Biobased alginate/castor oil edible films for active food packaging

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ABSTRACT

In this study, we aim to prepare novel bioactive edible films based on sodium alginate and castor oil (CO). The chemical structures and crystallinity were investigated using FTIR and XRD, respectively. Thermal stability by TGA was improved after CO addition. Addition of CO to alginate resulted in better mechanical properties when compared with neat alginate. The water vapour permeability was significantly reduced (p < 0.05) while the total colour difference was not significantly changed (p > 0.05) after CO incorporation. The antibacterial study proved a significant inhibitory effect of the films towards Gram-positive bacteria while no effect was observed for Gram-negative bacteria.

1. Introduction

Great efforts have been done in the recent years to increase food shelf-life by inhibiting the fungal and bacterial development on its surface (Gokkurt, Findik, Unal, & Mimaroglu, 2012). Usage of plastic packaging materials has a number of drawbacks like the generation of wastes associated with these materials. Edible films (EFs) prepared from biodegradable biopolymers may be an effective alternative to avoid the problems resulting from disposing of the conventional packaging materials (Nayak et al., 2008). Polysaccharides, proteins, and lipids are the commonly used polymeric ingredients to produce biodegradable packaging materials (Porta, Mariniello, Di Pierro, Sorrentino, & Giosafatto, 2011). Alginites (A), as biodegradable polymers, are widely utilized to prepare EFs owing to their novel properties as gel and film formation (Azeredo, Miranda, Rosa, Nascimento, & de Moura, 2012; Maizura, Fazilah, Norziah, & Karim, 2007; Riquelme, Herrera, & Matiacevich, 2017; Sipahi, Castell-Perez, Moreira, Gomes, & Castillo, 2013; Wang, Auty, & Kerry, 2010). Alginites are produced through the alkaline treatment of alginic acid which is extracted from Phaeophyceae plant (Lee & Mooney, 2012). Plasticizers are incorporated within alginites films to improve their mechanical properties. Among plasticizers, glycerol is the most extensively employed plasticizer in alginate films (Gontard, Guilbert, & CUQ, 1993). Recently, EFs are prepared on the way to achieve the term “active packaging” through the inclusion of specific compounds as antioxidants and antimicrobial materials. These active EFs undergo some additional functionalities, beside their packaging role, as carrier for antioxidants (Tien, Vachon, Mateescu, & Lacroix, 2001), antimicrobial agents (Millette, Le Tien, Smorigiewicz, & Lacroix, 2007) and other components (Ozdemir & Floros, 2004; Suppakul, Milzt, Sonneveld, & Bigger, 2003), and consequently extend the shelf-life of the food. Alginate films have good resistance to oil and fats transfer but they are poor water barriers because alginites are water-soluble polymers (Wang, Khor, & Lim, 2001). The superior valuable feature of alginites is the capability to yield a stable gel and insoluble polymer when they react through their carboxylate groups with polyvalent metal cations, especially Ca2+ ions (Grant; Morris, Rees, Smith, & Thom, 1973; King, 1983; Zhu, Zhang, Tang, & Kou, 2014). Natural antimicrobial materials like essential oils are frequently incorporated into alginate to prepare active packaging EFs that can inhibit the bacterial growth and thereby prevent the spoilage of food (Azarakhsh, Osman, Ghazali, Tan, & Adzahan, 2014; Pranoto, Salokhe, & Rakshit, 2005; Rojas-Graü et al., 2007). Castor oil (CO) was recognized as the best vegetable oil attributable to its inherent biodegradability, renewability, low cost, and abundant availability (Sathiskumar & Madras, 2011, Abdel Aziz, Elsoholy, & Saad, 2018). Castor oil is the triglyceride of ricinoleic acid which contains reactive secondary hydroxyl groups (Abdel Aziz, Elsoholy, & Saad, 2018). CO exhibits a significant microbial inhibition towards the human pathogen bacteria (Totaro et al., 2014), and this property can be beneficial in the production of antimicrobial EFs.

In this study, we report the preparation of edible films from alginate and castor oil. The effect of CO content on the chemical, physical and antimicrobial properties of the prepared films will be studied.
2. Experimental

2.1. Materials

Sodium alginate (A) was purchased from Alpha-Chemika (Mumbai, India). Castor oil, glycerol, and CaCl₂ were supplied from Sigma-Aldrich (Steinheim, Germany).

