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Preparation and performance evaluation of glucomannan–chitosan–nisin ternary antimicrobial blend film

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Abstract

The material behaviour and antimicrobial effect of konjac glucomannan edible film incorporating chitosan and nisin at various ratio or concentrations is discussed. This activity was tested against food pathogenic bacteria namely *Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes*, and *Bacillus cereus*. Mechanical and physical properties were determined and the results indicated that the blend film KC2 (mixing ratio konjac glucomannan 80/chitosan 20) showed the maximum tensile strength (102.8 ± 3.8 MPa) and a good transparency, water solubility, water vapor transmission ratio. The differential scanning calorimetry (DSC), Fourier transform infrared spectroscopy (FTIR), etc. were used to characterize the structural change of the blend films. The results showed that the strong intermolecular hydrogen bonds took place between chitosan and konjac glucomannan. Incorporation of nisin at 42,000 IU/g of film for the selected blend film KC2 was found to have antimicrobial activity against *S. aureus*, *L. monocytogenes*, and *B. cereus*. The antimicrobial effect of chitosan or KC2 incorporating nisin was much better than that of konjac glucomannan incorporating nisin at each corresponding concentration and existed significant difference ($p < 0.05$), however, there was no significant difference on the antimicrobial effect between chitosan and KC2 both incorporating nisin. At all these levels, the ternary blend film KC2-nisin had a satisfactory mechanical, physical properties and antimicrobial activity, and could be applied as a potential 'active' packaging material.

Keywords: Konjac glucomannan; Chitosan; Nisin; Antimicrobial film; Film physical properties

1. Introduction

Antimicrobial packaging has been touted as a major focus in the next generation of 'active' packaging (Brody, 2001). Food quality and safety are also the major concerns in the food industry, as consumers prefer fresher and minimally processed products. In particular, bacterial contamination of ready to eat products is of concern to human health. Antibacterial sprays or dips have been made to overcome those contaminations (Ouattara, Simard, Piette, Begin, & Holley, 2000). However, direct surface application of antibacterial substances has some limitations because the active substances can be neutralized, evaporated or diffused inadequately into the bulk of food (Siragusa & Dickson, 1992; Torres, Motoki, & Karel, 1985).

Edible films or coatings have been investigated for their abilities to retard moisture, oxygen, aromas, and solute transports (Gennadios & Weller, 1990). It is one of the most effective methods of maintaining food quality. This is further improved by film carrying food additives such as antioxidants, antimicrobial, colorants, flavors, fortified nutrient, and spices (Pena & Torres, 1991). In many cases, the agents being carried are slowly released into the food surface and therefore remain at high concentrations for extended periods of time (Coma, Sebti, Pardon, Deschamps, & Pichavant, 2001).

Konjac glucomannan, a heteropolysaccharide derived from the konjac tuber, consists of 1,4-linked β-D-mannose

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and β-D-glucose units in a molar ratio of 1.6:1 with a low degree of acetyl groups at the side chain C-6 position and having an average molecular weight of 0.67–1.9 million (Li & Xie, 2004). It has been generally used in food, film-former, and also been used in biomedical applications, for example, drug delivery (Pathak & Barman, 2003; Wang & He, 2000), cellular therapy (Slepian & Massia, 2001), etc., especially the edible packing films have been studied widely (Pang, 2003; Pang & Li, 2004). However, most of the films do not have either the practicable mechanical properties or antimicrobial activity.

Chitosan, a polymer of 1,4 linked β-D-glucosamine and *N*-acetyl glucosamine units, is prepared by deacetylation of chitin. Chitosan has been proved to be nontoxic, biodegradable, biofunctional, biocompatible and have antimicrobial characteristics (Darmadji & Izumimoto, 1994; Jongrittiporn, Kungsuwan, & Rakshit, 2001; Wang, 1992). Chitosan films are easily prepared by evaporating from dilute acid solutions (Park, Marsh, & Rhim, 2002). A number of studies on the antimicrobial characteristics of films made from chitosan have been carried out earlier (Chen, Yeh, & Chiang, 1996; Coma, Martial-Gros, Garreau, Copinet, & Deschamps, 2002; Ouattara et al., 2000).

