Chitosan-Key Lime Film for Food Preservation

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Abstract—In the present work, environmental friendly films were prepared by incorporating key lime (\textit{Citrus aurantifolia}) extract to chitosan biopolymer. The chitosan-key lime (CKL) films were synthesised using two concentrations of key lime, namely 25\% and 50\% (v/v). The physical and chemical properties of CKL films were characterised using several analytical instruments such as Fourier Transform Infrared Spectrometer (FTIR), UV-Visible Spectrophotometer (UV-Vis), Scanning Electron Microscope (SEM), Differential Scanning Calorimeter (DSC) and Universal Testing Machine. The addition of key lime extract reduced the transparency value of biopolymer films. A pronounced effect was obtained for CKL 50\% (v/v) film, of which the opacity value was decreased from 1.06 (chitosan film) to 0.54. The elongation at break of the films was increased from 13.34\% (chitosan film) to 76.06\% (CKL 25\% v/v) and 154.69\% (CKL 50\% v/v), respectively. Meanwhile, the tensile strength of the films was decreased from 34.09 MPa (chitosan film) to 10.28 MPa (CKL 25\% v/v) and 3.02 MPa (CKL 50\% v/v), respectively. Gram-positive bacteria (\textit{Staphylococcus aureus}) and Gram-negative bacteria (\textit{Escherichia coli}) were used to test the antimicrobial activity of the films. The CKL films had a superior antimicrobial property against \textit{S. aureus} and \textit{E. coli} as compared to chitosan film. Based on preservation study, CKL films were able to control the weight loss and browning index of cherry tomatoes. Overall, results from this study suggest that CKL films have great potential to preserve food products.

Keywords: biopolymer film, chitosan, food preservation, key lime

I. INTRODUCTION

One of the challenges in food industry is related to the packaging of food products with a short shelf-life period [1]. Although conventional packaging materials such as plastics and their derivatives are effective to preserve food products, they create serious environmental problems that continue to present the food industry as a source of pollution [1,2]. For this reason, all stakeholders in food industry as well as scientists specialising in the food engineering and packaging are required to seek alternatives to overcome this serious problem.

In recent years, there is a growing interest in food industry primarily on product innovation that satisfies consumers’ demand for high quality and healthy food products. A preference for food products with natural instead of synthetic additives is also significantly related to health concerns [3]. Edible films from biopolymers offer an application of the active food packaging [4]. Biopolymers can be used as vehicles for additives like antioxidant and/or antimicrobial agents, vitamins, flavours and pigments [5]. This will improve the quality and increase the shelf-life of the foods.

In this context, chitosan (\(\beta\)-(1.4)-2-amino-2-deoxy-\(\beta\)-glucose) has attracted much attention. Chitosan has excellent film forming properties, and derives from chitin, the second most abundant natural polymer in nature after cellulose [6]. It is normally produced from waste bio-products of shellfish industry. Chitosan can also be obtained from the chitin component of fungal cells walls [7]. Chitosan has cationic groups along its backbone, which has been shown to have antimicrobial properties against bacteria, yeasts, mould and fungi [1,6,7]. In addition, chitosan posses several unique properties such as biodegradability, biocompatibility, non-toxicity and it is renewable [7].

Chitosan can be applied in two forms, namely coatings and films [7]. The formulation of chitosan coatings and films usually involve acetic acid as a solvent. The use of acetic acid even at low concentration (1\% v/v) will produce an unpleasant smell. This scenario has reduced its application in food preservation. Therefore, this research was devised as a direct response to the aforementioned issue. We hypothesised that
key lime (Citrus aurantifolia) extract can be used as an alternative to acetic acid, producing both environmental and user friendly films for food preservation.

II. MATERIALS AND METHODS

A. Chemicals

Chitosan with low molecular weight (85% degree of deacetylation) was purchased from Sigma-Aldrich. All other chemicals were of analytical grade obtained from Merck and Fisher Scientific. Key lime (C. aurantifolia) was purchased from a local market. Deionised water was used for the experiments.