2.2. Preparation of antibacterial edible films

1 g of A was dissolved in distilled water (100 mL) upon stirring then the mixture was mixed with glycerol (0.4 mL), as a plasticizer. Diluted solution of castor oil (10 mL/100 mL) from absolute ethanol was then added to the alginate solution in different concentrations 0 mL/100 g, 1.0 mL/100 g, 2.0 mL/100 g and 3.0 mL/100 g. The mixtures were stirred for 3 h at room temperature, cast into polyacrylic plates (12 × 16 cm), then dried at 40°C for 24 h in a vacuum oven. The unpeeled films were soaked in 45 mL of aqueous CaCl₂ solution (1 g/100 g), then the films were dried in a vacuum for 2 h, then the films were peeled off, and washed with deionized water to remove the unreacted CaCl₂ (Pranoto et al., 2005). The sample labels were CA, CA/1% CO, CA/2% CO and CA/3% CO, for films containing CO with 0 mL/100 g, 1.0 mL/100 g, 2.0 mL/100 g and 3.0 mL/100 g, respectively.

2.3. Characterization

2.3.1. FTIR

FTIR was measured through a Perkin-Elmer B25 spectrophotometer using dry KBr pellet method. For each sample, 10 scans were done from 400 to 4000/cm with a resolution of 4/cm.

2.3.2. Wide-angle X-ray diffraction (XRD)

XRD was studied using a Philips Xpert MPD Pro diffractometer with Ni-filter and Cu-Kα radiation source at an accelerating voltage/current of 50 kV/40 mA. The samples were measured in the range 2θ varying from 3° to 70° at rate 2°/min.

2.3.3. Thermogravimetric analysis (TGA)

TGA was measured using Shimadzu TGA-50 H Thermal Analyzer under a nitrogen atmosphere at rate 10 K/min. The samples were scanned from the room temperature to 700°C. From such TGA thermograms, the integral procedural decomposition temperature (IPDT) which can be used to relatively compare the whole thermal stability (Doyle, 1961) is calculated from Eq. (1):

\[
\text{IPDT} = A \cdot K (T_i - T_f) + T_i
\]

where

\[
A = \frac{S_1 + S_2}{S_i + S_2 + S_3}
\] (2)

\[
K = \frac{S_1 + S_2}{S_i}
\] (3)

where \(T_i\) (°C) and \(T_f\) (°C) are the initial and final experimental temperatures, respectively. Graphical illustration of the areas \(S_1\), \(S_2\) and \(S_3\) are illustrated in the supplementary figure (Suppl. 1).

2.3.4. Mechanical testing

Mechanical testing was studied using the Zwick 1445 (Germany) according to ASTM D882-2010 standard (2010). Films samples of 0.2 ± 0.02 mm thickness were cut into strips with 10 ± 0.2 mm length and 5 ± 0.2 mm width. According to this standard, five strips of each sample were measured independently at a drawing speed of 10 mm/min then the measured values were averaged.

\[\] 2.3.5. The water vapour permeability (WVP)

WVP was determined according to ASTM E96-92 (1990). In this technique, a permeation cell filled with water (100% RH; 2.337 kPa vapour pressure at 20°C) was sealed by the edible films. The permeation cell was placed in a desiccator (20°C, 0% RH, 0 kPa vapour pressure). The mass loss of the permeation cell as a result of transferring the water to the desiccator was recorded every 2 h over 24 h. The WVP is calculated using Eq. (4) (Sobral, Menegalli, Hubinger, & Roques, 2001):

\[
\frac{Wx}{\Delta P} = WVP \times t
\]

where \(x\) (mm) is the film thickness, \(A\) (m²) is the permeation area, \(\Delta P\) (kPa) is water vapour partial pressure difference across films, \(t\) (day) is time and \(g\) (g) is the mass loss. The WVP measurements were repeated for three films of the same sample, and the obtained values were averaged.

2.3.6. The colour properties

The colour properties of the film surfaces were studied using a CR 400, Minolta colorimeter (Japan). The resulted parameters are the lightness (L) and chromatic parameters: \(a^*\) (green (-a) to red (+a)) and the \(b^*\) (blue (-b) to yellow (+b)) colours. Each listed value represented the average of measuring three different areas of the same film. Based on the measured parameters, the total colour difference (\(\Delta E\)) is calculated using Eq. (5):

\[
\Delta E = \sqrt{(L^* - L)^2 + (a^* - a)^2 + (b^* - b)^2}
\]

where \(L^* = 95.6\), \(a^* = +0.11\) and \(b^* = +1.7\) are the values of a standard white plate. \(L\), \(a\) and \(b\) are the measured values of the prepared films.

