J. Microbiol. Biotechnol. (2007), **17**(3), 520–523

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

Physical Properties of Nisin-Incorporated Gelatin and Corn Zein Films and Antimicrobial Activity Against *Listeria monocytogenes*

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Received: September 5, 2006
Accepted: November 30, 2006

Abstract Edible films of gelatin and corn zein were prepared by incorporating nisin to the film-forming solutions. Corn zein film with nisin of 12,000 IU/ml had an increase of 11.6 MPa in tensile strength compared with the control, whereas gelatin film had a slight increase with the increase of nisin concentration added. Water vapor permeability for both corn zein and gelatin films decreased with the increase of nisin concentration, thus providing a better barrier against water. Antimicrobial activity against *Listeria monocytogenes* increased with the increase of nisin concentration, resulting in 1.4 log cycle reduction for corn zein film and 0.6 log cycle reduction for gelatin film at 12,000 IU/ml. These results suggest that incorporation of nisin into corn zein and gelatin films improve the physical properties of the films as well as antimicrobial activity against pathogenic bacteria during storage, resulting in extension of the shelf life of food products by providing with antimicrobial edible packaging films.

Keywords: Edible films, nisin, *Listeria monocytogenes*, gelatin, corn zein

New natural biodegradable and edible packaging films have been studied for environmental concern, and edible coating to inhibit the growth of pathogenic bacteria in food products is an active research interest in the food science field [5, 9, 10]. In that aspect, antimicrobial protein films as biodegradable packaging offer environmental compatibility as well as longer shelf life of foods [14, 18].

Consumers demand foods without chemical preservatives. Use of antimicrobial protein films is appropriate for that purpose if an antimicrobial agent is incorporated into foods [3]. Antimicrobial compounds used for food packaging include organic acid, bacteriocins, enzymes, and plant extracts [13]. Nisin has been tested as a natural antimicrobial substance that can be added for packaging films [11]. Nisin is a 3,500-Da peptide produced by *Lactococcus lactis*, and inhibits Gram-positive bacteria, in particular, an effective inhibitor of *Listeria monocytogenes* [12].

Gelatin is a biodegradable polymer that is used in biomedical fields. Corn zein may be used for protein film in food application. Thus, gelatin and corn zein were selected as suitable proteins for edible films blended with nisin. Therefore, in this study, to develop edible food packaging films having antimicrobial activity, we prepared corn zein and gelatin films containing nisin, and examined their physical properties and antimicrobial activities against *Listeria monocytogenes*.

Corn zein film-forming solutions were prepared by heating and stirring 10% corn zein (Sigma Chemical Co., St. Louis, MO, U.S.A.) in 100 ml of 95% ethanol. As plasticizers, 3% glycerol was added, and varying amounts (4,000, 8,000, 12,000 IU/ml of film solution) of nisin (Sigma Chemical Co., St. Louis, MO, U.S.A.) were immediately incorporated into the film-forming solution at pH 5.0. Film-forming solutions were heated in a water bath at 75°C for 20 min. Gelatin film-forming solutions were also prepared by heating and stirring 10% gelatin (Sigma Chemical Co., St. Louis, MO, U.S.A.) in 100 ml of distilled water. Glycerol and varying amounts of nisin were also added, and then heated in a water bath at 90°C for 30 min. Film-forming solutions were then strained through cheese cloth and cast on flat, Teflon-coated glass plates (24 cm×30 cm). Uniform film thickness was maintained by casting the same amount of film-forming solution on each plate. Plates were dried at 25°C for 24 h. Dried films were peeled intact from the casting surface. Specimens were cut for water vapor permeability (2 cm×2 cm) and tensile strength (2.54 cm×10 cm) measurements. Film thickness was measured with a micrometer (Mitutoyo, Model No. 2046-08, Tokyo, Japan), and there was no significant change in thickness (data not shown).

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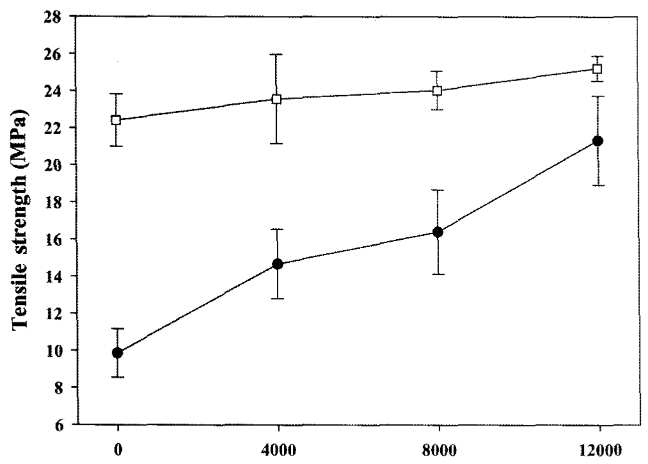

Fig. 1. Effect of nisin concentrations on the tensile strength of corn zein and gelatin films (□, Gelatin films; ●, Corn Zein films).

