Physical and Chemical Properties of Edible Films Containing Nisin and Their Action Against Listeria Monocytogenes

S. Ko, M.E. Janes, N.S. Hettiarchchy, and M.G. Johnson

ABSTRACT: The effects of hydrophobicity/hydrophilicity of edible films against Listeria monocytogenes strain V7 by various nisin concentrations (4.0 - 160 IU/film disk) and pH values ranging from 2.0 to 8.0 were determined and the mechanical properties and water vapor permeability of films prepared with or without nisin were compared. Surface hydrophobicities (446, 282, 232 and 142, respectively) of whey protein isolates, soy protein isolates, egg albumen and wheat gluten were determined. As the nisin concentration increased, the amount of inhibition progressively increased in all tested films. Using nisin, edible films with higher hydrophobicity values of 280 to 450 units under an acidic environment exerted a greater inhibitory effect against L. monocytogenes. Different interactions of nisin with the proteins of the different films resulted in variation in the mechanical properties and water vapor permeabilities of the films tested.

Keywords: edible films, nisin, Listeria monocytogenes, surface hydrophobicity, mechanical properties

Introduction

As a result of increasing concern over environmental safety of nondegradable synthetic product and food safety, there is a demand for natural degradable packaging material from renewable sources as an alternative to synthetic polymers and natural antimicrobial agents. Edible films can increase the shelf-life of foods by providing barrier properties against migration of moisture, gases, and vapor (Kunte and others 1997), functioning as a carrier of food ingredients, additives, and antimicrobial agents, and offering mechanical protection to foods (Torres and others 1985). The interaction between the added antimicrobials and film-forming materials may affect the mechanical properties and water vapor permeability of the resulting films.

Components of edible films and coatings can be divided into three categories, including hydrocolloids, lipids and composites. Suitable hydrocolloids include proteins and carbohydrates. Protein, lipid, polysaccharide, and composite are based on the nature of the material, used for film production (Guilbert 1986). Each film or coating type provides its own unique functional characteristics and is best suited to a specific food application.

Pure lipid films have extremely low water vapor permeability because of their hydrophobic characteristics. Protein and polysaccharide films exhibited poor water barrier properties because they have hydrophilic properties (Krochta 1994). Highly polar polymers, such as proteins and polysaccharides having large degrees of hydrogen bonding and containing hydroxyl groups, have extremely low gas permeability values and poor moisture barrier properties. However, when lipids were incorporated into these films, water vapor permeability could be reduced. The water vapor permeability of edible films can be improved by the addition of lipids. This can be achieved by laminating a hydrophilic film containing a lipid layer—making a composite film where both hydrophilic and hydrophobic components are dispersed in a solvent—and either drying or emulsifying the lipid within the hydrophilic phase (Debeaufort and others 1993; Park and others 1994; Gontard and others 1994; McHugh and Krochta 1994).

The bacteriocin nisin, a 3500-Da peptide produced by Lactococcus lactis subsp. lactis inhibits gram-positive organisms (for example Listeria monocytogenes, Staphylococcus aureus, and Clostridium botulinum) (Nettles and Barefoot 1993). Nisin is a hydrophobic protein (Klaenhammer 1993), and has GRAS (generally recognized as safe) status for use with certain cheese products in the USA.

While several researchers have previously reported on the potential benefits of using antimicrobials in films produced from natural food materials (Ming and others 1997; Orr and others 1996; Dawson and others 1995; Hoffman and others 1998; Natrajan 1997; Janes and others 1999; Chen and others 1996), none of these studies reported on the possible effects of nisin immobilized in films with different hydrophobicities.

This study was conducted to determine the effects of different hydrophobic/hydrophilic edible films on the activity of nisin against Listeria monocytogenes strain V7 and to compare the mechanical properties and water vapor permeabilities of films prepared with or without nisin.