2.3.7. Antibacterial properties

The antibacterial activity of the prepared films was evaluated against S. aureus (RCMB 010010) and B. subtilis (RCMB 010067), as Gram-positive bacteria, E. coli (RCMB 010052) and S. typhi (RCMB 010072), as Gram-negative bacteria. Agar well diffusion method was applied with using Ampicillin and gentamicin standards are for Gram-positive and Gram-negative, respectively (Miles & Ampyes, 1996). The films were cut into discs of diameter 17 mm then immersed in Mueller Hinton agar plates containing approximately 10⁵–10⁶ CFU/mL of bacteria, followed by incubation for 24 h at 37°C. The zone of inhibition was measured in mm as the diameter of the inhibition zone of the bacterial growth. The reported values of the zone of inhibition represent the average of three independent experiments.

2.3.8. Statistical analysis

Experimental data were statistically analyzed by analysis of variance (ANOVA) using SPSS® 16 software. All the measurements were done in three independent triplicates and the values were averaged and expressed as the mean ± standard error. Comparison of means was performed using T-Test (significant level at \(p < 0.05\)).

3. Results and discussions

3.1. FTIR spectroscopy

FTIR spectra of A, CA, CA/1% CO, CA/2% CO and CA/3% CO are shown in Fig. 1. Alginate exhibited the characteristic bands for O-H stretching vibration (broad at 3446/cm), asymmetric and symmetric stretching modes for -CH₂- and -CH- at 2929/cm and 2820/cm, respectively, symmetric and asymmetric stretching for the carboxylate group at 1419/cm and 1615/cm, respectively (Angadi, Manjeshwar, & Aminabhavi, 2012; Su et al., 2011). For calcium alginate (CA), the bands was decreased. This indicated the presence of an electrostatic

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attraction between alginate and Ca\(^{2+}\) ions (Alcantara, Aranda, Darder, & Ruiz-Hitzky, 2010; Hua, Ma, Li, Yang, & Wang, 2010; Sartori, Finch, Ralph, & Gilding, 1997). The FTIR spectra of CA/CO showed no change in the positions of the bands of CA due to the presence of CO in low content and the relative similarities of the function groups.

### 3.2. Wide angle X-ray diffraction

Fig. 2 depicts the XRD patterns of alginate, CA and CA/castor oil films. Alginate showed two peaks at 2\(\theta\) = 13.4° and 21.6° (Fan et al., 2005). For CA, the diffraction peak at 13.4° was disappeared while the intensity of the peak at 21.6° was decreased indicating the presence of a defective crystallinity resulting from entrance of Ca\(^{2+}\) ions inside the chains of alginate leading to the formation of microcrystals (Fan et al., 2005). In addition, a broad diffused peak at 2\(\theta\) = 40° has appeared which is characteristic for calcium alginate (Bajpai, Chand, & Chaurasia, 2012). For CA/CO films, the diffraction peak at 21.6° was disappeared while the intensity of the peak at 40° was reduced with increasing CO. This change might result from altering the packing of alginate chains by the CO due to the formation of strong interaction, namely hydrogen bonding and electrostatic, between alginate and CO. As a result, close packing is prevented, and thus regular crystalline structures was not formed (Dong, Wang, & Du, 2006).

### 3.3. Thermal stability

The thermal stability is very important for the materials used in food packaging applications (Pathak, Yun, Lee, & Paeng, 2010). Fig. 3 illustrates the TG and DTG of the prepared films. The degradation of sodium alginate occurred mainly over three stages. The first mass loss occurred in the temperature range 50–192 °C (ca. 17.5% mass loss) is due to the water removal. The second degradation step staring from 192 °C to 486 °C with a maximum degradation rate at 246 °C and accompanied by 44% mass loss might be due to the decomposition of the glycosidic bonds, the loss of the hydroxyl groups and evolution of CO\(_2\) (Pongjanyakul, Priprem, & Puttipipatkhanchorn, 2005; Soares, Santos, Chierice, & Cavaleiro, 2004; Tripathy & Singh, 2001; Yang et al., 2016).

For the calcium alginate and calcium alginate/castor oil films, the first step due to evaporation of moisture occurred in the temperature range 50–180 °C. The second step occurred around 200 °C was primarily due to the decomposition of glycerol (plasticizer) (Shankar & Rhim, 2015). The third step occurred in temperature range 237–430 °C, in which the maximum degradation rate temperatures were 261 °C, 266 °C, 267 °C and 268.5 °C with mass loss of 22%, 20%, 20% and 18.5% for CA, CA/1% CO, CA/2% CO and CA/3% CO, respectively.

