Characterization of Chitosan Films Incorporated with Different Substances of Konjac Glucomannan, Cassava Starch, Maltodextrin and Gelatin, and Application in Mongolian Cheese Packaging

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Abstract: Four kinds of edible composite films based on chitosan combined with additional substances (konjac glucomannan, cassava starch, maltodextrin and gelatin) and the addition of lysozyme were prepared and used as packaging materials for Mongolian cheese. The prepared composite films were evaluated using scanning electron microscopy and Fourier transform infrared spectroscopy. The physicochemical properties of all chitosan composite films, including thickness, viscosity, opacity, color, moisture content, water vapor permeability, tensile strength and elongation at break, were measured. The results show that Konjac glucomannan–chitosan composite film presented the strongest mechanical property and highest transparency. The cassava starch–chitosan composite film presented the highest water barrier property. The study on the storage characteristics of Mongolian cheese was evaluated at 4 °C. The results show that the cheese packaging by cassava starch–chitosan composite film presented better treatment performance in maintaining the quality, reducing weight loss and delaying microbial growth.

Keywords: edible chitosan composite films; konjac glucomannan; cassava starch; maltodextrin; gelatin; Mongolian cheese

1. Introduction

The packaging is a significant step for food products in extending their shelf life, reducing interaction with spoilage factors and ensuring their safety during marketing [1]. As packaging materials, plastics and petroleum-based polymers are non-biodegradable and nonrenewable, which lead to environmental pollution and cause serious ecological problems [2]. Therefore, edible packaging (edible films and coatings) has been attracting much attention for replacing traditional plastic packages in food packaging applications, due to its advantages of non-toxicity, health and safety and pollution-free [3]. Polysaccharides, proteins and lipids are the frequent components of edible films and coatings [4–6]. Chitosan could be used to create such films due to its excellent properties (e.g., film-forming properties, antibacterial property, nontoxicity, biodegradability and biocompatibility) [7]. However, compared with a single component film, the composite film prepared by chitosan combined with additional film-forming substances could obtain better properties [8].

Konjac glucomannan (KGM) is a natural polysaccharide which has been explored in the preparation of edible films due to its excellent film-forming ability, nontoxicity and biodegradability [9,10]. In previous work, the increase of KGM content could increase the...
tensile strength (TS) of the blend film [11]. Starch is a non-toxic natural polysaccharide, usually used to construct packaging film [12,13]. The blend of cassava starch with chitosan has successfully improved the water vapor permeability of films [14]. Maltodextrin is a polysaccharide substance utilized in the food and pharmaceutical industry [15]. The dissolution of the composite film can be improved by adding maltodextrin into the substrate [16]. Gelatin is an animal protein obtained from partial hydrolysis of collagen [17]. Due to its good film-forming ability and excellent barrier properties, gelatin is an effective blending material in composite film formation with polysaccharides (e.g., chitosan and starch) [18,19]. Moreover, compared with a single component film, the mechanical properties and barrier properties of the gelatin–chitosan blend film are improved [20]. However, there are few reports on the performance comparison of composite films prepared by combining chitosan with different kinds of substances.

Besides, the addition of antimicrobial agents in edible films and coatings could improve their functionalities. Lysozyme has been used as an antimicrobial agent in edible films and coatings [21]. The incorporation of antibacterial agents in the edible film can effectively reduce the growth of microorganisms on the food surface and extend the shelf life of the packaged products. However, little information is known on the difference in antibacterial properties of lysozyme in different film substrates.

Mongolian cheese, a white to light yellow color with distinctive flavors, is a fermented dairy product produced and consumed by the people of Inner Mongolia, China [22,23]. As a fresh cheese, Mongolian cheese has a limited shelf-life due to textural changes and mold growth. Therefore, the preservation of Mongolian cheese requires novel practices to extend its shelf life. However, there are few reports on the preservation of Mongolian cheese in the edible film with antibacterial agents.

In this study, we developed edible composite films based on chitosan incorporated with the addition of lysozyme and additional film-forming substances such as KGM, cassava starch, maltodextrin and gelatin. The present research aimed to compare the properties of these films, including physical, chemical and mechanical properties. The prepared composite films were used for packaging Mongolian cheese for 30 days, and the water migration (by low-field nuclear magnetic resonance) and microbiological changes of Mongolian cheese after the storage period were evaluated.

2. Materials and Methods

2.1. Materials

Mongolian cheese, purchased from Zhenglanqi Lifeng Dairy Factory (Xilinhot, China), was vacuum-packed and transported to the lab by air within 24 h at 4 °C. Chitosan (Degree of deacetylation ≥ 95%) and gelatin were purchased from Shanghai Mackling Biochemical Co., Ltd (Shanghai, China). Konjac glucomannan (food grade) was supplied by Konson Konjac Co., Ltd (Wuhan, China). Maltodextrin with an average dextrose equivalent of 18 was purchased Shandong Xiwang Food Co., Ltd (Binzhou, China). Food grade cassava starch and lysozyme were obtained from Henan Wan Bang Industrial Co., Ltd (Shanghai, China). Acetic acid and glycerol were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China).

