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Characterization of konjac glucomannan–gellan gum blend films and their suitability for release of nisin incorporated therein

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Abstract

The structure and properties of dried blend films of konjac glucomannan and gellan gum prepared by a solvent-casting technique with different blending ratios of the two polymers have been studied by using Fourier transform infrared spectroscopy (FT-IR), wide-angle X-ray diffraction (WAXD), differential scanning calorimetry (DSC), scanning electron microscopy (SEM), transparence analysis, and strength tests. The crystallinities of the blend films decreased with the increase of the konjac glucomannan proportion. The DSC and FT-IR showed the existence of interaction between two kinds of polysaccharides. The tensile strength of the blend films achieved its maximum 17.5 MPa when the konjac glucomannan content in the blend films was around 70 wt% (KG7). The section morphology of the blend films observed by SEM also displayed the best miscibility at this ratio. The structure analysis indicated that there was a hydrogen interaction between konjac glucomannan and gellan gum. Antimicrobial films incorporating nisin were found to have antimicrobial activity against *Staphylococcus aureus* particularly with higher contents of gellan gum. With the increase of the content of gellan gum, the antimicrobial effects enhance. These results suggest that the blend films KG7 could be applied as a potential food package material for releasing the active agent such as nisin.

Keywords: Konjac glucomannan; Gellan gum; Blend films; Miscibility; Active package

1. Introduction

In the last 20 years, antimicrobial release systems have been used mainly in pharmaceutical applications (Bezemer et al., 2000; Colombo, Bettini, Santi, De Ascentiis, & Peppas, 1996; Conte, Colombo, Gazzaniga, Sangalli, & La Manna, 1988; Kuijpers et al., 1998), while their use in food packaging is still restricted (Floros, Nielsen, & Farkas, 2000), although it is expected to grow due to the increase of the concept of 'active packaging'. Methods to deliver antimicrobials to food can vary from direct addition to incorporation into packaging materials. There have been remarkable developments in recent years in the polymeric packaging films incorporating antimicrobial agents for improving the preservation of packaged foods. Nisin has been recognized by the US Food and Drug Administration (FDA) as generally recognized as safe (GRAS) and has a relatively broad spectrum of antibacterial activity against food-borne pathogens and spoilage organisms (Breukink & de Kruijff, 1999).

Lately, a lot of research has been done on bacteriocins in food packaging. Ming, Weber, Ayres, and Sandine (1997) applied nisin and pediocin to cellulose casings to reduce *Listeria monocytogenes* in meats and poultry. Nisin was incorporated into polyethylene-based plastic films to inhibit the surface growth of *Lactobacillus helveticus* and *Brochothrix thermosphacta* on meats (Siragusa, Cutter, & Willett, 1999). Natrajan and Sheldon (2000a, 2000b) applied nisin-coated polymer (PVC, LLDP, nylon) films and protein and polysaccharide-based films to inactivate *Salmonella typhimurium* on fresh broiler skin.

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Konjac glucomannan is a high molecular weight water-soluble non-ionic heteropolysaccharide found in tubers of the *Amorphophallus konjac* plant. Konjac glucomannan is a linear random copolymer of (1 → 4)-β-D-glucopyranose and β-D-mannopyranose, having glucose and mannose units in a molar ratio of 1:1.6 with a low degree of acetyl groups (approximately 1 acetyl group per 19 residues) at the C-6 position (Nutricol, 1993; Williams, Foster, Martin, & Norton, 2000). When konjac flour is dissolved in alkaline coagulant (such as calcium hydroxide, sodium or potassium carbonate), the deacetylation reaction occurs and a thermally stable gel is formed (Thomas, 1997). The rate of gel formation is dependent upon pH and processing temperature. The research work on konjac glucomannan was successful in fields such as pharmaceuticals, food and chemical engineering. Several articles reported blend films of konjac glucomannan and other natural or synthetic polymers, such as chitosan (Li, Kennedy, Jiang, & Xie, 2006; Xiao, Gao, Wang, & Zhang, 2000; Ye, Kennedy, Li, & Xie, 2006), gelatin (Li et al., 2006; Xiao, Lu, Gao, & Zhang, 2001; Li, Kennedy, Peng, Yie, & Xie, 2006), and PVA (Li & Xie, 2004). As for the blend films of konjac glucomannan and chitosan, the mechanical properties achieved their maximum when the weight ratio of konjac glucomannan to chitosan was 8:2, and strong intermolecular hydrogen bonds took place between the amino groups of chitosan and the hydroxyl groups of konjac glucomannan (Ye et al., 2006).