Antimicrobial agents such as organic acids, bacteriocins and spice extracts have been tested for their ability to control meat spoilage (Abugroun, Cousin, & Judge, 1993; Hotchkiss, 1995; Miller, Call, & Whiting, 1993). Nisin is a bacteriocin produced by *Lactococcus lactis* subsp. lactis. It has antimicrobial activity against a broad spectrum of Gram-positive bacteria. Nisin has widely been used in the food industry as a safe and natural preservative and has been studied of its suitability to be incorporated into cellulose, whey protein isolate, soy protein isolate, egg albumin, wheat gluten, hydroxypropyl methylcellulose and zein film (Coma et al., 2001; Janes, Kooshesh, & Johnson, 2002; Ko, Janes, Hettiarachchy, & Johnson, 2001). The development of complementary methods to inhibit the growth of pathogenic bacteria such as packaging material-associated antimicrobial agents is an active area of research.

This study was done to improve antimicrobial efficacy of edible film based on konjac glucomannan by incorporating chitosan and nisin. Mechanical and physical properties were characterized, and antimicrobial efficacy was assessed against four food pathogenic bacteria. The molecular interaction, thermal stability, and mechanical properties of the blend films were studied by FTIR, DSC and electron tensile test etc. The relationship between the structure and their physical properties and antimicrobial activity is also discussed.

2. Experimental

2.1. Materials

Konjac glucomannan was prepared as our previous work (Li & Xie, 2004) and the viscosity-average molecular weight (M_v) of the konjac glucomannan was 9.89×10^5 according to the Mark-Houwink equation $[\eta] = 5.96 \times 10^2 M_v^{0.73}$ at 25 °C.

Chitosan was purchased from Wuhan Tianyuan Biomaterial Co. (Wuhan, China). Its degree of deacetylation was measured to be 85% by the method of Jiang (2002), in which the degree of deacetylation of chitosan was determined directly by first derivative UV-spectrophotometry using hydrochloric acid as solvent and *N*-acetyl-D-glucosamine (ACGLSM) as standard. Good linearity between the values of first derivative ($Da/d\lambda$) and concentrations of ACGLSM in the range of 0.0025–0.0400 mg ml^{-1} was obtained. The linear regression equation obtained was given as follows: $dA/d\lambda = 2.349C + 0.0008$ ($r = 0.9996$). The M_v of the chitosan was 1.68×10^5 according to the Mark-Houwink equation $[\eta] = 1.424 \times 10^{-3} M_v^{0.96}$ at 25 °C.

2.2. Preparation of blend films

Konjac glucomannan was dissolved in distilled water, and the insoluble residue was filtered out leaving a concentration of 1% w/w. Chitosan was dissolved in 0.8% w/w aqueous acetic acid to prepare a 1% w/w solution. The solutions of konjac glucomannan and chitosan with different mixing ratios [9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9 konjac glucomannan/chitosan (w/w)] were cast onto polystyrene plates and dried at room temperature and then subjected to vacuum drying for 48 h to obtain the dried films. A series of blend films were obtained and coded as KC1, KC2, KC3, KC4, KC5, KC6, KC7, KC8, and KC9, respectively. The films obtained from pure konjac glucomannan and chitosan were coded as KGM and CHI.

Nisin, having an activity of 1050 IU/mg (Sigma–Aldrich Chemie GmbH, Steinheim, Germany), was incorporated into pure konjac glucomannan, chitosan film or the selected blend film (KC2) forming solution at various levels to obtain antimicrobial films, and coded as konjac glucomannan-N, chitosan-N, KC2-N, respectively. The dry films obtained were peeled off and stored in a chamber at 50% RH and 25 °C until evaluation.

2.3. Characterization of films

The powdered films were blended with potassium bromide and laminated, and the IR spectra was recorded with a Nicolet Nexus 470 FTIR spectrometer (Thermo Nicolet Corporation, Madison, WI, USA). The DSC of the film samples (ca. 10 mg each) was performed on a DSC model 200PC (NETZSCH, Germany) under a nitrogen atmosphere with a flow capacity of 25 ml/min from 30 to 400 °C at a heating rate of 10 °C/min. The percentage of light transmittance (T) of the films was measured by using a Shimadzu UV-160A spectroscope (Shimadzu, Kyoto, Japan) at 480 and 210 nm. The tensile strength (σ_b) and elongation at break (ε_b) of the films were measured on an electron tensile tester CMT-6104 (Shenzhen Sans Test Machine Co., Ltd., China) according to the Chinese standard method GB/T4456-96 (Polyethylene Blown film for

packaging, 1996), in which the film-strips were cut into 120 × 15 mm strips, gauge length (i.e., the distance between the two clamps) was set at 80 mm, with a tensile rate (i.e., the rate of extending travel of the clamp) of 250 mm/min, a return rate (i.e., the rate of return travel of the clamp) of 200 mm/min and the breaking load 200 N.

