Effects of clay type and content on mechanical, water barrier and antimicrobial properties of agar-based nanocomposite films

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ABSTRACT
Brittleness and other properties such as low thermal stability, medium gas barrier properties and low solvent resistance (e.g., against water) of the pure biopolymer film are often insufficient for food packaging applications. The nanoscale distribution of nanoclays (such as montmorillonite, saponite or hectorite), with a high aspect ratio (100-1,500) and extremely high surface-to-volume ratio (700-800 m\textsuperscript{2}/g) established significant improvements to the polymer matrix in terms of mechanical, gas barrier, and optical properties at low filler content (less than 5% by weight). Agar-based composite films with different types of nanoclays including Cloisite Na\textsuperscript{+}, Cloisite 30B and Cloisite 20A were prepared using a solvent casting method and their tensile, water vapour barrier and antimicrobial properties were tested. Tensile strength (TS), elongation at break (E), and water vapour permeability (WVP) of control agar film were 29.7±1.7 MPa, 45.3±9.6\%, and \((2.22±0.19)×10^{-9}\) g·m/m\textsuperscript{2}·s·Pa, respectively. All the film properties tested including transmittance, tensile properties, WVP, and XRD patterns indicated that Cloisite Na\textsuperscript{+} was the most compatible with agar matrix. TS of the nanocomposite films prepared with 5% Cloisite Na\textsuperscript{+} increased by 18%, while WVP of the nanocomposite films decreased by 24% through nanoclay compounding. Among the agar/clay nanocomposite films tested, only agar/Cloisite 30B composite film showed a bacteriostatic function against \textit{Listeria monocytogenes}. The effect of clay concentration tested using Cloisite Na\textsuperscript{+} showed a significant decrease in TS and WVP, with increases in clay content. Packaging film properties can be improved by choosing the proper type of nanoclay and its optimum concentration. The increased barrier property against water vapour transmission suggests a great potential of the agar/clay composite films in the application of food packaging.

Keywords: Agar; nanocomposite films; organoclays; physical properties; antimicrobial activity

INTRODUCTION
The growth of environmental concerns over non-biodegradable petrochemical-based plastic packaging materials has raised interest in packaging materials based on biopolymers derived from renewable sources. The use of biopolymer-based packaging materials is expected to solve the waste disposal problems to a certain extent. Such biopolymers include naturally occurring carbohydrates and proteins of plant and animal origin, and those synthesized chemically from naturally derived monomers such as lactic acid \cite{1}. Among the renewable source-based biodegradable plastic packaging materials, agar is one of the most interesting materials since it is thermoplastic, biodegradable, biocompatible and has high mechanical strength with moderate water resistance \cite{2,3}. Agar is extracted from two major commercial sources of red seaweed such as \textit{Gelidium sp.} and \textit{Gracilaria sp.}. It consists of a mixture of agarose (gelling fraction) and agarpectin (non-gelling fraction), which is slightly branched and sulfated. In the commercial manufacture of food grade agar, most of the agarpectin is removed during processing; hence, commercial agars are mainly composed of the agarose fraction \cite{4}. Agarose is a linear polymer with a molecular weight of about 120 kDa, and is composed of a repeating unit of agarobiose which is composed of 1,3-linked-D-galactose and 1,4-linked 3,6-anhydro-L-galactose. Most applications for agar are based on its gelling ability and the fact that it is more stable to low pH and high temperature conditions compared to other gelling systems. Due to its renewability and biodegradability with good film forming properties, agar has been tested to prepare environmentally-friendly packaging materials such as foams, films and coatings \cite{2}, and added to other biopolymers such as milk protein and starch to improve mechanical and water vapour barrier properties \cite{3}. However, brittleness and other properties such as low thermal stability, medium gas barrier properties and low solvent resistance (e.g., against water) of the pure polymer are often insufficient for food packaging applications \cite{5}. Recently, a nanocomposite has been suggested as a promising option to overcome such
shortcomings of hydrophilic biopolymer-based packaging films. Nanocomposite is a hybrid material consisting of polymer matrix reinforced with nano-scale fillers having at least one dimension in the nanometer range, for which purposes nanoclays with layered silicate structure are mainly used in the packaging application sector. The nanoscale distribution of nanoclays (such as montmorillonite, saponite or hectorite), with a high aspect ratio (100-1,500) and extremely high surface-to-volume ratio (700-800 m²/g) established significant improvements to the polymer matrix in terms of mechanical, gas barrier, and optical properties at low filler content (less than 5% by weight) [6-8]. Various natural biopolymers have been tested to exploit the improvement of film properties. However, to the best of our knowledge, study on the preparation of agar-based nanocomposite films has not shown in the literature yet.