As observed from these results, when CO was added to CA, the temperatures of the maximum decomposition rate was shifted to higher temperatures and the mass loss % was decreased, suggesting that the thermal stability was enhanced after incorporation of CO. Moreover, the \(T_{50}\)% (temperature at which 50% decomposition occurred) was found to be 266 °C, 356 °C, 399 °C, 433 °C, 486 °C for A, CA, CA/1% CO, CA/2% CO and CA/3% CO, respectively. The higher temperature stage (\(T > 500 °C\)) is associated with the degradation of the more thermally stable structure produced due to the cross-linking reaction.

The calculated IPDT values obtained from Eq. (1) were found to be 1.23 \(\times\) 10\(^7\), 1.34 \(\times\) 10\(^7\), 1.40 \(\times\) 10\(^7\), 1.44 \(\times\) 10\(^7\) and 1.54 \(\times\) 10\(^7\) for A, CA, CA/1% CO, CA/2% CO and CA/3% CO, respectively. Based on the IPDT values, incorporation of CO in alginate films enhances their thermal stability and thus resist the thermal decomposition over time when used in food packaging.

### 3.4. Mechanical properties

Average stress-strain curves of the prepared films are shown in Fig. 4, and Young's modulus (Y), tensile strength (TS) and elongation at break (EB) are summarized in Table 1. The TS was significantly increased (\(p < 0.05\)) upon the incorporation of castor oil due to increasing the interaction resulting from the formation of hydrogen bonds and electrostatic attraction between alginate and CO. However, further increasing of CO beyond 1.0% led to decreasing the TS values which might result from the interference of CO with the Ca\(^{2+}\) ions which are responsible for the ionic interaction and formation of the cross-linked network. EB was increased with increasing CO which acts as a plasticizing agent and thus facilitating the chains mobility. Young's modulus which is related the strength of the films was significantly increased (\(p < 0.05\)) upon adding CO with 1.0% while further increasing of CO leads to a reduction in its value. Based on the obtained results, CA/1% CO sample exhibited the best mechanical properties.

### 3.5. Water vapour permeability

WVP gives an indication about the water transfer from the food to its environment. EFs should have a lower WVP for reducing, as possible, the dehydration of food and thus keeping it fresh (Gontard, Guilbert, & CUQ, 1992). As seen from Table 2, the WVP of CA (21.53 g mm/m\(^2\) day kPa) was found to decrease significantly (\(p < 0.05\)) with incorporation and increasing the CO content. This behavior might result from the hydrophobicity of CO and the increased crosslinking density which hinder the water mobility across the films (Rojas-Graü et al., 2006).

### 3.6. Colour measurement

The appearance of the food product may affect the consumer...
Table 1
Mechanical testing parameters of alginate and alginate/castor oil films.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Young modulus (MPa)</th>
<th>Tensile strength (MPa)</th>
<th>Elongation at break (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA (control)</td>
<td>33.73 ± 0.79a</td>
<td>17.35 ± 4.36a</td>
<td>10.04 ± 5.10a</td>
</tr>
<tr>
<td>CA/1% CO</td>
<td>77.39 ± 0.55b</td>
<td>48.15 ± 1.25b</td>
<td>12.60 ± 0.48b</td>
</tr>
<tr>
<td>CA/2% CO</td>
<td>44.48 ± 0.89b</td>
<td>30.29 ± 0.16b</td>
<td>14.28 ± 8.13b</td>
</tr>
<tr>
<td>CA/3% CO</td>
<td>39.69 ± 0.80b</td>
<td>30.02 ± 3.42b</td>
<td>15.86 ± 11.12a</td>
</tr>
</tbody>
</table>

Results are represented as mean ± standard deviation of three replicates. Values with different superscript letters in each column are significantly different (p < 0.05).

Table 2
Colour variables and water vapour permeability of alginate/castor oil edible films.

<table>
<thead>
<tr>
<th>Sample</th>
<th>L</th>
<th>a</th>
<th>b</th>
<th>ΔE</th>
<th>WVP (g mm/m² day kPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA</td>
<td>85.04a</td>
<td>−8.71a</td>
<td>−2.26a</td>
<td>14.30a</td>
<td>21.53a</td>
</tr>
<tr>
<td>CA/1% CO</td>
<td>84.20a</td>
<td>−6.64a</td>
<td>−1.02a</td>
<td>14.05a</td>
<td>19.21b</td>
</tr>
<tr>
<td>CA/2% CO</td>
<td>83.10a</td>
<td>−4.57a</td>
<td>0.52a</td>
<td>13.40a</td>
<td>18.31b</td>
</tr>
<tr>
<td>CA/3% CO</td>
<td>82.50a</td>
<td>−1.43a</td>
<td>1.20a</td>
<td>13.20a</td>
<td>17.02a</td>
</tr>
</tbody>
</table>

Results are represented as mean ± standard deviation of three replicates. Values with different superscript letters in each column are significantly different (p < 0.05).