2.2. Film Preparation

Chitosan solution (2%, w/v) was prepared by dissolving chitosan in 1% (v/v) acetic acid solution. Cassava starch was dispersed in deionized water to obtain a 2% (w/v) starch solution, heated at 80 °C and kept for 60 min under stirring to accomplish a complete starch gelatinization. Gelatin was added to deionized water at 60 °C and stirred until completely dissolved; 2% (w/v) gelatin solution was obtained. Then, 2% (w/v) maltodextrin and 2% (w/v) KGM were dissolved in deionized water. The chitosan solution was mixed with the same volume of the cassava starch solution, the gelatin solution, the maltodextrin solution and the KGM solution, respectively, and 2% (w/v) of glycerol and 0.25% (w/v) of lysozyme were added to each solution [24,25]. All the film-forming solutions (CS: chitosan solution
containing 2% (w/w) glycerol and 0.25% (w/v) lysozyme; KGCL: KGM-chitosan solution containing 2% (w/w) glycerol and 0.25% (w/v) lysozyme; MCL: maltodextrin-chitosan solution containing 2% (w/w) glycerol and 0.25% (w/v) lysozyme; GCL: gelatin-chitosan solution containing 2% (w/w) glycerol and 0.25% (w/v) lysozyme; and CSCL: cassava starch-chitosan solution containing 2% (w/w) glycerol and 0.25% (w/v) lysozyme) were prepared. The vacuum created by a pump removes the vapor from each film-forming solution in degassing applications for 1 h. Fifteen milliliters of film-forming solutions were poured into a Teflon dish (90 mm) and kept still at room temperature for 72 h to evaporate the solvent and form the film. The formed films were peeled from the dishes. All the films were stabilized in a controlled humidity chamber at 50% relative humidity (RH) for three days at room temperature (25 °C) before analysis.

2.3. Film Properties

2.3.1. Viscosity and Thickness

The viscosities of the film-forming solutions were measured using a DV2T Brookfield viscometer (Shanghai vitron instruments Co., Ltd., Shanghai, China). Film thickness was determined according to the method described by previous studies with slight modifications [26]. The thickness of 10 different points in a film was measured in triplicate using a handheld digital micrometer (Nscing Es/Su Measurement, Nanjing, China) with a resolution of 0.001 mm.

2.3.2. Color

According to the method described by Zhong and his colleagues with slight modifications [27,28], a CR-400 color aberration meter (Konica Minolta Holdings, Inc., Tokyo, Japan) was used to measure film color. Measurements were operated in triplicates. The CIE Lab-scale was used. The measure of lightness (L* values, range 0–100) represents black to white, the redness measurement (a* values) describes green (−) to red (+) and the yellowness measurement (b* values) represents blue (−) to yellow (+). Total color difference (ΔE*) and whiteness (WI) were calculated using Equations (1) and (2), respectively.

\[
\Delta E^* = \left[ (\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2 \right]^{0.5}
\]

\[
WI = 100 - \sqrt{(100 - L^*)^2 + (a^*)^2 + (b^*)^2}
\]

2.3.3. Opacity

Opacity was determined according to the method described by a previous study with slight modification [29]. Opacity was determined by measuring the absorbance of film samples at 600 nm using a UV spectrophotometer (UV-7504 single-beam UV-Vis spectrophotometer, Shanghai, China). The measurements were operated in triplicates. All film samples were cut into a rectangular shape of 10 mm × 30 mm and placed into the test cell of the UV Spectrophotometer. An empty test cell was used as the control. The opacity of the film samples was calculated with the Equation (3).

\[
\text{Opacity} = \frac{\text{Abs}_{600}}{X}
\]

where Abs_{600} is the absorbance value at 600 nm and X is the thickness (mm) of films. All measurements were performed in triplicate.

2.3.4. Moisture Content

The moisture content was determined according to the methods described by previous studies with slight modifications [30]. Film samples were cut into a rectangular shape of
4 cm × 4 cm, and then dried in an oven at 105 °C for 24 h. All samples were weighed in triplicate before and after drying. Moisture content was calculated by Equation (4).

\[
\text{Moisture content(\%)} = \frac{M_0 - M}{M_0} \times 100\% \tag{4}
\]

where \( M_0 \) and \( M \) are the masses of the film samples before and after drying, respectively.

2.3.5. Mechanical Properties

Mechanical properties were determined according to the methods described by previous studies with some modifications [31]. Mechanical properties were measured by using a computer-controlled tensile testing machine (DCP-KZ300; Chengdu Mingchi Instruments Co., Ltd., Chengdu, China). Samples were clamped between the tensile grips with an initial distance of 50 mm. The force and deformation were documented during extension at 100 mm/min. The experiment was operated in triplicate. The tensile strength (TS) was expressed in MPa and calculated by Equation (5).

\[
\text{TS} = \frac{F_{\text{max}}}{L \times W} \tag{5}
\]

where \( F_{\text{max}} \) is the maximum tension during extension, \( L \) is the width of the film sample and \( W \) is the thickness of the film sample.

The elongation at break (EAB) was calculated by Equation (6) and expressed in percent.

\[
\text{EAB} = \frac{L_1 - L_0}{L_0} \tag{6}
\]

where \( L_0 \) is the initial length of the film and \( L_1 \) is the length of the test film after breakage.

2.3.6. Water Vapor Permeability (WVP)

WVP was determined according to the methods described by previous studies with some modifications [32]. The WVP was measured with a WVP tester (Labstone, B-31E, Shanghai, China). Films were placed on the top of the containers containing 5 mL water and then the edges were sealed by a lid tightened by screws. Then, the samples were placed in the cell of the equipment at 38 °C and 10% RH. Data were recorded every 2 h for 12 h. All measurements were performed in triplicate.

2.3.7. Fourier Transform Infrared (FTIR) Spectroscopy

The FTIR spectroscopy was determined according to the method described by a previous study with slight modifications [33]. Films were cut into a rectangular shape of 2 cm × 2 cm and dried in an oven at 40 °C for 12 h before the measurement. The infrared spectra of films were analyzed using an ATR/FTIR spectrometer (Thermo Fisher, Nicolet iS5, Waltham, MA, USA). Spectra were recorded in the range of 500–4000 cm\(^{-1}\), with 64 scans and a 4 cm\(^{-1}\) spectra resolution.