The water vapor transmission was determined according to method ASTM E96-80 (ASTM, 1989). A container with silica gel was closed with a sample of edible film firmly fixed on top. Then, the container was placed in a desiccator with distilled water at a temperature of 25 °C. The films were weighed daily on a Mettler analytical balance for 10 days. The water vapor transmission was calculated according to the following equation:

$$\mathrm{WVT} = w \cdot x/A,$$

where WVT is the water vapor transmission (g H_2O mm cm^{-2}), x is the average thickness of the film (0.050 ± 0.005 mm) and A is the permeation area (12.57 cm^2).

The water vapor transmission rate (WVTR, g H_2O mm h^{-1} cm^{-2}) was calculated according to the following equation:

$$\mathrm{WVTR} = w \cdot x/A \cdot t.$$

The term x/t was calculated by linear regression from the points of weight gain and time, during a constant transmission rate period.

The 80 mm × 80 mm sheet film was soaked in 25 ± 1 °C water for 5 min; the weights of the dried samples (W_0) were measured directly. After drying the swollen films in desiccators with silica gel until the weights were constant (W_1), the water solubilities (W_s) were calculated using the following equation:

$$W_s(W_0 - W_1)/W_0 \times 100\%.$$

All the tests were carried out in triplicate.

2.4. Antimicrobial assay

Antimicrobial activity tests of edible films were carried out using agar diffusion method. Edible films were cut into a disc form of 17 mm diameter using a circular knife. Film cuts were placed on Mueller Hinton agar (Merck, Darmstadt, Germany) plates, which had been previously seeded with 0.1 ml of inoculum containing indicator microorganisms in the range of 10^5–10^6 CFU/ml. The plates were then incubated at 37 °C for 24 h. The diameters of inhibitory zones surrounding film discs as well as the contact areas of edible films with agar surface were then measured.

2.5. Statistical analysis

The data were analyzed using the statistical analysis system (SAS Institute, Inc., 1995). Significance was determined at the 5% level.

3. Results and discussion

3.1. Physical properties

3.1.1. Mechanical properties of films

Because polymer materials, such as films, may be subjected to various kinds of stress during use, the determination of the mechanical properties involves not only scientific but also technological and practical aspects (Freddi, Romano, Massafra, & Tsukada, 1995). The dependence of the tensile strength on the chitosan content for the blend films is shown in Fig. 1. The tensile strength of the pure konjac glucomannan film was 88.1 ± 1.2 MPa and much higher than that of chitosan (61.0 ± 3.6 MPa). When the chitosan was added into the konjac glucomannan solution, the tensile strength of the resulting blend film increased with increase of chitosan content and reached a maximum point at about 20 wt% chitosan content (KC2), achieving 102.8 ± 3.8 MPa. The remarkable increase in the tensile strength of the blend films indicated the presence of intermolecular interactions between konjac glucomannan and chitosan molecules in the blend films. The dependence of the elongation at break on the chitosan content for the blend films is shown in Fig. 2; there was no significant difference between the blend films and the pure konjac glucomannan or chitosan film, the reason might be that

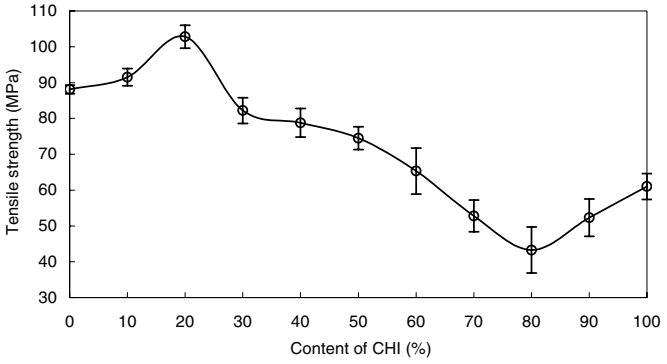

Fig. 1. The dependence of the tensile strength on the chitosan content for glucomannan–chitosan blend films.