Gellan gum is an extracellular polysaccharide produced by fermentation of *Sphingomonas paucimobilis* (formerly *Pseudomonas elodea*) (Kang, Veeder, Mirrasoul, Kaneko, & Cottrell, 1982). The polymer is a linear, anionic heteropolysaccharide based on a tetrasaccharide repeat unit of β-D-glucose, β-D-glucuronic acid, and α-L-rhamnose units in molar ratios of 2:1:1 (Sanderson, 1990). High-acyl gellan, which forms only weak gels, is recovered directly from the fermentation broth, whereas low-acyl gellan is obtained by deacylation with alkali treatment (Sanderson, 1990) and forms strong gels with the proper formulation of polymer and cation concentrations. The addition of a small amount of salt readily promotes conformational transition into an ordered double-helix chain (Crescenzi, 1995). The double-helix formation and sol–gel transition occur almost simultaneously in concentrated gellan solution (Miyoshi, Takaya, & Nishinari, 1995). Because of its good film-forming (Lee, Shim, & Lee, 2004; Sudhamania, Prasada, & Sankar, 2003) and outstanding drug release (Iulian, Marcel, Anca, Silvia, & Valentina, 2006; Kedzierewicz, Lombry, Rios, Hoffman, & Maincent, 1999; Miyazaki, Aoyama, Kawasaki, Kubo, & Attwood, 1999; Santucci et al., 1996; Sunil, Sheetal, & Tejraj, 2006) properties gellan gum has been widely used in manufactured biomaterials.

The synergistic effects between konjac glucomannan and gellan gum mixed hydrosol or gel had been studied in the past ten years (Miyoshi, Takaya, Williams, & Nishinari, 1996; Nishinari, Miyosbi, Takaya, & Williams, 1996). However, little information has been focused on the characterization of konjac glucomannan–gellan gum blend films. The aim of this paper is to explain the structure, miscibility and mechanical property of blend films by FT-IR, XRD, DSC, SEM, and strength tests for food package using. Moreover, the practical release ability of nisin from the blend films for antimicrobial purposes is also discussed.

2. Materials and methods

2.1. Materials

Konjac glucomannan was prepared as our previous work (Li & Xie, 2004). The viscosity-average molecular weight (Mv) of the konjac glucomannan was determined by viscometry to be 1.32×10^6 according to the Mark–Houwink equation $[\eta] = 5.96 \times 10^{-2}\ \mathrm{Mv}^{0.73}$ at 25 °C.

Deacetylated gellan polymer powder, Kelcogel, was provided by Kelco Inc. (San Diego, CA, USA), which was used without further purification.

2.2. Preparation of blend films

Solutions of konjac glucomannan/gellan gum at various ratios were prepared by mixing the appropriate amounts of each powder and dispersing in distilled water to a total concentration of 1 wt%. The solutions of mixtures were prepared by stirring at 70 °C for 2 h and then heated at 90 °C for 1 h (Nishinari et al., 1996). The solutions were cast on polystyrene plates and dried at 60 °C for 12 h. By changing the weight ratio of konjac glucomannan to gellan gum 1:9, 3:7, 5:5, 7:3, and 9:1, a series of blend films coded as KG1, KG3, KG5, KG7, and KG9 were prepared, respectively. The films obtained from pure konjac glucomannan and gellan gum were coded as KGM and GG.

Nisin, with an activity of 1050 IU/mg (Sigma–Aldrich Chemie GmbH, Steinheim, Germany), was incorporated into pure and blend films in the antimicrobial assay.