2.3.8. Scanning Electron Microscopy (SEM)

The SEM was determined according to the method described by a previous study with slight modification [1]. The film samples were detected using a Hitachi S-3400N scanning electron microscope (Techtomp Co., Ltd., Beijing, China) with an accelerating voltage of 5 kV at 500 magnification (surfaces and cross sections). Before observation, the films were coated with a thin gold layer.

2.4. The Application of the Edible Films on Mongolian Cheese

Mongolian cheese was cut into cubes (2 cm × 2 cm × 2 cm) and then wrapped by the prepared films. Each coated cheese cube was tightly wrapped in aluminum foil to enable a more direct contact between the film and the surface of the cheese cube. All samples were
then stored at 4 °C. The cheese samples were subjected to one of the following treatments accordingly: (1) untreated (control); (2) CS treatments, cheese samples wrapped with CS films; (3) KGCL treatments, cheese samples wrapped with KGCL films; (4) MCL treatments: cheese samples wrapped with MCL films; (5) GCL treatments, cheese samples wrapped with GCL films; and (6) CSCL treatments, cheese samples wrapped with CSCL films.

2.4.1. Microbiological Analysis

The microbiological analysis was determined according to the methods described by previous studies with slight modifications [34]. The total viable counts (TVCs) and total Yeast and Mold counts (TYMCs) were tested. Two-gram cheese samples were ground and serially diluted in aseptic saline (0.9%, w/v) in a ratio of 1:10. All solutions were vortex-mixed for 1 min. After serial dilutions, 0.1 mL of each dilution were spread on selective agar medium. The plate count agar medium was used to evaluate the TVCs and potato dextrose agar medium was used for the TYMCs. TVCs were enumerated using plate count agar medium after the plates were incubated at 37 °C for 48 h. TYMCs were enumerated using potato dextrose agar after the plates were incubated at 28 °C for 72 h.

2.4.2. Weight Loss

All cheese samples were weighed the film coating treatments and after the storage period and data calculated by Equation (7).

\[ W\% = \frac{m_i - m_t}{m_t} \times 100\% \quad (7) \]

where \( m_i \) is the initial weight of the cheese and \( m_t \) is the weight after a storage time of \( t \).

2.4.3. Low-Field Nuclear Magnetic Resonance (LFNMR) and Magnetic Resonance Imaging (MRI)

LFNMR measurement was determined according to the method described by a previous study with slight modification [35]. Cheese samples were placed in a 70 mm tube coil of the LFNMR analyzer (MesoMR23-060H-I, Shanghai Niumag Analytical Instruments Co., Shanghai, China). \( T_2 \) measurements were performed according to the Carr-Purcell-Meiboom-Gill (CPMG) sequence with echo time (TE) of 0.4 ms. The repetition time (TR) was set to 2000 ms. The \( T_2 \) measurements were performed with a time delay between the 90° pulse and 180° pulses (\( \tau \)) of 35.00 µs. MR imaging of cheese samples was obtained from a low-field nuclear magnetic resonance imaging analyzer (MesoMR23-060H-I, Shanghai Niumag Analytical Instruments Co., Shanghai, China). A spin-echo (SE) pulse sequence was used with 20 ms echo time (TE) and 500 ms repetition time (TR).

2.5. Statistical Analysis

The parametric distribution of the data was evaluated initially by the Shapiro-Wilk test for the normality. If the data showed normal distribution, then statistical comparisons were made by one-way analysis of variance (ANOVA) followed by a Duncan multiple range tests. For the data without a normal distribution, non-parametric Kruskal-Wallis test was performed followed by the Dunn’s multiple comparisons test. All analyses were carried out using the SPSS Statistics 22 (IBM Corp., New York, NY, USA). The results were presented as the mean ± standard deviation (SD). Differences are considered to be significant when the \( p \)-values are under 0.05.

3. Results and Discussion

3.1. Film Appearance and Thickness

All films were transparent, as observed by naked eye observation, homogeneous and flexible (Figure 1). The thicknesses of the films are shown in Table 1. There were no significant differences between the thickness values of each film (\( p > 0.05 \)), which indicated good compatibility among the film components. Similar results were also found in other
studies [36]. The difference in thickness was not significant in all comparisons of films made from soy protein incorporated with addition of two types of starch and maltodextrin in the study by Galus. They showed that differences in thickness of films depended on preparation methods and amount of added substances [36].

![Image of films](image)