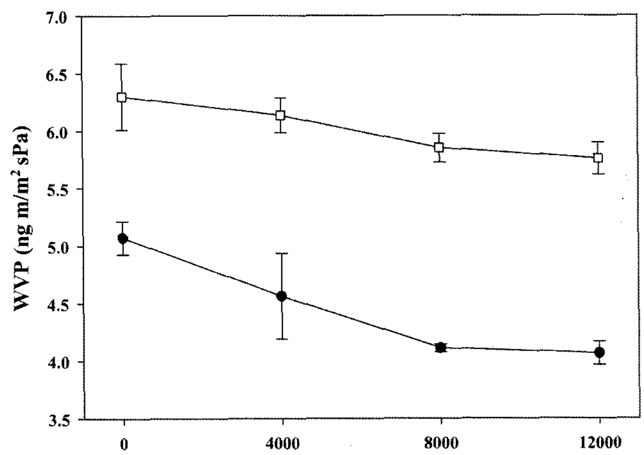

Fig. 2. Effect of nisin concentrations on the water vapor permeability of corn zein and gelatin films (□, Gelatin films; ●, Corn Zein films).

Film tensile strength (TS) was determined with an Instron Universal Testing Machine (Model 4484, Instron Co., Canton, MA, U.S.A.) according to ASTM Standard Method D882-91 [1]. Film specimens were conditioned in an environmental chamber at 25°C and 50% RH for 2 days. Initial grip distance of 5 cm and cross-head speed of 50 cm/min were used. TS was calculated by dividing the maximum load by initial cross-sectional area of a specimen. Five replicates of each film were tested.

TS of corn zein film increased with the increase of nisin concentration, whereas there was no significant change for gelatin films (Fig. 1). Corn zein film had the tensile strength of 10.98 MPa for the control, and 22.58 MPa with nisin of 12,000 IU/ml. This difference could be explained by rearrangement of the disulfide bonds and hydrophobic interactions among zein molecules [12]. Incorporation of nisin into corn zein film-forming solutions might have caused the increase of TS by forming a film network between nisin molecules and protein molecules. For gelatin film, the TS was 24.95 MPa at nisin of 4,000 IU/ml, and 22.18 MPa at 12,000 IU/ml, compared with the control of 24.66 MPa. This negligible difference could be due to a lack of interaction between hydrophilic gelatin molecules and hydrophobic nisin molecules. However, it should be noted that the gelatin film had higher TS than that of corn zein film. The reason may be the property of gelatin molecules to recover the triple helix structure of the collagen, having a more organized network [7].

Water vapor permeability (WVP) is one of the most important functional properties of protein films. The WVP was determined according to the modified ASTM E 96-95 method [2] at 25°C and 50% RH using a polymethylacrylate cup [16]. The cup was filled to 1 cm with distilled water and covered with a film specimen. Film specimens were conditioned in an environmental chamber at 25°C, 50% RH for 2 days. The weight loss of cups with time was measured. A linear regression analysis was performed to calculate a slope. WVP (ng m/m^{2}s Pa) values were then calculated from the following equation:

WVP=(WVTRL)/Δp, where the water vapor transmission rate (WVTR, g/m^{2}s) was calculated by dividing the slope by the open area of the cup. L is mean thickness (m), and Δp is corrected partial vapor pressure difference (Pa) across the film specimen.

The WVP for both corn zein and gelatin films decreased with the increase of nisin concentration (Fig 2). For corn zein film, the control had 5.23 ng m/m^{2}sPa, whereas the WVP of the film at nisin of 12,000 IU/ml decreased by more than 1 ng m/m^{2}sPa. In the case of gelatin film, the control had 6.46 ng m/m^{2}sPa, which is higher than that of corn zein film. This can be explained by the greater hydrophobic property of corn zein molecules. It has been known that corn zein is hydrophobic since it contains lots of nonpolar amino acids such as leucine (15.4%) or proline (10.0%) [19]. Incorporation of nisin at 12,000 IU/ml decresed the WVP of gelatin film by 0.4 ng m/m^{2}sPa, which is less than that for corn zein. The lower WVP by the addition of nisin may be explained by interstructural bridges between the protein molecules and nisin molecules, resulting in the formation of denser three-dimensional networks [17]. In the case of the glucomannan-chitosan-nisin ternary antimicrobial blend film, WVP decreased with the increase of chitosan concentration [15].