Materials & Methods

Material for film

Commercial soy protein isolates (SPI) (Ardex D) and spray-dried wheat gluten (WG) were obtained from Archer Daniels Midland Co. (Decatur, Ill., U.S.A.) and Midwest Grain Products, Inc. (Atchison, Kans., U.S.A.), respectively. Glycerol used as a plasticizer (Fisher Scientific, Fair Lawn, N.J., U.S.A.). Egg albumen (EA) protein was supplied by Henningsen Foods, Inc. (Omaha, Nebr., U.S.A.). Whey protein isolate (WPI) was obtained from New Zealand Milk Products, Inc. (Santa Rosa, Calif., U.S.A.).

Microorganisms and media

Listeria monocytogenes V7 serotype (1/2a) FDA was obtained from M.G. Johnson’s research laboratory at the Univ.
Edible Films Containing Nisin . . .

Table 1a—Effect of nisin on the thickness, mechanical properties, and water vapor permeability of WPI films

<table>
<thead>
<tr>
<th>Thickness (μm)</th>
<th>WPI film</th>
<th>+ Nisin</th>
<th>- Nisin</th>
</tr>
</thead>
<tbody>
<tr>
<td>244.1 ± 12.0</td>
<td>244.4 ± 8.5</td>
<td>244.1 ± 12.0</td>
<td>244.4 ± 8.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tensile strength (MPa)</th>
<th>1.95 ± 0.9</th>
<th>3.50 ± 0.34</th>
</tr>
</thead>
<tbody>
<tr>
<td>Puncture strength (N)</td>
<td>2.57 ± 0.25</td>
<td>2.06 ± 0.09</td>
</tr>
<tr>
<td>WVP (g-mm/m²-h-kPa)</td>
<td>1.45 ± 0.05</td>
<td>1.59 ± 0.12</td>
</tr>
</tbody>
</table>

Table 1b—Effect of nisin on the thickness, mechanical properties, and water vapor permeability of SPI films

<table>
<thead>
<tr>
<th>Thickness (μm)</th>
<th>SPI film</th>
<th>+ Nisin</th>
<th>- Nisin</th>
</tr>
</thead>
<tbody>
<tr>
<td>156.4 ± 3.87</td>
<td>169.3 ± 3.63</td>
<td>156.4 ± 3.87</td>
<td>169.3 ± 3.63</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tensile strength(MPa)</th>
<th>8.59 ± 0.94</th>
<th>10.43 ± 2.53</th>
</tr>
</thead>
<tbody>
<tr>
<td>Puncture strength (N)</td>
<td>5.55 ± 0.41</td>
<td>7.22 ± 0.55</td>
</tr>
<tr>
<td>WVP (g-mm/m²-h-kPa)</td>
<td>1.72 ± 0.07</td>
<td>1.77 ± 0.02</td>
</tr>
</tbody>
</table>

of Arkansas, Fayetteville, AR. 72701. Brain Heart Infusion broth medium (BHI) (Difco, Detroit, Mich., U.S.A.) was used to grow the microorganism for all experiments. *Listeria monocytogenes* 1/2a (LM) was incubated overnight in BHI broth at 37 °C and serially diluted in PBS. Counts (CFU/ml) were determined by plating duplicate decimal dilutions of samples on total plate count agar (Difco, Detroit, Mich., U.S.A.) for 24 h at 37 °C.

**Determination of hydrophobicity of film forming solutions**

Surface hydrophobicity of WPI, SPI, WG, and EA film-forming solutions was measured to determine the correlation between the activity of nisin incorporated in edible films for inhibition of *L. monocytogenes* and the hydrophobicity of films. Surface hydrophobicity measurement of the SPI, WPI, WG, and EA film-forming solutions was performed using a hydrophobic fluorescence probe 1-anilino-8-naphthalene sulfonate (ANS) (Hayakawa and Nakai 1985). Each solution (WPI, SPI, and EA film-forming solutions) was diluted to 0.01% to 0.02% in 0.01M phosphate buffer (pH 7.0). Ten μL of 8.0 mM ANS in 0.01M phosphate buffer, pH 7.0 were added into each 4.0 mL of protein solution. Fluorescence intensity was measured with a spectrophluorometer (Kontron model SFM23/B spectrophluorometer, Kontron Ltd., Zurich, Switzerland) at 390 nm (excitation) and 484 nm (emission). The slope of fluorescence intensity versus protein concentration plot was calculated by linear regression analysis and used as an index of protein surface hydrophobicity.