**Figure 1.** The appearance images of films: (a) CS film; (b) KGCL film; (c) MCL film; (d) GCL film; and (e) CSCL film.

**Table 1.** Physicochemical and mechanical properties of CS, KGCL, MCL, GCL and CSCL films.

<table>
<thead>
<tr>
<th>Films</th>
<th>Thickness (µm)</th>
<th>Moisture Contents (%)</th>
<th>Viscosity (Pa·s)</th>
<th>Water Vapor Permeability ( \times 10^{-12} ) g/(m·s·Pa)</th>
<th>Tensile Strength (MPa)</th>
<th>Elongation at Break (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CS</td>
<td>100.04 ± 7.53 (^a)</td>
<td>36.19 ± 0.88 (^e)</td>
<td>3.12 ± 0.01 (^BC)</td>
<td>8.24 ± 0.09 (^b)</td>
<td>3.36 ± 0.06 (^BC)</td>
<td>29.20 ± 1.51 (^AB)</td>
</tr>
<tr>
<td>KGCL</td>
<td>99.30 ± 5.40 (^a)</td>
<td>32.99 ± 0.75 (^c)</td>
<td>4.71 ± 0.03 (^C)</td>
<td>8.17 ± 0.39 (^b)</td>
<td>3.57 ± 0.18 (^C)</td>
<td>68.14 ± 7.91 (^C)</td>
</tr>
<tr>
<td>MCL</td>
<td>102.60 ± 2.95 (^a)</td>
<td>34.84 ± 0.26 (^d)</td>
<td>0.16 ± 0.02 (^A)</td>
<td>7.91 ± 0.33 (^b)</td>
<td>0.58 ± 0.03 (^AB)</td>
<td>26.99 ± 0.18 (^A)</td>
</tr>
<tr>
<td>GCL</td>
<td>98.80 ± 4.13 (^a)</td>
<td>26.93 ± 0.60 (^a)</td>
<td>0.20 ± 0.02 (^AB)</td>
<td>8.00 ± 0.05 (^b)</td>
<td>0.44 ± 0.01 (^A)</td>
<td>36.60 ± 5.77 (^ABC)</td>
</tr>
<tr>
<td>CSCL</td>
<td>103.50 ± 5.19 (^a)</td>
<td>28.43 ± 1.29 (^b)</td>
<td>0.81 ± 0.03 (^ABC)</td>
<td>7.01 ± 0.31 (^a)</td>
<td>3.04 ± 0.45 (^BC)</td>
<td>58.20 ± 4.66 (^BC)</td>
</tr>
</tbody>
</table>

CS: chitosan–lysozyme film; KGCL: konjac glucomannan–chitosan–lysozyme film; MCL: maltodextrin–chitosan–lysozyme film; GCL: gelatin–chitosan–lysozyme film; CSCL: cassava starch–chitosan–lysozyme film. The results represent means ± standard deviation of three replicates. Different capital letters (A–C) in the same column indicate significant differences using Dunn’s test (\( p < 0.05 \)). Different small letters (a–e) in the same column indicate significant differences using Duncan test (\( p < 0.05 \)).

3.2. Film Color and Opacity

Film color was an important index of overall appearance, which influenced consumer acceptance. \( \Delta E^* \) indicated the degree of total color difference from the standard color plate, and WI indicated the degree of whiteness, which can better describe the differences among all of the films [37]. \( L^*, a^*, b^* \), \( \Delta E^* \) and WI of all the film samples are shown in Table 2. The results show that the CSCL film had the lowest \( L^* \) and \( b^* \) values among all of the film
samples ($p < 0.05$), which indicated CSCL film was relatively darker than other kinds of films. The possible reason for this phenomenon might be due to a small amount of starch was hydrolyzed into glucose, which could react with chitosan by the Maillard reaction during the film-forming process [27]. The $b^*$ value of KGCL film was the highest, followed by MCL film, GCL film, CS film and CSCL film ($p < 0.05$). The $\Delta E^*$ value of KGCL film was 0.48, 0.48, 0.51 and 0.24 times higher than CS film, MCL film, GCL film and CSCL film ($p < 0.05$), respectively. Clearly, the KGCL film had a more yellowish color compared to other films. The higher $\Delta E^*$ value of KGCL film showed that its color was deeper than other films. There was no significant difference in color among CS film, MCL film and GCL film. Compared to GCL film and CSCL film, these three films had higher $L^*$ values and WI values and lower $a^*$ values and $\Delta E^*$ values, which indicated that these films were whiter, yellower and greener.

Table 2. Colorimetric characteristics of CS, KGCL, MCL, GCL and CSCL films.

| Films          | $L^*$ $\pm$ 0.04 | $a^*$ $\pm$ 0.15 | $b^*$ $\pm$ 0.04 | $\Delta E^*$ | Whiteness (WI) | Opacity  \\
<table>
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<tbody>
<tr>
<td>CS</td>
<td>88.92 $\pm$ 0.43</td>
<td>$-1.61 \pm 0.15$</td>
<td>9.05 $\pm$ 0.17</td>
<td>8.91 $\pm$ 0.41</td>
<td>85.60 $\pm$ 0.42</td>
<td>0.58 $\pm$ 0.02</td>
</tr>
<tr>
<td>KGCL</td>
<td>87.62 $\pm$ 0.52</td>
<td>$-0.93 \pm 0.05$</td>
<td>13.72 $\pm$ 0.62</td>
<td>13.17 $\pm$ 0.80</td>
<td>81.50 $\pm$ 0.79</td>
<td>1.12 $\pm$ 0.03</td>
</tr>
<tr>
<td>MCL</td>
<td>89.30 $\pm$ 0.13</td>
<td>$-1.68 \pm 0.08$</td>
<td>9.51 $\pm$ 0.46</td>
<td>8.92 $\pm$ 0.43</td>
<td>85.59 $\pm$ 0.41</td>
<td>0.36 $\pm$ 0.01</td>
</tr>
<tr>
<td>GCL</td>
<td>89.42 $\pm$ 0.20</td>
<td>$-1.20 \pm 0.07$</td>
<td>9.45 $\pm$ 0.33</td>
<td>8.75 $\pm$ 0.38</td>
<td>85.76 $\pm$ 0.37</td>
<td>0.44 $\pm$ 0.03</td>
</tr>
<tr>
<td>CSCL</td>
<td>86.11 $\pm$ 0.06</td>
<td>$-0.76 \pm 0.04$</td>
<td>8.19 $\pm$ 0.06</td>
<td>10.59 $\pm$ 0.07</td>
<td>83.86 $\pm$ 0.07</td>
<td>0.36 $\pm$ 0.01</td>
</tr>
</tbody>
</table>

CS: chitosan–lysozyme film; KGCL: konjac glucomannan–chitosan–lysozyme film; MCL: maltodextrin–chitosan–lysozyme film; GCL: cassava starch–chitosan–lysozyme film. The results represent means ± standard deviation of three replicates. Different capital letters (A–C) in the same column indicate significant differences using Dunn’s test ($p < 0.05$). Different small letters (a–d) in the same column indicate significant differences using Duncan test ($p < 0.05$).