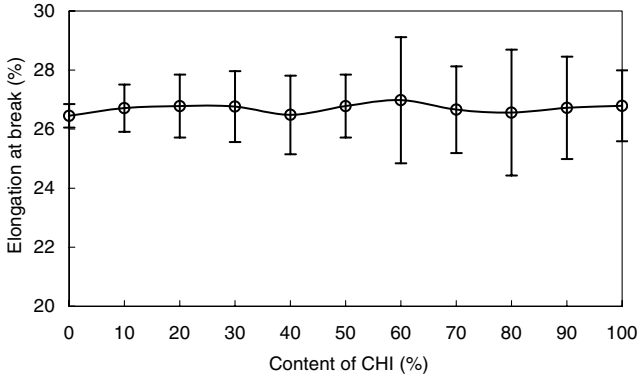

Fig. 2. The dependence of the elongation at break on the chitosan content for glucomannan–chitosan blend films.

the konjac glucomannan and chitosan polysaccharide molecular chains have approximately equal molecular flexibility.

3.1.2. Water vapor transmission rate (WVTR)

WVTR is a measure of ease of the moisture to penetrate and pass through a material and has great influence on the food shelf life. The film WVTR was calculated and the results were shown in (Fig. 3) indicate that an increase in chitosan decreased WVTR in the whole, however, the film KC2 (the chitosan content was 20 wt%) had a low WVTR value, even lower than that of KC3. This might indicate the existence of intermolecular interactions and a decrease of the mobility of both the konjac glucomannan and chitosan macromolecules when the mixing ratio was 8:2.

3.1.3. Water-solubility of films

It was obvious that the KC2 blend film had the highest water (Fig. 4), as a result, the antimicrobial agents in the blend film could be released more rapidly when the blend film was in contact with the surface of the food containing water. Generally, the short-range structure, long-range structure and condensed state structure of the polymers could affect the water absorbability of film. Taking the short-range structure into account, the reason why the blending (KC2) could largely increase the water absorbability might be that both of the two polysaccharides molecular chains could be more expanding and could be soaked by water more easily.

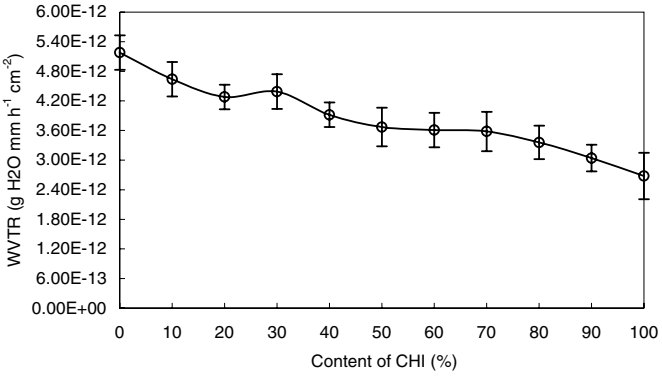

Fig. 3. The dependence of the water vapor transmission rate (WVTR) on the chitosan content of glucomannan–chitosan blend films.

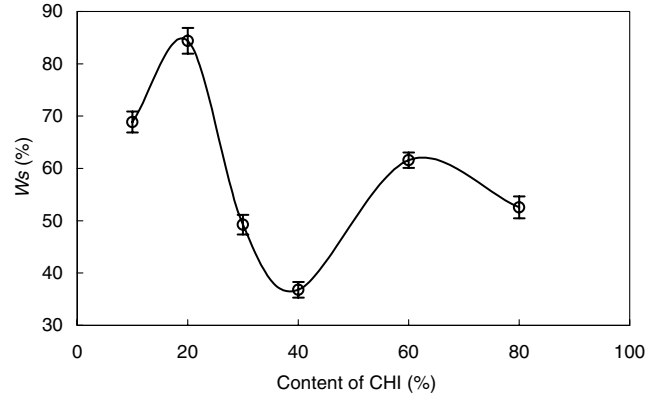

Fig. 4. The dependence of the water-solubility on the chitosan content of glucomannan–chitosan blend films.