**Preparation of antimicrobial films**

Films with and without nisin were prepared using SPI, WPI, EA and WG as shown below. Nisaplin, a commercial source of nisin, was obtained from Aplin & Barrett Ltd., Trowbridge, Wiltshire, England.

Twenty-three portions of SPI (5.0 g) were separately mixed with 95 mL of distilled water and stirred until uniform suspensions were obtained. Glycerol (2.0 g) was added into the above suspension to prevent film brittleness. The pH values of the solutions were adjusted from 2.0 to 8.0 at pH 1.0 increments using 0.1N or 1.0N hydrochloric acid and 0.1N or 1.0N sodium hydroxide, and each product (100 mL) was heated at 90 °C for 30 min in a water bath to form a film matrix by denaturation of protein. Varying amounts (3,000, 30,000, 60,000, 90,000, and 120,000 IU/15 mL of film solution) of nisin were immediately incorporated into one half (50 mL) of each film-forming solution at a pH of 2.0, 3.0, 6.0, 7.0, or 8.0 at which films could be produced, keeping the other half (50 mL) as a control without nisin. Three portions of 15 mL each of film forming solution from each half of solution were poured into 8.6 cm² polystyrene weighing boats (Fisher Scientific) and air-dried (24 to 48 h) at ambient temperature in a laminar flow hood to prevent microbial contamination. After drying, films were peeled from the polystyrene boats and stored at 5 °C until used.

Solutions of 7% WPI (mixed with 93 mL of distilled water), 7% EA (mixed with 93 mL of distilled water), and 5% WG (mixed with 40 mL of 70% EtOH to dissolve the WG and 55 mL of distilled water) were prepared because they yielded the best freestanding films. These were adjusted to different pH values (2.0 to 8.0) at 1.0 pH unit increments as described above. The solutions (100 mL) of WPI and EA were heated at 90 °C for 30 min in a water bath, while the solutions of WG were heated at 40 °C for 30 min. Glycerol (3.5 g, 2.5 g, and 1.5 g) was then added to the solution of WPI, EA, and WG as a plasticizer. Varying amounts (3,000, 30,000, 60,000, 90,000, and 120,000 IU/15 mL of film solution) of nisin were immediately incorporated into one half of each of film-forming solution at a pH where films could be produced, keeping the other half (50 mL) as a control without nisin. Three portions of fifteen mL each of solutions were pipetted into 8.6 cm² polystyrene weighing boats (Fisher Scientific) and dried as described earlier.

**Film Thickness**

The thickness of WPI, SPI, EA, and WG films with and without nisin was measured with an electronic digital micrometer (Digitigrig Mark II, Japan). Film strips were placed between the jaws of the micrometer and the gap reduced until first indication of contact was noted. Measurements were taken at nine random positions and the mean thickness in μm was used to calculate apparent water vapor permeability.

**Apparent Water Vapor Permeability (WVP)**

The WVP metal cups received from Kansas State Univ. with an open mouth area of 33 cm² were filled with distilled water with an air gap of 2.5 cm from the film specimen. Film samples were placed over the open mouth of metal cups and tightly sealed symmetrically with six screws onto the cups. Cup weights were recorded fifteen times at 30 min intervals for seven hours in a Mettler AE 163 balance. WVP was measured in chamber at 23 °C, and at 55% relative humidity. The linear regression-derived slopes were used to estimate water vapor transmission rate and WVP measurements were performed as described by McHugh and others (1993).
Place nisin in a film formulation at various pH values on inhibition of *L. monocytogenes*.