2.3. Characterization of films

Transmission infrared spectra of the films were recorded at room temperature using a Nicolet (USA) Nexus 470 FT-IR spectrometer at a resolution of 2 cm^{-1} in the range 400–4000 cm^{-1}. The films were mounted directly in the sample holder. The X-ray diffraction (XRD) curves of the films were recorded with a Rigaku (Japan) D/max-RB X-ray diffractometer and used a CuKα target at 40 kV and 50 mA. The diffraction angle ranged from 10° to 60°. The crystallinities of the films were calculated by $X_c = F_c/(F_c + F_a) \times 100\%$, where F_c and F_a are the areas of crystal and non-crystalline regions, respectively. The differential scanning calorimetry of the film samples (5 mg) was performed under a nitrogen atmosphere with a flow capacity of 25 ml/min by a DSC 204-F1 (NETZSCH, Germany) from 30 to 400 °C at a heating rate of 10 °C/min. Film samples of about 100-μm thickness were coated with gold in 0.1τ vacuum degree. The cross-section morphologies were observed on a Hitachi X-650 SEM. The transmittance (T%) of films about 20 μm in the wavelength of 480 nm was measured by using a Shimadzu UV-160A (Japan) spectroscope. The tensile strength (σb) and breaking elongation (εb) of the films were measured on an electron tensile tester RGT-2 (Shenzhen reger instrument Co., Ltd., China) with a tensile rate of 250 mm/min according to the Chinese standard method (GB/13022-91). The moisture uptake of the films was calculated as $W = [(W_1 - W_0)/W_0] \times 100\%$, where W is the moisture uptake, and W_1 and W_0 are the weights of the films in soaked and dried states, respectively. The films were dried at 60 °C for 12 h before all the measurements.

2.4. Antimicrobial assay (release ability on nisin)

Antimicrobial activity tests of films were carried out using an agar diffusion method. All films were cut into a disc form of 10 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 inoculums 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.

3. Results and discussion

3.1. Structure and miscibility

FT-IR is of importance in the study of the molecular structure. The width and intensity of the spectrum bands, as well as the position of the peaks, are all sensitive to environmental changes and to the conformations of macromolecules at the molecular level. Intermolecular interactions occur when different polymers are compatible, so the FT-IR spectrum of the blend is different from those of pure polymers, which is advantageous to the study of the extent of polymer compatibility.

Fig. 1 shows the FT-IR spectra for the films of gellan gum, the blends (KG3, KG5, KG7), and konjac glucomannan in the wave number range of 4000–400 cm^{-1}. The absorption band at 3364 cm^{-1} and the peaks at 2876 cm^{-1} are assigned to the stretching of –OH groups and C–H of methyl in konjac glucomannan; the stretching peaks of the carbonyl at 1722 cm^{-1} are assigned to the aceto groups in konjac glucomannan; the broad peak at 1642 cm^{-1} was assigned to stretching of C–O of associate hydroxy groups; the characteristic absorption bands of mannose in konjac glucomannan appeared at 873 and 799 cm^{-1}. The peaks at 1605 and 1404 cm^{-1} are assigned to the characteristic absorption band of carboxyl in gellan gum. The major bands centered at 3200–3500 cm^{-1} showed significant changes, which broadened and shifted to a higher wave number. For the blend film KG7, the strong absorption band at 3364 cm^{-1} assigned to the stretching of intermolecular hydrogen bonds in konjac glucomannan and shifted to 3276 cm^{-1}. Furthermore, the peak at 1722 cm^{-1} for konjac glucomannan disappeared in the spectra of the blends and the peaks at 1642 cm^{-1} shifted to 1404 cm^{-1} with the increase of gellan gum. Based on the results of FT-IR, it can be conclude that the miscibility between konjac glucomannan and gellan gum was due to the formation of intermolecular hydrogen bonds. The hydrogen bondings mainly resulted from the –COO$^-$ of carboxyl groups and –OH groups and –COCH$_3$ groups in konjac glucomannan, respectively.

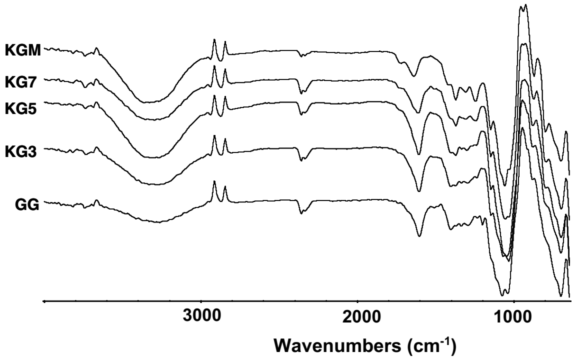

Fig. 1. FT-IR spectra of the GG, KG3, KG5, KG7, and KGM films.