B. Preparation of Chitosan and Chitosan-Key Lime Films

Films were prepared using casting procedure. Exactly 100 mL of acetic acid (1% v/v) was added to 2 g of chitosan powder. The mixture was stirred and heated at 60 ºC for 30 min using a magnetic stirrer to avoid lumps and obtain homogeneous solution. The solution was underwent vacuum for 20 s to eliminate bubbles and poured onto disposable polystyrene petri dishes (85 mm x 15 mm). Petri dishes were left to cool for 30 min at room temperature and air-dried in a convection oven at 40 ºC for 24 h. The films obtained were cooled at room temperature, carefully peeled from petri dishes and stored in a vacuum desiccator.

The chitosan-key lime (CKL) films were prepared using a similar procedure for preparation of chitosan film. For this purpose, exactly 100 mL of key lime extracts (25% and 50% v/v) were added separately to two sets of 2 g of chitosan powder. The mixtures were stirred, heated and dried in an oven as described earlier. The films obtained were kept in a desiccator until ready for analysis.

C. Characterisation Studies

Several analytical instruments such as Fourier Transform Infrared Spectrometer (FTIR), UV-Visible Spectrophotometer (UV-Vis), Scanning Electron Microscope (SEM), Differential Scanning Calorimeter (DSC) and Universal Testing Machine (UTM) were used to characterise the physical and chemical properties of chitosan and CKL films.

The FTIR spectra of the films were recorded in the wavenumber range between 4000 and 400 cm$^{-1}$ with over 25 cumulative scans using a Thermo Nicolet 6700 FTIR.

The transmission of UV and visible light in each film was recorded from 200 to 800 nm using an Agilent Cary 60 UV-Vis. The transparency of the film was calculated using the following equation [8]:

$$\text{Transparency (A/mm)} = \frac{-\log T}{x}$$

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 surface morphology of the films was observed using a Hitachi SU 8020 UHR SEM. The films were first coated using platinum to avoid charging.

The thermal stability of the films was studied using a PerkinElmer Diamond DSC. The films were heated within temperature range of -10 to 600 ºC at a rate of 10 ºC/min under a nitrogen flow.

The tensile strength and elongation at break of the films were determined using an Instron 5067 UTM.

D. Antimicrobial Properties

The antimicrobial activity of chitosan-key lime films was determined by the agar disc diffusion method against Gram-positive bacteria (Staphylococcus aureus) and Gram-negative bacteria (Escherichia coli). Bacteria were incubated at 37 ºC for 24 h. After incubation, the inhibition area which considered as a measure of the antimicrobial activity will be measured.

E. Preservation Study

Fresh cherry tomatoes were used in this study. Three cherry tomatoes were randomly selected, washed using deionised water and dried. The weight of each cherry tomato was measured. The fruits were then wrapped using the key-lime films and kept at room temperature. Two equations, namely weight loss (%) and browning index are involved in preservation study.

The weight loss (%) was calculated using the following equation [9]:

$$\text{Weight Loss} = \frac{W_0 - W}{W_0} \times 100$$

where $W_0$ is the initial weight and $W$ is the final weight of the sample.
The browning index was calculated using the following equation [10]:

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

III. RESULTS AND DISCUSSION

A. Characterisation Studies

FTIR Analysis

In this study, FTIR analysis was performed in order to determine the chemical structure of chitosan and CKL films. The FTIR spectra of chitosan and CKL films are shown in Figure 1. From Fig. 1(a), chitosan shows characteristic peaks at 3368 cm\(^{-1}\) (\(-\text{OH}\) and \(-\text{NH}_2\) stretching), 2873 cm\(^{-1}\) (\(-\text{CH}\) stretching), 1632 cm\(^{-1}\) (amide I), 1550 cm\(^{-1}\) (amide II), 1409 cm\(^{-1}\) (CH\(_2\) symmetrical stretching band) and 1075 cm\(^{-1}\) (C-O-C asymmetric stretching) [11].