3.2. Structure characterization

The main changes in the IR spectra of the films of KC1, KC2, KC3, KC4, KC6, KC8, konjac glucomannan and chitosan also shown in Figs. 5a and b. The absorption band around 3440 cm^{-1} broadened and shifted to a lower wave number with the decrease of chitosan content, indicating increase of intermolecular hydrogen bonds between chitosan and konjac glucomannan especially the chitosan content at 20 wt%. The stretching of carbonyl at 1723 cm^{-1} of konjac glucomannan disappeared; and the stretching of intramolecular hydrogen bonds at 1638 cm^{-1} in konjac glucomannan coupled and shifted to a lower wave number, suggesting that new hydrogen bonds between chitosan and konjac glucomannan molecules were formed in the blend films.

The position of the exothermal peak of the pure and blend films in the DSC curves of the konjac glucomannan, KC1, KC2, KC4, KC7 and chitosan films are shown in Fig. 6. Most of the konjac glucomannan/chitosan blend films showed a decomposition temperature lower than of the pure chitosan or konjac glucomannan film. However, the decomposition temperature reached 265.89 °C of KC2 which in larger than that of KC1 (260.80 °C) or KC4 (263.20 °C), which proved that a stronger interaction between the konjac glucomannan and the chitosan in KC2 had occurred.

Generally, transparency of films is an auxiliary criterion to judge the miscibility of two or more polymer mixed films. The best optical transparence (T%) at 480 nm for the blend films was shown by KC2 (Fig. 7) and achieved 88.6 ± 0.8%, indicating the best miscibility between konjac glucomannan and chitosan among all the blend films. In addition, the transparency at 210 nm decreased evidently with increase of the chitosan content, which means the blend films also had a better preventative ability against ultraviolet radiation than the pure konjac glucomannan film.

All this discussion on the structure and miscibility has revealed that the new hydrogen bonds between chitosan and konjac glucomannan molecules in the blend films had taken place in the blend film KC2, and it resulted in the changes of the physical properties of the blend films. Due to the outstanding tensile strength, transparency, water solubility and water vapor transmission, the KC2 film was selected to carry the antimicrobial assessment.

3.3. Antimicrobial activity

The details of antimicrobial activity of konjac glucomannan edible films incorporated with chitosan and nisin

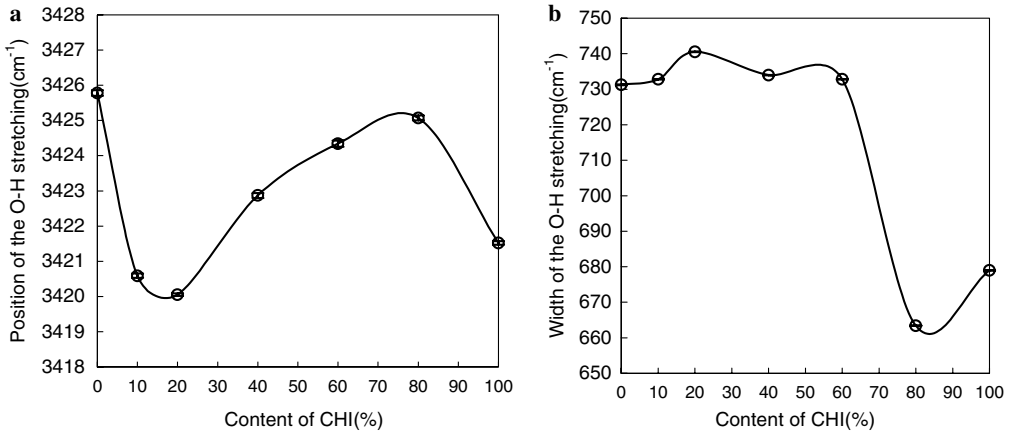

Fig. 5. Peak position (a) and width (b) for the O–H stretching of glucomannan–chitosan blend films.

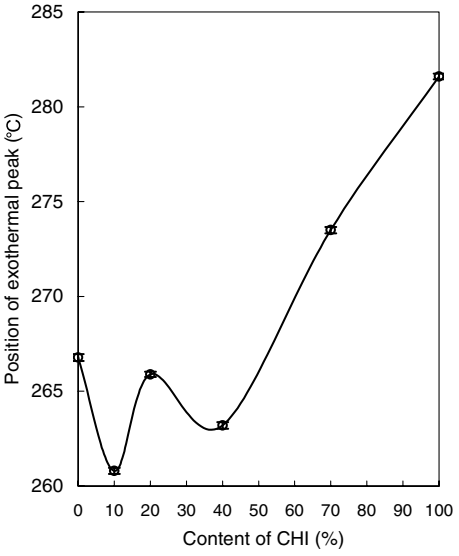

Fig. 6. Position of exothermal peak of glucomannan–chitosan pure and blend films.