The main objectives of this study were to prepare agar/clay nanocomposite films using a solvent casting method and to investigate the effect of type of nanoclays on the packaging film properties such as the mechanical, water vapor barrier, water resistance, and antimicrobial activity of the prepared agar-based nanocomposite films.

MATERIALS & METHODS

Food grade agar was obtained from Fine Agar Co., Ltd. (Damyang, Jeonnam, Korea). Three types of nanoclays including two organically modified montmorillonite (Cloisite 20A and Cloisite 30B), and one unmodified montmorillonite (Cloisite Na+) were purchased from Southern Clay (Gonzales, TX, USA). Agar and agar-based nanocomposite films were prepared using a solvent casting method. To test the effect of type of nanoclay for the preparation of agar/nanoclay composite films, 5% of agar/nanoclay films were prepared with the different types of nanoclay.

Film thickness was measured using a micrometer (dial thickness gauge 7301, Mitutoyo, Tokyo, Japan) at an accuracy of 0.01-mm. All film samples were preconditioned in a constant temperature humidity chamber set at 25°C and 50% RH for at least 48 h before further test. Color values of the films were measured using a Chroma meter (CR-400, Konica-Minolta Holdings, Inc., Tokyo, Japan). Film transparency was determined by measuring the percent transmittance at 660 nm using a UV/VIS spectrophotometer (Model 8451A, Hewlett-Packard Co., Santa Clara, CA, USA) [9]. Morphology of both surface and cross-section of agar and agar/clay nanocomposite films without coating were observed using a FE-SEM (S-4800, Hitachi Co., Ltd., Matsuda, Japan) operated at V_{acc} = 1 kV and I_{e} = 10 μA. Three dimensional images of the films were observed using an AFM (XE-100, Park AFM Co, Ltd., Suwon, Korea) in non-contact mode with scan size of 20 μm. XRD patterns were taken using a PANalytical Xper t pro MRD diffractometer (Amsterdam, Netherland), operated at 40 kV and 30 mA. Samples were scanned over the range of diffraction angle of 2θ =1-10° with a scanning rate of 0.4°/min at room temperature. Tensile strength (TS) and elongation at break (E) of each film were evaluated with a Model 5565 Instron Universal Testing Machine (Instron Engineering Corporation, Canton, MA, USA) following an ASTM Method D 882-88. Initial grip separation was set at 50 mm and cross-head speed at 50 mm/min. Water vapour permeability (WVP) was determined gravimetrically using a modified ASTM Method E96-95. The contact angle (CA) of water in air on the film surface was measured using a CA analyzer (Phenix 150 model, Surface Electro Optics Co., Ltd., Kunpo, Korea) after a water drop of ca. 10 μL was placed on the surface of film using a microsyringe [9]. Water solubility (WS) of films was determined as the percentage of soluble matter to initial dry matter of film sample [10]. Swelling ratio (SR) of films was also determined gravimetrically. Water vapour uptake ratio (WVUR) was determined following the method of Tunc et al. [10]. Two typical food pathogens including Gram-positive bacteria, Listeria monocytogenes ATCC-19111, and Gram-negative bacteria, E. coli O157:H7 ATCC-11775, were used to test the antimicrobial activity of agar films using a viable cell count method [9].

RESULTS & DISCUSSION

The type of nanoclays greatly influenced the degree of dispersion in the film forming solution. Among the clays tested, the natural MMT (Cloisite Na˚), which is hydrophilic, was dispersed best in the film forming solution followed by less hydrophobic Cloisite 30B by a simple mixing with a magnetic stirrer, however, the hydrophobic Cloisite 20A was hardly dispersed in the film forming solution without further treatment such as high shear mixing homogenization and ultrasonication. The resulting agar and agar/clay nanocomposite films obtained by the solvent casting method were flexible and free-standing. Apparently, neat agar film and agar/Cloisite Na˚ nanocomposite films were transparent, while agar/Cloisite 30B and 20A nanocomposite films were slightly translucent. Generally, lightness of the films (L-value) decreased, while b-value (yellowness) increased in all the nanocomposite films, consequently increased in total color difference (ΔE)
value (Table 1). The degree of change in Hunter $L$, $b$, and $\Delta E$ values was more significant in the agar/Cloisite 20A nanocomposite films than the other films. This may be attributed to incomplete dispersion of Cloisite 20A in the polymer matrix due to its high surface hydrophobicity [11].