The opacity values of all films are shown in Table 2. The results show that the opacity of CS, KGCL, MCL, GCL and CSCL films were 0.58, 1.12, 0.36, 0.44 and 0.36, respectively (Table 2). The MCL and CSCL films were more transparent than other films. A previous study reported that cassava starch/chitosan film were clear and transparent, indicating homogenous distribution of components [13]. All films show good transparency, indicating a good compatibility among different components [29]. Edible films should have good transparency in order to keep the original color of the food [38]. Therefore, MCL and CSCL films with good transparency showed potential for food packaging applications.

### 3.3. Moisture Content

The moisture content of the film is shown in Table 1. The highest moisture content was observed for CS film, which was 1.09, 1.04, 1.34 and 1.28 times that of KGCL film, MCL film, GCL film and CSCL film ($p < 0.05$), respectively. The moisture content is related to the total void volume occupied by water molecules in the network microstructure of the film [39]. There was significant difference in moisture content of different films (Table 1), indicating that there was variation of the total void volume. Compared with other films, the GCL film exhibited the lowest moisture content. It might be due to the interaction of functional groups (–OH, –NH$_2$, etc.) between gelatin and chitosan, which decreased the moisture absorption of film. Moisture content was an important index since a high moisture level improved adhesion of the film on aqueous foodstuffs, but a film with low moisture was more suitable for coating fatty foodstuffs [40]. Since Mongolian cheese is a high-fat food [41], CSCL film and GCL film are more suitable for wrapping Mongolian cheese than other films. A previous study also reported that chitosan and fish gelatin films containing phenolic acids could be suitable for fatty food applications [19].

### 3.4. WVP

WVP was directly related to the permeability of water through the films. Low WVP values improved the mechanical properties and structural integrity of the wrapped product, and they also might reduce water loss [30]. As shown in Table 1, the WVP of CSCL film is 85.07%, 87.62%, 88.62% and 85.80% of CS, KGCL, MCL and GCL films ($p < 0.05$).
respectively. This phenomenon might be due to CSCL film possessed better water vapor barrier performance [42]. An important factor affecting the WVP of a film is the hydrophilic–hydrophobic ratio of a film constituent. Water vapor could transfer through the hydrophilic part of the film. When the hydrophobic compounds of the film increase, the WVP decreases [34]. Compared with other films, CSGL possessed fewer free hydrophilic groups, which effectively reduced the adsorption and transfer of water vapor.

3.5. Mechanical Properties

The mechanical properties are favorable properties of packaging materials, which contributed to maintaining film integrity and stability when the film withstood stresses during storage, handling and transportation [30]. The mechanical properties, namely, the TS and EAB, are shown in Table 1. As shown in Table 1, the EAB of CS film was 42.85% and 50.17% of KGCL film and CSCL film, respectively. Clearly, the limited mechanical properties of CS film were not very fit for packaging food [1]. Compared with CS film, the films adding KGM and cassava starch can obviously improve mechanical properties of film. KGCL film exhibited the highest TS, which was 1.06, 6.16, 8.11 and 1.17 times those of CS, MCL, GC, and CSCL film, respectively (p < 0.05). The EAB of KGCL film was also 2.33, 2.52, 1.86 and 1.17 times those of CS, MCL, GCL and CSCL film (p < 0.05). Previous studies reported that the EAB of chitosan–KGM composite film was 63% [43]. The increased TS and EAB might result from hydrogen bonding between molecules [44]. The higher TS of KGCL film indicated that more hydrogen bonds were formed between chitosan and KGM while lower EAB of MCL film might result from the weakening of the hydrogen bonds between chitosan and maltodextrin molecules. The viscosities of the film-forming solutions are listed in Table 1. The viscosity of the KGCL film-forming solution was 1.51, 29.43, 22.43 and 5.81 times that of CS, MCL, GCL and CSCL films, respectively (p < 0.05). The mechanical properties of the film are related to the viscosity of the film-forming solution [27]. As the viscosity increased, the TS of the film becomes higher. An edible film with desirable mechanical properties should have high TS and EAB [26]. Therefore, the mechanical properties of KGCL and CSCL films are better for packaging.

3.6. FTIR

FTIR was used to determine the functional group characteristics and the intermolecular interactions of the films in this study. The FTIR results are shown in Figure 2 and Table 3. The main peaks of all the films were similar but some of the peak intensities varied. All spectra show three main spectral regions. (i) A broad asymmetric band between 3500 and 2800 cm\(^{-1}\): The peaks near 2930, 2880 cm\(^{-1}\) were stretching vibration characteristic peaks of CH that were related to the methylene hydrogen atom. A broad peak in the range of 3000–3500 cm\(^{-1}\) resulted from the stretching vibrations of the \(-\text{OH}\) groups, which were overlapped the N–H stretching in the same region [45]. (ii) A region between 1700 and 1100 cm\(^{-1}\) characteristic of the amide groups: All films exhibited characteristic absorption peaks at around 1647, 1567 and 1413 cm\(^{-1}\), which correspond to C=O stretching, N–H bending, C–N stretching. (iii) A strong absorption region between 800 and 1200 cm\(^{-1}\) characteristic of the chitosan saccharide structure: The bands at 1020–1035 cm\(^{-1}\) were ether-based C–O stretching vibrations [46]. The formation of chitosan composite films was carried out through the interaction mechanism of hydrogen bond formation. The NH\(^3+\) group of the chitosan molecule and the free \(-\text{OH}\) groups were able to easily form intramolecular and intermolecular hydrogen to constitute the overall structure of the film [47]. In the spectra of all films, the presence of the characteristics peak of each biopolymer used was observed, as well as a displacement of these bands, which is indicative of the interaction between the components in the film matrix [48]. As a result, these interactions will affect the thickness of the film, mechanical properties, barriers, opacity and morphology [48]. Besides, for GCL film, the higher intensity in the peaks in the region of amine and amides groups, at 1643 and 1547 cm\(^{-1}\), was noticed. The interaction between chitosan and gelatin might form a hydrogen bond in the GCL film, which increased the bond energy, leading to
an increase in peak intensity \[39,49\]. Due to the interaction between the functional groups (–OH, –NH\(_2\), etc.) between chitosan and gelatin, the moisture absorption of GCL film is reduced \[29\].