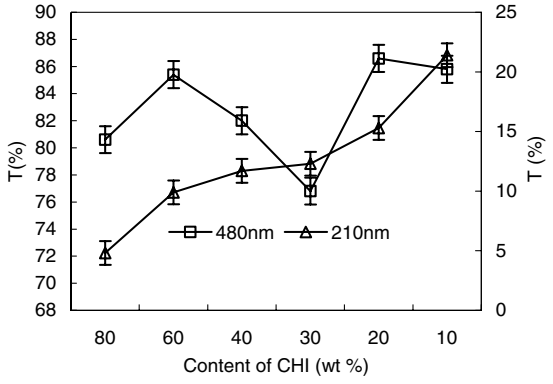

Fig. 7. The optical transmittance of glucomannan–chitosan blend films at 210 and 480 nm.

against *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus typhimurium*, *Listeria monocytogenes* and *Bacillus cereus*, which are common meat product contaminants are shown in Table 1. The inhibitory activity was measured based on the diameter of the clear inhibition zone. If there was no clear zone surrounding, it was assumed that there was no inhibitory zone, and was assigned a value of 0.00. Contact area was used to evaluate growth inhibition underneath film discs in direct contact with target microorganisms in agar.

In terms of the surrounding clear inhibition zone, in which no flora existed, the konjac glucomannan film did not show inhibitory effect against any tested microorganisms, and it was a pity that the chitosan film and the KC2 also did not show the inhibitory effect. Incorporating nisin into konjac glucomannan, chitosan and KC2 film did not show an inhibitory zone with *E. coli.* However, nisin-incorporated films revealed growth inhibition underneath film discs on these organisms. Among the inhibited microorganisms, *L. monocytogenes* was the most sensitive and susceptible to nisin. Incorporation of nisin for KC2 film at the lowest level of 42,000 IU/g of film already showed a clear inhibitory zone of 28.99 mm diametre. However, increasing the level of nisin at higher concentration did not reveal significant an increased inhibitory for KC2 with incorporated nisin, KC2-N. All the microorganisms underneath the films were inhibited when nisin was incorporated into konjac glucomannan, chitosan and KC2 films. Also, the antimicrobial agents were obviously more effective against Gram-positive bacteria than the Gram-negative bacteria studied.

As for as the three kinds of films incorporating nisin were concerned, it was obvious that significant difference ($p < 0.05$) existed between the chitosan-N series and KGM-N series after significance test, and between the KC2-N series and KGM-N series. The antimicrobial effect of chitosan-N series and KC2-N series were much better than the KGM-N series at each corresponding concentration, and the lower concentration of 42,000 IU/g of konjac glucomannan did not show inhibitory effect against *S. aureus* and *B. cereus.* Such a result revealed effectively that incorporating chitosan into the konjac glucomannan film (KC2) improved not only the physical properties but also

Table 1
Antimicrobial activity of konjac glucomannan film containing chitosan and nisin against food pathogenic bacteria of *E. coli, S. aureus, L. monocytogenes,* and *B. cereus*[a]