<table>
<thead>
<tr>
<th>Films</th>
<th>$L$</th>
<th>$a$</th>
<th>$b$</th>
<th>$\Delta E$</th>
<th>$T_{660 \text{ nm}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neat Agar</td>
<td>94.57±0.62$^a$</td>
<td>-0.16±0.02$^b$</td>
<td>6.17±0.50$^c$</td>
<td>4.80±0.72$^c$</td>
<td>72.2±1.5$^a$</td>
</tr>
<tr>
<td>Agar/Cloisite Na$^+$</td>
<td>94.14±0.19$^b$</td>
<td>-0.28±0.00$^c$</td>
<td>6.66±0.21$^a$</td>
<td>5.43±0.26$^bc$</td>
<td>62.6±1.0$^b$</td>
</tr>
<tr>
<td>Agar/Cloisite 30B</td>
<td>93.62±0.35$^{bc}$</td>
<td>-0.15±0.02$^b$</td>
<td>7.59±0.46$^b$</td>
<td>6.47±0.57$^b$</td>
<td>2.4±0.1$^c$</td>
</tr>
<tr>
<td>Agar/Cloisite 20A</td>
<td>92.92±0.19$^c$</td>
<td>-0.18±0.01$^b$</td>
<td>8.59±0.53$^a$</td>
<td>7.69±0.55$^a$</td>
<td>2.8±1.0$^e$</td>
</tr>
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</table>

Transparency of film determined by transmittance was affected greatly on the clay types used. The transmittance decreased significantly after incorporation of nanoclay (Table 1) and the degree of decrease in the transmittance was strongly dependent on the clay types used. A possible explanation for the different effect of clay types on the transmittance of the composite films may be due to the lipid moieties of quaternary ammonium salts incorporated in the organoclays [12]. In addition, the difference in the transmittance of the agar films may be due to the different miscibility between clay and polymer matrix. In that sense, Cloisite Na$^+$ seems to be more compatible with agar compared with other types of clay. Generally, it is known that the optical property of a well-developed nanocomposite film is not significantly changed when the clay platelets with about one nm thick are well dispersed through the polymer matrix, since such clay platelets with sizes less than the wavelength of visible light do not hinder light’s passage [13]. However, the large decrease in the transmittance of the nanocomposite films indirectly indicates that the clays are not completely dispersed in the polymer matrix. Such different effect of clays on the transparency of nanocomposite films may be attributed to not only types of polymer matrix and clay but also the compatibility between the polymer matrix and the clay [11].

For a better insight in the homogeneity and in the microstructure of dried films, SEM analysis was carried out. SEM images indicate clay particles are relatively well dispersed in the polymer matrix and they also show that the neat agar film has smoother surface than the other nanocomposite films (Fig. 1). So AFM images were used to study detailed structure of the surface of film samples. The 3-D AFM images clearly show the difference in surface roughness between films (Fig. 2). The result of average ($R_a$) and root mean square roughness ($R_q$) of the film samples indicates the surface roughness is dependent on the clay types used (Table 2). Though it is not statistically significant, the surface roughness of agar/Cloisite Na$^+$ nanocomposite film increased slightly, while the other two nanocomposite films composites with organoclays significantly increased in the surface roughness. This may be also attributed to the compatibility between nanoclay and polymer matrix. The XRD patterns of Cloisite Na$^+$, Cloisite 30B and Cloisite 20A revealed the diffraction peaks at $2\theta = 7.15^\circ$, $2\theta = 4.71^\circ$ and $2\theta = 3.43^\circ$, respectively (Fig. 3), which suggests that the layer distance of Cloisite Na$^+$, Cloisite 30B and Cloisite 20A were 1.24 nm, 1.88 nm and 2.58 nm, respectively. The $d_{001}$ peak of the clays was shifted to lower angle of 4.82$^\circ$, 4.53$^\circ$, and 3.17$^\circ$, corresponding to an increase in $d$-spacing to 1.83 nm, 1.95 nm and 2.79 nm for agar/Cloisite Na$^+$, agar/Cloisite 30B and agar/Cloisite 20A nanocomposite samples, respectively. The higher basal spacings of clays in the nanocomposites, as compared to the pristine clays, are due to the intercalation of polymer chains inside the clay layers. The XRD results indicate that the degree of intercalation was higher in Cloisite Na$^+$ nanocomposite films compared with other types of nanoclay. This result also indicates that Cloisite Na$^+$ interacts better with agar than the other type of clays. TS and E of pure agar film were 29.7±1.7 MPa and 45.3±9.6%, respectively (Table 2). Tensile test results indicate that the TS of the composite films except agar/Cloisite Na$^+$ films did not change significantly after incorporation of nanoclay, but TS of agar/Cloisite Na$^+$ films increased about 18% after incorporation of nanoclay. However, the E of nanocomposite films did not show any significant change. Usually, a well-developed polymer/clay nanocomposite results in highly increased mechanical strength compared to the pure polymer matrix since uniform dispersion of the nano-sized clay particles produces an ultra-high interfacial interaction and ionic bonds between the nanoclay and host polymer [2,6-8]. However, compounding the MMT clays reduced the TS in the present study, indicating that interaction between the organoclays and polymer matrix is not well developed probably due to the organoclays are not well dispersed in the polymer matrix. Ogata et al. [14] prepared the solvent cast agar/organoclay nanocomposite and found through small and wide-angle X-ray scattering measurements that the silicate layers could not be individually well-dispersed in the agar/clay blend. The mechanical strength of agar/clay nanocomposite films can be tuned.
through proper choice of clay type and processing method to form an intercalation or exfoliation structure. Among the nanoclays tested, Cloisite Na⁺ was more effective in maintaining TS property than the other nanoclays, indicating that Cloisite Na⁺ is more compatible with agar, as compared to the other types of clay.