**Figure 2.** FTIR spectra of CS, KGCL, MCL, GCL and CSCL films.

**Table 3.** Functional group analysis of CS, KGCL, MCL, GCL and CSCL films.

<table>
<thead>
<tr>
<th>Films</th>
<th>Peak at cm(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>CS</td>
<td>3290 ± 1.00 (\text{A}^{\text{B}})</td>
</tr>
<tr>
<td>KGCL</td>
<td>3291 ± 0.58 (\text{B})</td>
</tr>
<tr>
<td>MCL</td>
<td>3290 ± 0.00 (\text{B})</td>
</tr>
<tr>
<td>GCL</td>
<td>3283 ± 1.53 (\text{B})</td>
</tr>
<tr>
<td>CSCL</td>
<td>3290 ± 0.09 (\text{B})</td>
</tr>
</tbody>
</table>

Assignment OH CH CH C=O N–H C–N C–O

CS: chitosan–lysozyme film; KGCL: konjac glucomannan–chitosan–lysozyme film; MCL: maltodextrin–chitosan–lysozyme film; GCL: gelatin–chitosan–lysozyme film; CSCL: cassava starch–chitosan–lysozyme film. The results represent means ± standard deviation of three replicates. Different capital letters (A–C) in the same column indicate significant differences using Dunn’s test \((p < 0.05)\). CH and C=O data were found non-significant (ns) according to Dunn’s test \((p > 0.05)\).

3.7. SEM

SEM images of the surfaces and cross sections of the films are shown in Figure 3. The cross-section of the composite films varied in the roughness of appearances. The CS and KGCL films were continuous and compact and also had a smoother texture without pores. In Sun’s report, konjac glucomannan/chitosan film sample was relatively homogeneous and dense \[10\]. The CSCL film had a homogenous surface even though the cross-section appeared wrinkled. The GCL film had a rougher surface, and the fractured surface was irregular. The surface of MCL film was less homogeneous with a discontinuous void or visible cracks cross-section. As shown in Figure 3, KGCL film exhibited a good microstructure in its cross-sectional surface, which may be related to the uniform dispersion of chitosan and KGM matrix and their good miscibility and compatibility, resulting in strong interaction and adhesion at the interface between chitosan and KGM \[9\]. The homogenous surface of CSCL film might be due to the complete dissolution of the starch and chitosan in its film-forming solution \[50\]. Therefore, as a packaging material for Mongolian cheese, KGCL and CSCL films possessed a better microstructure.
3.8. Weight Loss

Compared with the control samples, the weight losses of film-wrapped cheese showed a significant decrease during storage ($p < 0.05$) (Table 4). The values of weight loss of the cheese wrapped with CS, KGCL, MCL, GCL and CSCL films reached 73.49%, 50.60%, 72.29%, 63.86% and 54.22% of the control samples after 30 days of storage, respectively ($p < 0.05$). A previous study reported that chitosan-based coating allowed a decrease in weight loss of the cheeses [34]. In this study, although the cheese was wrapped, all films still reduced weight loss in cheese during storage. The weight loss of cheese was influenced by film type and especially the water permeability, which controlled the water release of cheese [51]. The results show that KGCL film and CSCL film more effectively reduced the weight loss of Mongolian cheese during storage than other treatments. This
phenomenon might be due to the fact that CSCL film and KGCL film exhibited the good barrier properties of water vapor (Table 1).

Table 4. Weight losses of Mongolian cheese (percent of initial weight) during storage.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Control</th>
<th>CS</th>
<th>KGCL</th>
<th>MCL</th>
<th>GCL</th>
<th>CSCL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 15</td>
<td>0.34 ± 0.08 b</td>
<td>0.16 ± 0.01 a</td>
<td>0.12 ± 0.02 a</td>
<td>0.15 ± 0.08 a</td>
<td>0.16 ± 0.13 a</td>
<td>0.12 ± 0.06 a</td>
</tr>
<tr>
<td>Day 30</td>
<td>0.83 ± 0.04 b</td>
<td>0.61 ± 0.12 a</td>
<td>0.42 ± 0.11 a</td>
<td>0.60 ± 0.05 a</td>
<td>0.53 ± 0.10 a</td>
<td>0.45 ± 0.20 a</td>
</tr>
</tbody>
</table>

Control: untreated Mongolian cheese; CS: Mongolian cheese was wrapped with CS film; KGCL: Mongolian cheese was wrapped with KGCL film; MCL: Mongolian cheese was wrapped with MCL film; GCL: Mongolian cheese was wrapped with GCL film; CSCL: Mongolian cheese was wrapped with CSCL film. The results represent means ± standard deviation of three replicates. Different small letters in the same row indicate significant differences using Duncan test (p < 0.05).