Following reaction with key lime extracts, the \(-\text{OH}\) and \(-\text{NH}_2\) band was shifted from 3368 to 3396 cm\(^{-1}\), while the \(-\text{CH}\) band was shifted from 2873 to 2920 and 2933 cm\(^{-1}\) (Figs. 1(b) and 1(c)). A similar observation was reported by Aresta et al. [12]. They reported that the broad band which represents \(-\text{OH}\) and \(-\text{NH}_2\) groups was shifted from 3410 to 3310 cm\(^{-1}\) after incorporation of ascorbic acid into chitosan nanoparticles. A new absorption band was observed at 1715 cm\(^{-1}\), which corresponds to C=O stretching. It is known that limes are an excellent source of ascorbic acid (C\(_6\)H\(_8\)O\(_6\)) [13]. Therefore, the appearance of C=O stretch at 1715 cm\(^{-1}\) can be related to the presence of ascorbic acid in the CKL films. Interaction of chitosan and key lime extract has led to combination of the absorption bands of amide I (1632 cm\(^{-1}\)) and amide II (1550 cm\(^{-1}\)), producing a new absorption band at 1617 cm\(^{-1}\). The CH\(_3\) stretching band was shifted from 1409 cm\(^{-1}\) to 1393 cm\(^{-1}\) with a significant reduction in absorption intensity. In contrast, the wavenumber of C-O stretch band was shifted from 1150 cm\(^{-1}\) to 1216 cm\(^{-1}\) with a considerable increase in absorption intensity.

Overall, the change in wavenumber and absorption intensity could be attributed to interaction between \(-\text{OH}\) and \(-\text{NH}_2\) groups of chitosan and \(-\text{OH}\) and C=O groups of ascorbic acid. In general, the aforementioned functional groups are capable of forming hydrogen bonds [14].

![FIGURE 1. FTIR spectra of (a) Chitosan, (b) CKL 25%, and (c) CKL 50%.](image_url)

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

where \(W_i\) and \(W_f\) are the initial and final weights of the fruits, respectively.
**UV-Vis Analysis**

Transparency of films is important due to their great impact on the appearance of the food products [2,15]. Since consumers prefer to see foods, higher transparency would have an advantage. Light transmission and transparency of chitosan and CKL films at selected wavelength are given in Table 1. The transmission of UV light in chitosan and CKL films was very low at 200-280 nm. In fact, it was negligible at 200 nm. This suggests that the films have excellent UV barrier properties, which induces lipid oxidation in the food system [1,15].

As shown in Table 1, the transmission of visible light was between 8.35 to 82.84% at 350-500 nm, and was greater than 80% at 600-800 for all films. In visible range (350-800 nm), both CKL films (25 and 50% v/v) exhibited lower light transmission than the chitosan film (control). The addition of key lime extract to chitosan reduced the transparency of CKL films. The opaque appearance of the CKL films reflects the visible light, thereby hinders light transmission through the films. The transparency value (A/mm) decreased from 1.06 (chitosan film) to 0.79 (CKL 25%) and 0.54 (CKL 50%), respectively. The transparency of films was greatly affected by concentration of key lime extract, of which higher concentration caused greater reduction in transparency. The effect of extract lime on reducing the transparency of chitosan film is similar to the effect of cellulose nanoparticles on transparency of hydroxypropyl methylcellulose (HPMC) films [16]. The transparency value of chitosan film obtained from this study was higher than those reported by Hosseini et al. [8] for chitosan (0.95 A/mm) and gelatin (0.56 A/mm) films, respectively.

**TABLE 1.** Light transmission (%) and transparency of chitosan and CKL films.

<table>
<thead>
<tr>
<th>Film</th>
<th>Light transmission at different wavelength (%)</th>
<th>Transparency (A/mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>200</td>
<td>280</td>
</tr>
<tr>
<td>Chitosan</td>
<td>0.04</td>
<td>21.96</td>
</tr>
<tr>
<td>CKL 25%</td>
<td>0.02</td>
<td>4.52</td>
</tr>
<tr>
<td>CKL 50%</td>
<td>0.01</td>
<td>2.14</td>
</tr>
</tbody>
</table>

**SEM Analysis**

The morphology and homogeneity of the matrix affect the permeability of a film [17]. SEM analysis was carried out to understand the effect of key lime extract on surface morphology of chitosan film. The SEM images of chitosan and CKL films are shown in Fig. 2. From Fig. 2, chitosan and CKL films display a smooth and homogeneous surface texture, without pores. When viewed the films at 5 μm, there was no significant different between chitosan and CKL films. In fact, the application of key lime extract at different concentrations produced a similar surface texture (Figs. 2(b) and 2(c)). This implies that concentrations of key lime did not influence the surface morphology of the films.