![Figure 1. Surface (upper) and cross-sectional (lower) SEM images of agar-based nanocomposite films.](image1)

![Figure 2. Three dimensional AFM images of agar-based nanocomposite films](image2)

![Figure 3. XRD patterns of three different types of nanoclays and agar-based nanocomposite films.](image3)

Table 2. Effect of clay type on surface roughness and tensile properties of agar-based nanocomposite films

<table>
<thead>
<tr>
<th>Films</th>
<th>$R_a$ (nm)</th>
<th>$R_q$ (nm)</th>
<th>Thickness (μm)</th>
<th>TS (MPa)</th>
<th>E (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neat Agar</td>
<td>43.97±4.50a</td>
<td>60.34±4.29a</td>
<td>70.3±2.9a</td>
<td>29.7±1.7a</td>
<td>45.3±9.6a</td>
</tr>
<tr>
<td>Agar/Cloisite Na⁺</td>
<td>60.28±12.77a</td>
<td>80.68±21.55a</td>
<td>67.0±3.5a</td>
<td>35.0±2.1b</td>
<td>44.6±0.2a</td>
</tr>
<tr>
<td>Agar/Cloisite 30B</td>
<td>153.35±46.41b</td>
<td>198.06±51.60b</td>
<td>75.0±3.0a</td>
<td>28.6±1.0a</td>
<td>49.8±4.9a</td>
</tr>
<tr>
<td>Agar/Cloisite 20A</td>
<td>127.14±37.90b</td>
<td>160.25±45.08b</td>
<td>79.2±3.2a</td>
<td>29.3±0.9a</td>
<td>57.4±7.4a</td>
</tr>
</tbody>
</table>
The WVP of nanocomposite films changed significantly depending on the type of nanoclays used (Table 3). The WVP of nanocomposite films compounded with natural MMT (Cloisite Na+) decreased, while those of films compounded with organically modified nanoclays (Cloisite 30B and 20A) increased slightly. This result is mainly attributed to the surface hydrophilicity or hydrophobicity of the clay types used. Expectedly, hydrophilic Cloisite Na+ is more compatible with hydrophilic agar polymer matrix than the other organoclays, resulting in well dispersed in the polymer matrix and indicating lower WVP with agar/Cloisite Na+ nanocomposite films. Between the organically modified nanoclays, Cloisite 30B was more effective in reducing WVP than Cloisite 20A, since the former is more hydrophilic than the latter. This result also indicates that Cloisite Na+ is more compatible with agar polymer matrix than the other clays. Sotthonwit et al. [11] also found the same trend of decrease in WVP of WPI/clay nanocomposite films, in which WPI/Cloisite Na+ showed the most decrease in WVP followed by WPI/Cloisite 30B, while WPI/Cloisite 20A did not show significant change in WVP compared to the neat WPI films. Their results are coincided with those of the present study. The increase in water vapour barrier property of polymer/clay nanocomposite films is mainly attributed to the tortuous path for water vapour diffusion due to the impermeable clay layers distributed in the polymer matrix consequently increasing the effective diffusion path length [15].