3.9. TVCs and TYMCs

Changes in TVCs and TYMCs of all the samples during storage are shown in Table 5. The TVCs and TYMCs of Mongolian cheese increased significantly during storage, and they showed similar trends. After 30 days of storage, the TVCs in the control sample were 1.04, 1.07, 1.05, 1.05 and 1.12 times those of CS, KGCL, MCL, GCL and CSCL treatments, respectively (p < 0.05); the TYMCs in the control sample were 1.05, 1.07, 1.06, 1.06 and 1.11 times those of CS, KGCL, MCL, GCL and CSCL treatments, respectively (p < 0.05). Clearly, compared with other treatments, TVCs and TYMCs in the control sample increased more quickly (Table 5). Previous studies reported that chitosan–lysozyme film exhibited good antibacterial properties [52] and lysozyme–chitosan coating could be used to extend shelf life of chicken eggs [53]. In Duan’s study, chitosan composite film containing lysozyme was effective in the control of microorganisms in Mozzarella cheese [54]. Duan et al. (2007) applied a chitosan–lysozyme composite film to Mozzarella cheese. Their study indicated that lysozyme in the film was effective in suppressing the growth of microorganisms up to 30 days [54]. Differences in the lysozyme interactions in different film matrices could affect its antimicrobial activity on the film surface [55]. CSCL film possesses a better antibacterial effect (Table 5). This phenomenon might be due to the fact of better protection of the lysozyme in the starch matrix [55].

Table 5. Total viable counts (TVCs) and total Yeast and Mold counts (TYMCs) in Mongolian cheese during storage.

<table>
<thead>
<tr>
<th>Samples</th>
<th>TVCs (log_{10} CFU/g)</th>
<th>TYMCs (log_{10} CFU/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fresh Day 15 Day 30</td>
<td>Fresh Day 15 Day 30</td>
</tr>
<tr>
<td>Control</td>
<td>5.28 ± 0.07 a 6.57 ± 0.08 c</td>
<td>8.32 ± 0.03 c 5.55 ± 0.05 a 6.79 ± 0.08 c</td>
</tr>
<tr>
<td>CS</td>
<td>5.28 ± 0.07 a 6.23 ± 0.05 b</td>
<td>7.97 ± 0.07 b 5.55 ± 0.05 a 6.54 ± 0.02 b</td>
</tr>
<tr>
<td>KGCL</td>
<td>5.28 ± 0.07 a 6.14 ± 0.07 b</td>
<td>7.80 ± 0.08 b 5.55 ± 0.05 a 6.45 ± 0.03 b</td>
</tr>
<tr>
<td>MCL</td>
<td>5.28 ± 0.07 a 6.23 ± 0.06 b</td>
<td>7.94 ± 0.01 b 5.55 ± 0.05 a 6.54 ± 0.00 b</td>
</tr>
<tr>
<td>GCL</td>
<td>5.28 ± 0.07 a 6.19 ± 0.10 b</td>
<td>7.90 ± 0.01 b 5.55 ± 0.05 a 6.51 ± 0.04 b</td>
</tr>
<tr>
<td>CSCL</td>
<td>5.28 ± 0.07 a 5.92 ± 0.03 a</td>
<td>7.40 ± 0.17 a 5.55 ± 0.05 a 6.31 ± 0.05 a</td>
</tr>
</tbody>
</table>

CFU: Colony-Forming Units; Control: untreated Mongolian cheese; CS: Mongolian cheese was wrapped with CS film; KGCL: Mongolian cheese was wrapped with KGCL film; MCL: Mongolian cheese was wrapped with MCL film; GCL: Mongolian cheese was wrapped with GCL film; CSCL: Mongolian cheese was wrapped with CSCL film. The results represent means ± standard deviation of three replicates. Different small letters in the same column indicate significant differences using Duncan test (p < 0.05).

3.10. LFNMR and MRI

LFNMR can effectively characterize the water structure and water mobility in food during storage [56]. The NMR relaxation in cheese is affected by the food network structure due to the fact that each given proton is affected by its surroundings [57]. It exhibited plenty of information about the water mobility, which can evaluate the mobility and distribution of water in food [35]. Due to the interaction between macromolecules and water, proton relaxation is affected by the cheese matrix. The spectra of different relaxations times are...
produced by water molecules in different states such as bound, immobilized, and free water [58].

The relaxation time ($T_{21}$, $T_{22}$ and $T_{23}$) and the corresponding percentage of peak area ($M_{21}$, $M_{22}$ and $M_{23}$) of all samples are shown in Table 6. In this study, three peaks were observed in the $T_2$ distribution spectrum of cheese samples. $T_{21}$ showed bound water; $T_{22}$ referred to water in the immobilized state; and $T_{23}$ denoted free water [58]. As shown in Table 6, the increase in $T_2$ relaxation times of cheese samples during storage indicates that the water within these cheese matrices becomes more mobile with time [59]. Compared with the fresh sample, there was no significant difference in $T_{21}$ of KGCL, GCL and CSCL samples on the 30th day. The relaxation time of $T_{22}$ and $T_{23}$ in control, CS and MCL samples were higher than other samples, suggesting higher mobility of water molecules in control, CS and MCL samples. The higher transverse relaxation times indicated higher mobility of water [35]. The indistinctive difference in $T_{22}$ and $T_{23}$ of KGCL, GCL and CSCL samples compared with fresh samples indicated these three treatments could hold water and keep its contents constant. As shown in Table 6, the $T_{22}$ peak area percentage ($M_{22}$) was the highest, indicating that the main water in the sample was the water entrapped inside the protein gel network within the cheese matrix [60]. After 30 days of storage, the $M_{22}$ in control, CS and MCL samples were significantly lower ($p < 0.05$), indicating that there was more immobilized water loss in control, CS and MCL samples than other samples. Therefore, the results suggest that the KGCL, GCL and CSCL treatments are more effective for maintaining the quality of the Mongolian cheese during storage.