XRD is another important method to characterize the miscibility of two polymers; generally, when the crystalline component and the non-crystalline component in a composite material have good miscibility, its crystallinity is lower than that of the individual crystalline components. The crystallinities of gellan gum, KG1, KG3, KG5, KG7, KG9, and konjac glucomannan based on WXRD were 34.5%, 46.7%, 41.8%, 35.9%, 31.3%, 38.1%, and 40.6%, respectively (Fig. 2). The pure konjac glucomannan film showed a non-crystalline state and only had a very broad peak around 19.86°. Two crystal peaks could be recognized at around 19.16° and 22.56° in the XRD pattern of gellan gum. With the increase of proportion of konjac

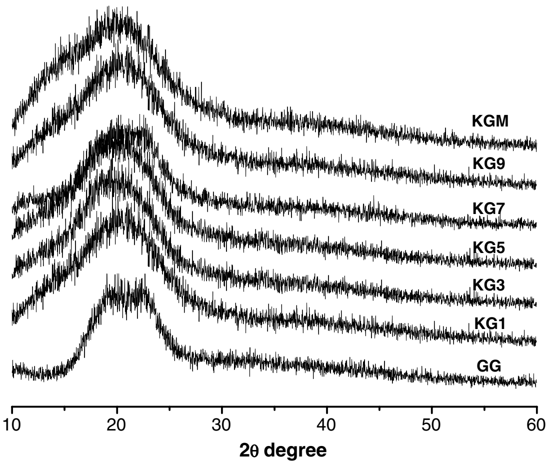

Fig. 2. WXRD curves of the GG, KG1, KG3, KG5, KG7, KG9, and KGM films.

glucomannan, the diffraction peak corresponding to $2\theta = 25.56°$ of gellan gum becomes gradually lowered, and the diffraction angle neared more and more to 19.16° accordingly. The changes of the diffraction patterns indicated that an intermolecular hydrogen bonding interaction occurred between the two polymers and destroyed the original crystalline domains of konjac glucomannan and gellan gum.

From the thermal behaviour of pure and blend films, as investigated by means of DSC measurements (Fig. 3), the films all show endothermic peaks around 70–100 °C, corresponding to the loss of crystalline water molecules in the films formed during drying progress. An exothermic peak appearing at about 326.0 °C was attributed to the greatest thermal degradation of konjac glucomannan. The DSC curve of gellan gum showed an exothermic peak at 250.3 °C, which was attributed to the disintegration of the molecular chains. Some changes appearing in the DSC curves of the blend films may suggest that hydrogen bonding interaction was established between the konjac glucomannan and gellan gum. It is worth noting that all films showed exothermic peaks at about 350–330 °C, which resulted from the greatest thermal degradation of the films. With the increase of konjac glucomannan content in the blend, the greatest thermal degradation peaks shifted to a higher temperature – which may indicate the existence of a certain degree of interaction between two kinds of macromolecules.

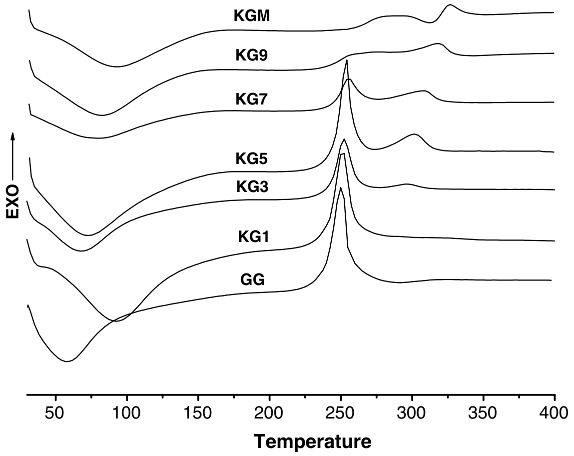

Fig. 3. The DSC curves of the GG, KG1, KG3, KG5, KG7, KG9, and KGM films.

In the SEM photographs of cross-section of the films (Fig. 4) the blend film KG7 showed a smooth and homogeneous surface morphology, suggesting high miscibility and blend homogeneity between konjac glucomannan and gellan gum at the composite ratio of konjac glucomannan to gellan gum to 7:3 by weight. This result supports the conclusion of miscibility between konjac glucomannan and gellan gum in the films and was in good agreement with those from FT-IR, XRD, and DSC.