The antimicrobial activities of corn zein and gelatin films containing nisin were determined according to the method in the literature [12]. *Listeria monocytogenes* was incubated at 37°C, 130 rpm in BHI (Brain Heart Infusion, Difco Co., Detroit, MI, U.S.A.) broth until it reached

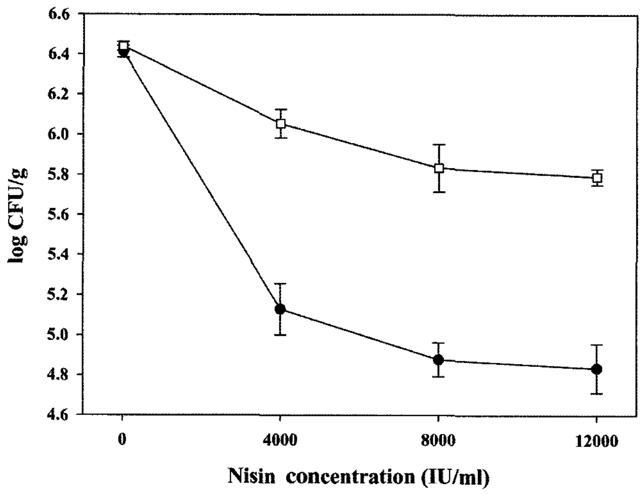

Fig. 3. Effect of nisin concentrations on the antimicrobial effect of corn zein and gelatin films against *Listeria monocytogenes* (□, Gelatin films; ●, Corn Zein films).

7.8×10^4 CFU/ml. Fifteen μl of the bacterial suspension was placed on prepared corn zein and gelatin film discs (0.02 g) containing nisin and without nisin. The film discs were incubated at room temperature for 60 min. After incubation, film discs were placed in 0.98 ml of 0.1% peptone water and then homogenized for 2 min. The solution was diluted with 0.1% peptone water and plated in plate count agar (PCA, Difco Co., Detorit, MI, U.S.A.) plates. All plates were incubated at 37°C for 24 h. Each microbial count was the mean of three determinations and microbial counts were expressed as colony forming unit (CFU)/g.

The antimicrobial activities of nisin-incorporated corn zein and gelatin films against *Listeria monocytogenes* are shown in Fig. 3. Incorporation of nisin into the corn zein and gelatin films inhibited growth of *Listeria monocytogenes*, and increase of nisin concentration increased the degree of inhibition against *Listeria monocytogenes*. For corn zein film, addition of nisin of 12,000 IU/ml decreased by 1.4 log CFU/g. This is in good agreement with the report for nisin-coated paper [13]. For gelatin film, incorporation of nisin of 12,000 IU/ml led to 5.81 log CFU/g, compared with the control, at 6.41 log CFU/g, resulting in decrease of 0.6 log CFU/g. The reason for the small inhibition may be due to a decrease of nisin activity by the hydrophilic nature of gelatin molecules. There would be another reason regarding treatment temperature in preparation of film. Hoffman *et al.* [6] reported that nisin is stable at 100°C in the absence of water and there was an increasing loss of nisin activity in polyethylene film forming at 149°C with heating times. Boziaris and Nychas [4] also reported that the inhibitory effect of nisin against *Listeria monocytogenes* was dependent on incubation temperature. Therefore, the relatively low antimicrobial activity of nisin for gelatin film, compared with zein film, could be due to the manufacturing condition of the film. Ko *et al.* [12] reported that the maximum concentration of nisin for antimicrobial activity against *Listeria monocytogenes* was 8,000 IU/ml. Our results showed a similar pattern since there was a drastic decrease in colony forming units at nisin of 8,000 IU/ml. This is in good agreement with the report of nisin-contained edible films that are made of starch [8].

In summary, incorporation of nisin into corn zein and gelatin film-forming solutions improved the physical properties of the films such as TS and WVP, as well as antimicrobial activity, providing a good candidate for a means of extending the shelf life as food packaging film.

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