<table>
<thead>
<tr>
<th>Films</th>
<th>WVP*</th>
<th>RH I (%)</th>
<th>CA (deg.)</th>
<th>WS (%)</th>
<th>SR (%)</th>
<th>WVUR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neat Agar</td>
<td>2.22±0.19&lt;sup&gt;b&lt;/sup&gt;</td>
<td>68.7±0.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>47.05±4.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.66±1.98&lt;sup&gt;b&lt;/sup&gt;</td>
<td>295.27±5.68&lt;sup&gt;b&lt;/sup&gt;</td>
<td>140.58±2.17&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Agar/Cloisite Na+</td>
<td>1.68±0.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>71.6±1.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>43.28±1.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>18.47±0.48&lt;sup&gt;a&lt;/sup&gt;</td>
<td>334.52±3.92&lt;sup&gt;a&lt;/sup&gt;</td>
<td>141.09±2.20&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Agar/Cloisite 30B</td>
<td>2.31±0.14&lt;sup&gt;b&lt;/sup&gt;</td>
<td>68.9±0.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>55.99±2.17&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.26±3.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>223.90±9.98&lt;sup&gt;b&lt;/sup&gt;</td>
<td>124.85±2.47&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Agar/Cloisite 20A</td>
<td>2.62±0.18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>68.2±0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>53.94±0.95&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.18±1.19&lt;sup&gt;b&lt;/sup&gt;</td>
<td>234.28±5.09&lt;sup&gt;c&lt;/sup&gt;</td>
<td>127.34±3.31&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
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</table>

*WVP (×10<sup>-9</sup> g.m/m².s.Pa)

The CA of water droplet on packaging film indicates surface hydrophobicity of the film, which is generally used to estimate the resistances of the film against liquid water [2]. The CA of neat agar film was 47.05º, and it decreased significantly in agar/Cloisite Na+ nanocomposite film and increased significantly in both agar/Cloisite 30B and 20A nanocomposite films (Table 3). This implies decrease in surface hydrophobicity in the former and increase in the latter films, which is mainly attributed to the hydrophilicity/hydrophobicity of the clay used. Water resistance properties of agar and agar/clay nanocomposite films were determined by water solubility (WS), swelling ratio (SR), and water vapour uptake ratio (WVUR) (Table 3). After formation of nanocomposite, agar/Cloisite Na+ nanocomposite films showed a significantly increase in the WS and SR, while the WVUR did not show any significant change. On the contrary, the SR and WVUR decreased significantly in both agar/Cloisite 30B and Cloisite 20A nanocomposite films, while the WS did not change significantly. The increase in the WS and SR of agar/Cloisite Na+ nanocomposite films may be attributed to the hydrophilic nature of the Cloisite Na+, while hydrophobicity of organoclays caused decrease in the SR and WVUR of the resulting nanocomposite films. It is noteworthy that the agar/Cloisite Na+ nanocomposite film has a high water holding capacity (high SR) with increased mechanical strength and moderate flexibility.

Figure 4. Antimicrobial activity of agar-based nanocomposite films against L. monocytogenes and E. coli O157:H7.
The antimicrobial activity of agar and agar/clay nanocomposite films was tested by the viable cell count method. It was previously demonstrated that organically modified clay powders, substituted with quaternary ammonium salt, especially Cloisite 30B, showed strong antimicrobial activity against both Gram-positive and negative bacteria with small amounts of clay [12]. However, when compounded with agar, noticeable antimicrobial activity was not observed against the test microorganisms, regardless of clay types, except agar/Cloisite 30 B nanocomposite film (Fig. 4). As expected, pure agar and agar/Cloisite Na+ films did not show any antimicrobial activity. Only agar composite films compounded with Cloisite 30B showed substantial antimicrobial activity against L. monocytogenes. The antimicrobial activity of nanocomposite films prepared with Cloisite 30B is mainly attributed to the quaternary ammonium salt in the organically modified MMT (Cloisite 30B) [9,11,12].

CONCLUSION

Intercalated agar/clay nanocomposite films were prepared by solvent-casting and the degree of intercalation was dependent on the clay type used. Tensile, water vapour barrier, and antimicrobial properties of agar-based nanocomposite films showed that the type of nanoclay. Hydrophilic natural montmorillonite, Cloisite Na+ showed better intercalation into and interaction with the agar matrix than organoclays such as Cloisite 30B or Cloisite 20A as evidenced by XRD patterns, as well as the transparency, TS, and WVP test results. Packaging film properties can be improved by choosing the proper type of nanoclay. The increased barrier property against water vapour transmission suggests a great potential of the agar/clay composite films in the application of food packaging.

REFERENCES