Generally, transparency of films is an auxiliary criterion to judge the miscibility of two or more polymer-mixed films (Li & Xie, 2004). The optical transmittance (T%) dependence of the wavelength (480 nm) for the pure and blend films is shown in Fig. 5. The low point at 90% seemed to be an exception, the examination was repeated for several times, but it did not change the result. The reason might be that the transparency of the pure konjac glucomannan was rather high, when it was added to by gellan gum, and the amount of gellan gum was still much lower than the amount needed for forming the hydrogen bond network with konjac glucomannan, so the transparency decreased. However, with the increase in the addition, the hydrogen bond interaction strengthened, and the transparency increased. The best optical transmittance of blend films appeared at the konjac glucomannan/gellan gum = 70/30 mixing ratio (KG7), indicating the best miscibility between konjac glucomannan and gellan gum.

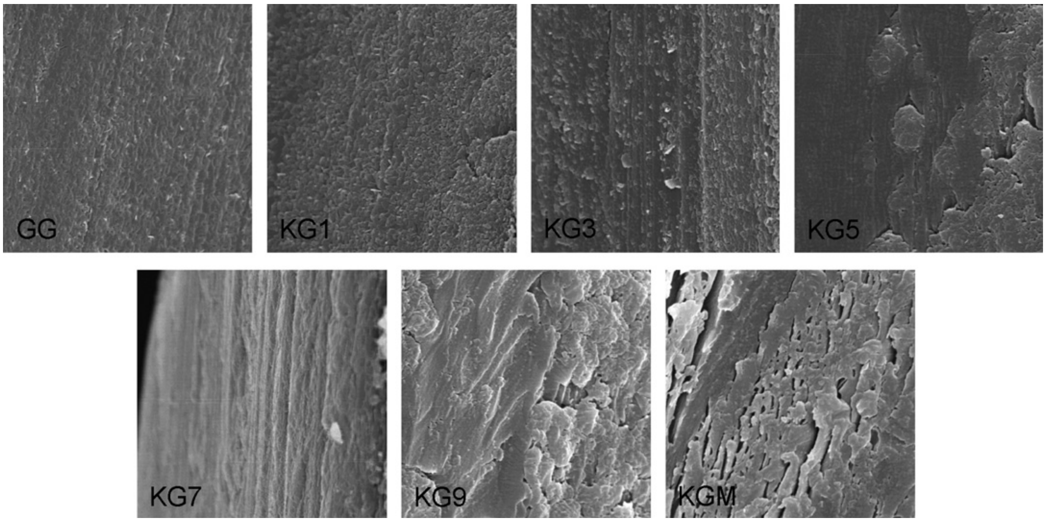

Fig. 4. SEM photographs of the cross-section of the GG, KG1, KG3, KG5, KG7, KG9, and KGM films (5000×).

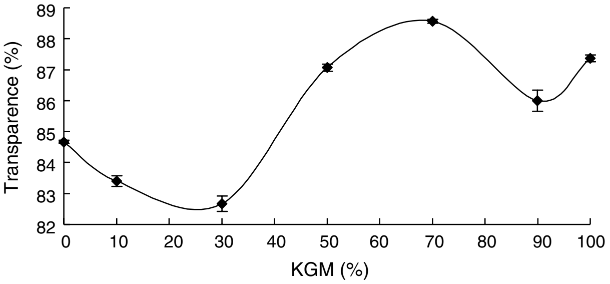

Fig. 5. The dependence of optical transmittance on the KGM content for the blend films.

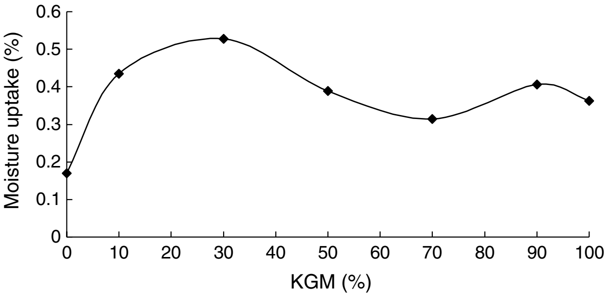

Fig. 7. The dependence of the moisture uptake on the KGM content for the blend films.