Depending on physical and chemical properties of the biopolymer and additives, the addition of additives may alter the surface morphology of biopolymer film. For example, Shittu et al. [18] observed a significant change in surface texture of chitosan film when pseudoboehmite alumina (BAH) was added at three rates, namely 1, 2 and 3 % (w/w). They reported that an increase in BAH content reduced the surface smoothness, as well as the formation of holes and crack texture on the surface of chitosan film. Hosseini et al. [15] studied the effects of chitosan nanoparticles addition on surface morphology of chitosan film. The application of chitosan nanoparticles at 2% (w/w) was reported to produce a composite film with a smooth and good particles distribution, as well as without formation of aggregates. No significant difference was reported when compared with chitosan film.

![FIGURE 2. SEM images of (a) Chitosan, (b) CKL 25%, and (c) CKL 50%.](image-url)
DSC Analysis

The changes of the thermal properties of chitosan film as a result of key lime extract incorporation were studied using DSC. The DSC thermograms of chitosan and CKL films are shown in Fig. 3. From Fig. 3(a), chitosan showed characteristic of weak exothermic peak at 151 °C and endothermic peak at 299 °C. Interaction of chitosan with key lime extract at 25 and 50% (v/v) concentrations has caused the exothermic peak at 151 °C to shift to 175 and 165 °C (Figs. 3(a) and 3(b)), respectively. This scenario could be attributed to formation of hydrogen bond between ascorbic acid and polymeric network of chitosan. A similar observation was obtained for chitosan-Vitamin C film studied by Aresta et al. [12]. The temperature for exothermic peak was shifted to a higher temperature following incorporation of Vitamin C into chitosan.

A significant change in thermal properties of chitosan was obtained when key lime extract was added at concentration of 50% (v/v). From Fig. 3(c), the DSC thermogram of CKL 50% (v/v) film exhibited a characteristic of exothermic at 165 °C, and three endothermic peaks at 139, 226 and 340 °C. This finding suggests that the thermal properties of chitosan were greatly influenced by concentration of key lime extract. The change in crystalline structure and thermal stability of chitosan would alter the permeability of chitosan film.

Mechanical Properties

Adequate mechanical strength and extensibility are key criteria for a packaging film [7,17]. Table 2 presents the percentage of elongation at break (% EAB) and tensile strength of chitosan and CKL films. The EAB of chitosan increased with addition of key lime extract. In fact, an increase in concentration of key lime extract had significantly increased the EAB of the films. A pronounced effect was obtained for CKL 50% film, of which the EAB was increased from 13.34% (chitosan film) to 154.69%. A lesser effect was observed for CKL 25%, with an increment of 62.72%. In contrast to EAB, the TS value (MPa) decreased from 34.09 to 10.28 and 3.02 for CKL 25% and CKL 50%, respectively. A similar trend of reduction was obtained by Pereda et al. [19]. They reported that the TS value for chitosan-bovine gelatin reduced with an increase in amount of bovine gelatin. Based on mechanical properties study, it can be concluded that the strength and flexibility of the chitosan-key lime films could be modified by changing the concentration of key lime extract.

TABLE 2. Elongation at break (EAB) and tensile strength (TS) of chitosan and CKL films.