<table>
<thead>
<tr>
<th>NMR Parameters</th>
<th>Fresh</th>
<th>Control</th>
<th>CS</th>
<th>KGCL</th>
<th>MCL</th>
<th>GCL</th>
<th>CSCL</th>
</tr>
</thead>
<tbody>
<tr>
<td>$M_{21}$ (%)</td>
<td>8.45 ± 0.27 $^a$</td>
<td>9.65 ± 0.12 $^c$</td>
<td>9.91 ± 0.22 $^c$</td>
<td>9.07 ± 0.17 $^b$</td>
<td>9.89 ± 0.14 $^c$</td>
<td>8.62 ± 0.39 $^a$</td>
<td>9.08 ± 0.27 $^b$</td>
</tr>
<tr>
<td>$M_{22}$ (%)</td>
<td>89.63 ± 0.23 $^c$</td>
<td>86.49 ± 0.26 $^a$</td>
<td>86.50 ± 0.15 $^a$</td>
<td>88.18 ± 0.17 $^b$</td>
<td>86.78 ± 0.17 $^a$</td>
<td>88.48 ± 0.19 $^b$</td>
<td>88.36 ± 0.13 $^b$</td>
</tr>
<tr>
<td>$M_{23}$ (%)</td>
<td>1.93 ± 0.22 $^a$</td>
<td>3.87 ± 0.14 $^d$</td>
<td>3.59 ± 0.14 $^d$</td>
<td>2.75 ± 0.26 $^b$</td>
<td>3.33 ± 0.16 $^c$</td>
<td>2.90 ± 0.23 $^b$</td>
<td>2.56 ± 0.33 $^b$</td>
</tr>
<tr>
<td>$T_{21}$ (ms)</td>
<td>0.78 ± 0.03 $^b$</td>
<td>1.35 ± 0.11 $^b$</td>
<td>1.27 ± 0.18 $^b$</td>
<td>0.92 ± 0.13 $^b$</td>
<td>1.24 ± 0.21 $^b$</td>
<td>0.83 ± 0.07 $^b$</td>
<td>0.93 ± 0.04 $^a$</td>
</tr>
<tr>
<td>$T_{22}$ (ms)</td>
<td>22.25 ± 0.42 $^a$</td>
<td>28.02 ± 1.14 $^c$</td>
<td>27.54 ± 0.16 $^c$</td>
<td>24.39 ± 0.99 $^b$</td>
<td>27.36 ± 0.06 $^c$</td>
<td>24.96 ± 0.12 $^b$</td>
<td>22.75 ± 0.92 $^a$</td>
</tr>
<tr>
<td>$T_{23}$ (ms)</td>
<td>191.16 ± 0.00 $^a$</td>
<td>235.43 ± 0.00 $^e$</td>
<td>223.74 ± 0.00 $^f$</td>
<td>204.91 ± 0.00 $^d$</td>
<td>219.64 ± 0.00 $^e$</td>
<td>200.66 ± 0.44 $^c$</td>
<td>195.75 ± 1.93 $^b$</td>
</tr>
</tbody>
</table>

Table 6. Water distribution and transverse relaxation time in Mongolian cheese during storage.

Fresh: untreated fresh Mongolian cheese; Control: untreated Mongolian cheese; KGCL: Mongolian cheese was wrapped with KGCL film; MCL: Mongolian cheese was wrapped with MCL film; GCL: Mongolian cheese was wrapped with GCL film; CSCL: Mongolian cheese was wrapped with CSCL film. The results represent means ± standard deviation of three replicates. Different small letters in the same row indicate significant differences using Duncan test ($p < 0.05$).

MR images of cheese samples of different treatments are shown in Figure 4. MR images are displayed on a color scale where red color indicates higher signal intensity and blue color indicates lower signal intensity. Red regions of the image of samples corresponded to higher signal intensity and were associated with greater water density [61]. The signal intensity of cheese samples reflected the moisture content in samples [62]. Initially, fresh cheese samples possessed higher signal intensity, which showed higher water content of cheese (Figure 4). As storage time increased, signal intensity decreased with the loss of moisture in the cheese. As shown in Figure 4, the change in signal intensity of the control samples was greatest, indicating the water content decreased significantly after 30 days of storage (Figure 4). Kuo reported that water in cheese exists mainly within the protein matrix [63]. The water loss of the control samples in the images might be attributed to the local dehydration of proteins [61]. Compared to fresh samples, the signal intensity changed little for KGCL, GCL and CSCL samples (Figure 4), indicating that there was no significant water loss. It also suggests that the KGCL, GCL and CSCL treatments could be effective in maintaining Mongolian cheese quality during storage. Lamanna reported that NMR technology could be a promising tool for evaluating the freshness preservation ability of packaging [64,65].
4. Conclusions

Four kinds of edible composite films (KGCL, MCL, GCL and CSCL) and chitosan film were prepared and compared. The film-forming components were compatible with each other as evidenced by FTIR spectra. SEM images presented that KGCL and CSCL films possessed a better microstructure compared with other films. KGCL film showed the strongest mechanical property and the highest transparency. CSCL film presented the highest water barrier property. When the composite films were used for packaging Mongolian cheese to improve the preservation, the results show that all the wrapped Mongolian cheeses reduced weight loss and delayed the growth of bacteria, yeast and mold compared with the uncoated Mongolian cheese. In comparison with other films, the CSCL film was more effective in maintaining the quality of Mongolian cheese during storage.

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Data Availability Statement: The data presented in this study are available in article.

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Conflicts of Interest: The authors declare no conflict of interest.
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