Film etc. material	*E. coli* (G^-)		*S. aureus* (G^+)		*L. monocytogenes* (G^+)		*B. cereus* (G^+)	
	Inhibition (diameter of the clear inhibition zone)	Contact (under the film disc)	Inhibition (diameter of the clear inhibition zone)	Contact (under the film disc)	Inhibition (diameter of the clear inhibition zone)	Contact (under the film disc)	Inhibition (diameter of the clear inhibition zone)	Contact (under the film disc)
Konjac glucomannan film (KGM)	0.00	−	0.00	−	0.00	−	0.00	−
Chitosan film (CHI)	0.00	+	0.00	+	0.00	±	0.00	−
KGM–CHI blend film (KC2)	0.00	±	0.00	+	0.00	+	0.00	−
Konjac glucomannan incorporating different content of nisin film (KGM-N, ×10³ IU nisin/g of film)								
42	0.00	±	0.00	−	21.08 ± 0.52	−	0.00	−
84	0.00	−	18.67 ± 0.76	−	22.74 ± 2.30	−	18.99 ± 1.57	−
168	0.00	−	22.03 ± 0.31	−	24.07 ± 0.66	−	21.15 ± 0.83	−
336	0.00	−	20.02 ± 0.73	−	22.97 ± 1.37	−	24.07 ± 0.53	−
Chitosan incorporating different content of nisin film (CHI-N, ×10³ IU nisin/g of film)								
42	0.00	±	21.78 ± 2.54	−	26.87 ± 2.13	−	21.31 ± 0.59	−
84	0.00	±	30.92 ± 2.87	−	33.92 ± 1.71	−	30.18 ± 2.28	−
168	0.00	−	33.78 ± 1.91	−	34.13 ± 0.93	−	34.35 ± 2.97	−
336	0.00	−	32.87 ± 2.49	−	39.99 ± 2.07	−	28.89 ± 0.67	−
KGM-CHI incorporating different content of nisin film (KC2-N, ×10³ IU nisin/g of film)								
42	0.00	±	23.63 ± 0.34	−	28.99 ± 1.31	−	22.29 ± 1.27	−
84	0.00	±	28.79 ± 0.56	−	31.27 ± 1.00	−	28.39 ± 0.73	−
168	0.00	±	30.23 ± 0.39	−	31.03 ± 0.28	−	32.93 ± 0.57	−
336	0.00	−	28.53 ± 0.51	−	32.27 ± 0.68	−	29.03 ± 0.37	−

Inhibition is the diameter in mm of the inhibitory zone surrounding film discs. Contact is the contact area under film discs on agar surface and (+) indicates growth in the area, (−) indicates no growth, and (±) indicates partial growth in the area.
[a] Mean ± standard deviation ($n = 3$).

the antimicrobial activity. In fact, one of the reasons for the antimicrobial character of chitosan is its positively charged amino group which interacts with negatively charged microbial cell membranes, leading to the leakage of proteinaceous and other intracellular constituents of the microorganisms (Shahidi, Arachchi, & Jeon, 1999). In the Gram-positive bacteria, the major constituent of its cell wall is peptidoglycan and there is very little protein. The cell wall of Gram-negative bacteria on the other hand is thinner but more complex and contains various polysaccharides, proteins and lipids beside peptidoglycan. The cell wall of Gram-negative bacteria also has an outer membrane, which constitutes the outer surface of the wall (Black, 1996).

Comparing the chitosan-N series with KC2-N series, it could be drawn that there was no significant difference after significance test on the antimicrobial effect between the two kinds of films at each corresponding concentration for *S. aureus* and *B. cereus.* However, as for the antimicrobial effect for *L. monocytogenes*, there was significant difference ($p < 0.05$) after significance test at the each corresponding concentration, and the chitosan-N was better than the KC2-N in the higher concentration. The latter presents itself as a stronger antimicrobial activity than the former in the lower concentration, which might be explained in that the blend film KC2 had a better water solubility and increased the release of nisin in the blend film.

4. Conclusions

The results of mechanical and physical properties tests showed that the blend film KC2 had the maximum tensile strength (102.8 ± 3.8 MPa) and a higher transparency (88.6 ± 0.8%), a greater water solubility (84.9 ± 1.9%) and a lower water vapor transmission ratio (4.64 ± 0.25 g H_2O mm h^{-1} cm^{-2}). This was to say incorporating the chitosan to the konjac glucomannan film enhanced the mechanical and physical properties remarkably. The analysis of DSC, FTIR, and transparency on the structural change of the blend films showed that strong intermolecular hydrogen bondings took place between chitosan and konjac glucomannan, and the interaction of the blend film KC2 was much greater than the others. This explained the enhancing of the physical properties.

Antimicrobial effect of konjac glucomannan edible film incorporating chitosan and nisin at various ratio or concentrations against food pathogenic bacteria namely *E. coli*, *S. aureus*, *L. monocytogenes*, and *B. cereus* was evaluated in detail. Incorporation of nisin at 42,000 IU/g of film for the selected blend film KC2 was found to have antimicrobial activity against *S. aureus*, *L. monocytogenes*, and *B. cereus*. The antimicrobial effect o f chitosan or KC2 incorporating nisin were much better than that of konjac glucomannan incorporating nisin at each corresponding concentration and existed significant difference ($p < 0.05$),

however, there was no significant difference on the antimicrobial effect between chitosan and KC2 incorporating nisin. Incorporating chitosan into the konjac glucomannan film (KC2) therefore improved not only the physical properties but also the antimicrobial activity.

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