<table>
<thead>
<tr>
<th>Film</th>
<th>EAB (%)</th>
<th>TS (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chitosan</td>
<td>13.34</td>
<td>34.09</td>
</tr>
<tr>
<td>CKL 25%</td>
<td>76.06</td>
<td>10.28</td>
</tr>
<tr>
<td>CKL 50%</td>
<td>154.69</td>
<td>3.02</td>
</tr>
</tbody>
</table>

B. Antimicrobial Properties

Antimicrobial film is important for active packaging systems that have been found highly effective in killing or inhibiting spoilage and pathogenic microorganisms that contaminate foods [20]. The antimicrobial activity of chitosan and CKL films was assessed using Gram-positive bacteria (S. aureus) and Gram-negative bacteria (E. coli). The inhibition zone of the films against S. aureus and E. coli was calculated based on the formation of bacterial inhibition zone around the test films, which is given in
Table 3. The addition of key lime extract improved antimicrobial property of chitosan. A significant inhibition effect was observed on *S. aureus*, of which the inhibition zone was increased from 7 mm (chitosan film) to 9 mm (CKL 25%) and 11 mm (CKL 50%), respectively. A slight increase in the inhibition zone was observed for *E. coli*, of which it was increased from 5 mm (chitosan film) to 7 mm (CKL 25%) and 8 mm (CKL 50%), respectively. Results suggest that an increase in concentration of key lime extract would enhance the antimicrobial activity of chitosan film. It is interesting to note that natural additive like key lime extract can be an alternative to synthetic additives for controlling food pathogens.

<table>
<thead>
<tr>
<th>Film</th>
<th><em>S. aureus</em> Inhibition zone (mm)</th>
<th><em>E. coli</em> Inhibition zone (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chitosan</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>CKL 25%</td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td>CKL 50%</td>
<td>11</td>
<td>8</td>
</tr>
</tbody>
</table>

C. Preservation Study

The effectiveness of chitosan and CKL films to preserve food was evaluated in a preservation study using cherry tomatoes. Unwrapped fruits were used as controls in this study. The changes in the appearance and fruit freshness were monitored in naked eyes. After 14 days of preservation study, the weight loss and browning index of cherry tomatoes were determined, which are presented in Table 4. From Table 4, it is clear that wrapping fruits with chitosan and CKL films reduced the weight loss and browning index. A significant effect was obtained for CKL 50% films, of which the weight loss was reduced from 45.17% (control) to 9.22%. Meanwhile, the browning index was successfully reduced from 400 (control) to 120.

Several studies have shown that ascorbic acid was able to reduce weight loss and browning of fruit and vegetables. Ayranci and Tunc [21] has studied the effects of ascorbic acid addition in methylcellulose film. The methylcellulose-ascorbic acid film was reported able to control browning and reduce vitamin loss in mushrooms and cauliflower. In another study, Perez-Gago et al. [22] had successfully control browning in fresh-cut potatoes and apples by using whey protein concentrate-ascorbic acid film. It is known that natural additives are normally rich which compounds that can act as antioxidants and antimicrobial activity [13,23]. Therefore, it can be concluded that the presence of ascorbic acid in key lime extract may act as antioxidant that prolonged the freshness of cherry tomatoes.
TABLE 4. Weight loss and browning indices of cherry tomatoes wrapped using chitosan and CKL films.

<table>
<thead>
<tr>
<th>Film</th>
<th>Weight loss (%)</th>
<th>Browning index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (no film)</td>
<td>45.17</td>
<td>400</td>
</tr>
<tr>
<td>Chitosan</td>
<td>26.67</td>
<td>330</td>
</tr>
<tr>
<td>CKL 25%</td>
<td>18.93</td>
<td>250</td>
</tr>
<tr>
<td>CKL 50%</td>
<td>9.22</td>
<td>120</td>
</tr>
</tbody>
</table>

IV. CONCLUSIONS

In this research, chitosan-key lime films have been successfully developed. The incorporation of key lime extract into chitosan, particularly at concentration of 50% (v/v), has a significant influence on transparency, thermal, mechanical and antimicrobial properties of chitosan film. The chitosan-key lime films have great UV barrier property. Chitosan-key lime films prolonged the freshness of cherry tomatoes. Overall, results from this study highlight the feasibility of key lime extract as an alternative to synthetic additives for food preservation. The key factor for this behaviour is the presence of ascorbic acid in key lime extract.

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