a significant increase in EAB value from 56.9 to 83.5% was measured after addition of BA to GL film. This observation could be due to enhancement in plasticiser’s effect when BA was added to GL film forming solution. Clarke et al. [23] reported that the addition of malic acid has provided plasticiser effect towards gelatin film which increased the EAB value of biopolymer film. The decrease in TS value may be due to discontinuities in polymer network caused by hydrophobic material. Although addition of BA decreased the TS value of GL film, it is interesting to note that the TS values of both GL (10.5 MPa) and GL-BA (8.70 MPa) films are technically accepted as packaging film because the conventional standard of TS for packaging film must be more than 3.50 MPa [9].

3.6. WVP and OP analyses

The WVP and OP values of films are presented in table 2. From table 2, GL film exhibits the highest WVP and OP values as compared to GL and CC films. The presence of large range of hydrophilic amino acid in GL film leads to more diffusion of water through the film [6]. The addition of BA successfully decreased the WVP value with decrement of 0.17 g m⁻¹ day⁻¹ atm⁻¹ and OP value with decrement of 0.07 cc m⁻¹ day⁻¹ atm⁻¹ of GL film. BA may increase the formation of hydrogen bond and decrease the free volume of the GL film. These phenomena are likely to improve the water and oxygen barrier properties [20]. Moreover, the improvement of barrier against water and oxygen can be related to hydrophobic effect of essential oil [13]. As discussed in SEM analysis section, the addition of BA has resulted in a significant changed in the surface morphology and texture of GL film. The smooth morphology of GL film became rough with grooves network texture following BA application, which may have an effect on WVP and OP. A study by Aljawish et al. [24] has found that the incorporation of ferulic acid and ethyl ferulate to chitosan film has resulted in a compact surface morphology, which has led to a significant decrease in OP value of the film. The WVP and OP values of GL-BA film are not comparable with those measured for CC film which has a better barrier against water vapour and oxygen. Overall, the findings of WVP and OP analyses suggest that the addition of BA essential oil improved the water and oxygen barrier of GL film.
Table 2. Tensile strength, elongation at break, WVP and OP of CC, GL and GL-BA films.

| Film      | Tensile strength (MPa) | Elongation at break (%) | WVP (g m⁻¹ day⁻¹ atm⁻¹) | OP (cc m⁻¹ day⁻¹ atm⁻¹) |
|-----------|------------------------|-------------------------|-------------------------|--------------------------|
| CC        | 39.7 ± 1.33            | 238 ± 2.06              | 0.86 ± 0.73             | 0.72 ± 0.43              |
| GL        | 10.5 ± 2.22            | 56.9 ± 2.74             | 1.73 ± 0.26             | 1.51 ± 0.30              |
| GL-BA     | 8.70 ± 2.52            | 83.5 ± 4.94             | 1.56 ± 0.12             | 1.44 ± 0.30              |

3.7. Antimicrobial study

Food safety and shelf-life of food can be improved by the addition of antimicrobial agent to bioactive film in order to prevent the growth of microorganism that can cause foodborne illness [25]. In this study, *Escherichia coli* (Gram-negative) and *Staphylococcus aureus* (Gram-positive) were used as tested microorganisms. Both food-borne pathogens can cause death [26]. As shown in table 3, there was no inhibition zone of GL against these pathogens. Clarke et al. [23] reported that GL had no antimicrobial activity against *E. coli* and *S. aureus*. After addition of BA, the inhibition zone increased from 0 mm to 10 mm against *S. aureus* and from 0 to 8 mm against *E. coli*. Based on table 3, it is clear that the addition of BA was more effective against *S. aureus* than *E. coli*. This is mainly due to structure of complex cell walls present in gram-negative bacteria [18]. Furthermore, plant essential oil consists of terpenoid and phenolic compounds that possess antibacterial properties [13]. Results from antimicrobial study suggest that the incorporation of banana leaf essential oil improved the antimicrobial property of gelatin film.

Table 3. Inhibition zone of GL and GL-BA against *S. aureus* and *E. coli*.

| Film   | *S. aureus* | Inhibition zone (mm) | *E. coli* | Inhibition zone (mm) |
|--------|-------------|----------------------|-----------|----------------------|
| GL     |             | 0                    | 0         |                      |
| GL-BA  |             | 10                   | 8         |                      |

3.8. Preservation study

Table 4 presents the weight loss and browning index of preserved cherry tomatoes for 14 days at room temperature. From table 4, the unwrapped fruits had the highest weight loss and browning index followed by GL, GL-BA and CC films. There are several factors that can affect the weight loss and browning index of food such as light, oxygen, water and microorganism activity [27, 28]. The unwrapped fruits were exposed to light, oxygen and water vapour without any protection, hence had the highest weight loss and browning index. It is apparent that the addition of BA reduced the weight loss and browning index of cherry tomatoes from 28.8 to 20.9% and from 110 to 90, respectively. In fact, the findings from FTIR, SEM, TGA, UV-Vis, WVP, OP and antimicrobial studies have proven that GL-BA film had a better protection against light, water, oxygen and antimicrobial as compared to GL film. As expected, the CC film exhibits the lowest weight loss and browning index of cherry tomatoes. Based on results obtained from preservation study, it can be concluded that the incorporation of BA improved the GL film to preserve cherry tomatoes.
Table 4. Weight loss and browning index of preserved cherry tomatoes after 14 days.

| Film    | Weight loss (%) | Browning index |
|---------|-----------------|----------------|
| Unwrapped | 32.3 ± 1.62      | 150            |
| CC       | 13.0 ± 2.52      | 45             |
| GL       | 28.8 ± 1.35      | 110            |
| GL-BA    | 20.9 ± 1.77      | 90             |

4. Conclusion
In conclusion, the results obtained from this research study suggest that the addition of banana leaf essential oil to gelatin film had significantly improved the light, water vapour and oxygen barrier of the film. This improvement had greatly influenced the ability of gelatin film to preserve cherry tomatoes. Furthermore, banana leaf essential oil provides an antimicrobial property to gelatin film, which has been proven able to inhibit *S. aureus* and *E. coli*.

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