4. Conclusions

Castor oil was successfully incorporated with different contents (0 mL/100 g, 1.0 mL/100 g, 2.0 mL/100 g and 3.0 mL/100 g) into alginate, and the structures of the prepared edible films were confirmed by FTIR. XRD confirmed the interaction between the castor oil and alginate as detected from the reduction of the crystallinity peaks of alginate with increasing the CO content. TGA revealed that the thermal stability was significantly enhanced after CO incorporation as mirrored by increasing the values of IPDT. CO improved the mechanical properties of the alginate films as the tensile strength and Young’s modulus were increased from 17.35 ± 4.36 MPa to 48.15 ± 1.25 MPa and from 33.73 ± 0.79 MPa to 77.39 ± 0.55 MPa, respectively, after CO incorporation of 1.0 mL/100. However, at higher CO content, both values of TS and Y were decreased although their values are higher than the alginate control. The water vapour permeability was decreased significantly (p < 0.05) while the total colour difference (ΔE) was not significantly changed (p > 0.05) after incorporation of CO in alginate films. Incorporation of CO into alginate edible film displayed a significant inhibitory effect against S. aureus and B. subtilis (Gram-positive bacteria) while no inhibitory effect was observed for S. typhi and E. coli preferences, and thus the colour of the packaging films has a great effect. The measured colour variables L, a, b and ΔE are collected in Table 2. The films tend to be darker with increasing the CO content as mirrored from the decreased values of L. The colour of the films became yellow as detected from the increase of both b and a with increasing the CO content. The total colour difference, ΔE, didn’t significantly change (p > 0.05), which denoted that the presence of CO didn’t change the film’s colour.

3.7. Antibacterial activity

EFs should have a significant bacterial inhibition in order to minimize the bacterial growth on the food surface. Table 3 summarizes the antibacterial activities of the investigated films. In this study, alginate, as control, didn’t possess any bacterial activity towards investigated bacteria. However, the bacterial inhibition was observed towards the Gram-positive bacteria after incorporation of CO, and the activity was increased with increasing the CO content. This inhibition might result from increasing the number of hydroxyl groups upon increasing the CO (Uscátegui, Arévalo, Díaz, Cobo, & Valero, 2016). The hydroxyl group increases the hydrophilic nature of the films, and thus help them to dissolve in the bacteria membrane and thus leading to their damage resulting from the uncontrolled transfer of substances across the membranes (Burt, 2004). Moreover, no activity towards Gram-negative was observed due to the presence of an additional external membrane around them which prevents any materials transfer across their membranes (Burt, 2004).
Table 3

Antibacterial activity of alginate and alginate/castor oil films.

<table>
<thead>
<tr>
<th>Samples code</th>
<th>Gram-positive bacteria</th>
<th>Gram-negative bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S. aureus</td>
<td>B. subtilis</td>
</tr>
<tr>
<td></td>
<td>Inhibition zone (mm)</td>
<td>Inhibition zone (mm)</td>
</tr>
<tr>
<td>CA (control)</td>
<td>0°</td>
<td>0°</td>
</tr>
<tr>
<td>CA/1% CO</td>
<td>14.54 ± 0.74ab</td>
<td>0°</td>
</tr>
<tr>
<td>CA/2% CO</td>
<td>15.39 ± 0.44bc</td>
<td>10.65 ± 0.55ab</td>
</tr>
<tr>
<td>CA/3% CO</td>
<td>16.97 ± 0.65bc</td>
<td>17.30 ± 0.25ab</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>23.80 ± 0.20</td>
<td>32.40 ± 0.30</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>22.30 ± 1.50</td>
<td>19.90 ± 0.30</td>
</tr>
</tbody>
</table>

Results are represented as mean ± standard deviation of three replicates. Values with different superscript letters in each column are significantly different (p < 0.05).

(Gram-negative bacteria). Based on the obtained results, the prepared alginate/castor oil films could be considered potential candidates in the field of edible films because of their improved thermal, mechanical and antibacterial properties.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.lwt.2018.05.049.

References