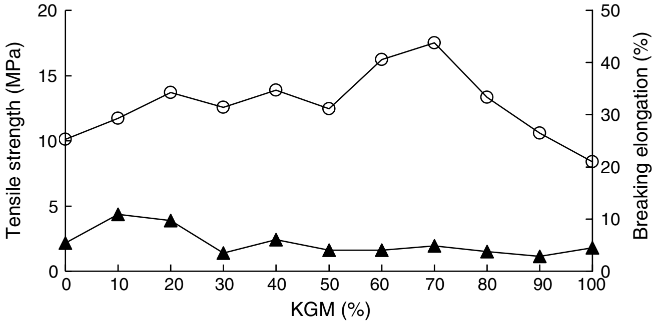

Fig. 6. The dependence of tensile strength and breaking elongation on the KGM content for the blend films.

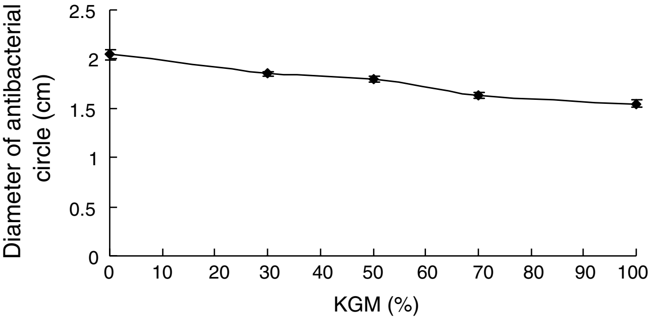

Fig. 8. The dependence of the antimicrobial activity on the KGM content for the blend films.

3.2. Physical properties

Tensile strength is the most important mechanical property for many applications. Fig. 6 shows the effect of konjac glucomannan/gellan gum ratio on the tensile strength and breaking elongation. The tensile strengths of the blend films increased with the increase of konjac glucomannan content, and the maximum value appeared at the konjac glucomannan/gellan gum = 70/30 mixing ratio (KG7) and achieved 17.51 MPa. The breaking elongation of the film was about 5.4% of average. The considerable enhancement in tensile strength of blend films indicated that intermolecular interactions between the konjac glucomannan and gellan in them.

As expected, from the dependence of the moisture uptake on the konjac glucomannan content for the blend films (Fig. 7) the addition of konjac glucomannan to the gellan gum resulted in a lower value at the mixing ratio konjac glucomannan/gellan gum = 70/30 (KG7). This result seems to due to konjac glucomannan containing a large number of hydrophilic hydroxyl groups.

3.3. Antimicrobial activity

The inhibitory activity was measured based on the diameter of the clear inhibition zone in the antimicrobial tests. In terms of the surrounding clear inhibition zone, in which no flora existed, the konjac glucomannan film did not show inhibitory effect against tested microorganisms. Incorporating nisin into pure and blend films show an inhibitory zone with *Staphylococcus aureus* (Fig. 8). As for the films of gellan gum, the blends (KG3, KG5, KG7), and konjac glucomannan incorporating nisin were concerned, it was obvious that significant difference ($p < 0.01$) existed. With the increase of the content of gellan gum, the diameter of the inhibition zone was enhanced. It is estimated that the release of nisin increased when gellan gum existed in the blend film.

4. Conclusions

The analysis by FT-IR, XRD, DSC, and transparency on the structural change of the blend films showed that intermolecular hydrogen bonding took place between konjac glucomannan and gellan gum, and the interaction of the blend film was much greater than that of the others, when the konjac glucomannan content in the blend films was around 70 wt% (KG7). In addition, the blend film (KG7) has a maximum tensile strength and lower moisture uptake value. This can be explained in terms of TERMS the enhancing of the interaction between two polysaccharides. Antimicrobial effects of konjac glucomannan–gellan gum film incorporating nisin at various ratios against food pathogenic bacteria namely *S. aureus* shows that the inhibitory activity has a positive relativity to the content of gellan gum. Results from the data of release rates show that the application of films including nisin has the potential to overcome the applications associated with short or long shelf-life food packages. Further research is necessary in order to determine if the ideal rate of antimicrobial release that provides the most effective inhibitory effect on pathogenic bacteria growth may depend on the length and temperature of storage.

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