<table>
<thead>
<tr>
<th>pH</th>
<th>Thickness (µm)</th>
<th>Tensile strength (MPa)</th>
<th>Puncture strength (N)</th>
<th>WVP (g-mm/m²-h-kPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0.24 ± 0.10</td>
<td>1.53 ± 0.10</td>
<td>2.20 ± 0.24</td>
<td>0.60 ± 1.96</td>
</tr>
<tr>
<td>3</td>
<td>0.26 ± 0.10</td>
<td>1.80 ± 0.60</td>
<td>2.15 ± 0.23</td>
<td>1.96 ± 0.76</td>
</tr>
<tr>
<td>4</td>
<td>0.22 ± 0.10</td>
<td>2.15 ± 0.23</td>
<td>2.15 ± 0.23</td>
<td>2.17 ± 0.44</td>
</tr>
<tr>
<td>5</td>
<td>0.23 ± 0.10</td>
<td>2.12 ± 0.44</td>
<td>2.15 ± 0.23</td>
<td>2.12 ± 0.44</td>
</tr>
<tr>
<td>6</td>
<td>0.24 ± 0.10</td>
<td>2.20 ± 0.24</td>
<td>2.15 ± 0.23</td>
<td>2.17 ± 0.44</td>
</tr>
<tr>
<td>7</td>
<td>0.23 ± 0.10</td>
<td>2.15 ± 0.23</td>
<td>2.15 ± 0.23</td>
<td>2.12 ± 0.44</td>
</tr>
<tr>
<td>8</td>
<td>0.22 ± 0.10</td>
<td>2.12 ± 0.44</td>
<td>2.15 ± 0.23</td>
<td>2.17 ± 0.44</td>
</tr>
</tbody>
</table>

**Note:** Means in each horizontal row followed by the same superscripts are not significantly different as determined by Duncan’s Multiple Range Test (P < 0.05). Thickness was a mean of 9 values ± standard error. Tensile strength was a mean of 3 values ± standard error. Puncture strength was a mean of 6 values ± standard error. Water vapor permeability was a mean of 3 values ± standard error.

**Table 1c—Effect of nisin on the thickness, mechanical properties, and water vapor permeability of EA film.**

**Table 1d—Effect of nisin on the thickness, mechanical properties, and water vapor permeability of WG films.**

Mechanical Properties of films

Tensile strength (TS) of WPI, SPI, EA, and WG films with and without nisin was measured with a texture analyzer (TA.XT2, Texture Technologies Corp., New York, N.Y., U.S.A.). Sample preparation and handling for texture analyses were carried out according to standard methods of ASTM D 882-91 (1991). Film samples were selected for lack of defects for textural test. Film samples were conditioned at ambient temperature and 50% RH for at least 48 h prior to textural analyses (ASTM 1991). Film specimens were prepared by cutting 40 mm long and 15 mm wide strips of films and these were mounted in the film-extension grips of the texture analyzer. The film strips were stretched 20 mm apart at a speed of 2 mm/sec in a tension mode. Tensile strength in MPa was calculated by dividing the peak load developed during the test by the film cross-sectional area. Puncture strengths of the films with/without nisin were measured on a texture analyzer by mounting circular film samples 16 mm diameter on a specially designed cup (12 mm diameter) and securing between a metal rim and rubber gasket by six screws placed symmetrically around the circumference. With a 3 mm probe (5 mm/s) in a Compression Mode, the films were punctured and the force (in Newton) at the point of rupture was recorded and expressed as puncture strength.

Ability of nisin incorporated into films to reduce *Listeria monocytogenes* counts

Fifteen µL of the bacterial suspension (10⁵ CFU/g) was placed on prepared SPI film discs (8.0 mm in diameter and 0.02 g in weight) containing nisin (120 IU/film disk) and on discs without nisin as controls. The films were incubated for 0, 30, 60, 90, and 120 min at ambient temperature. After incubation, the film discs were placed into stomacher bags and diluted with PBS (0.98 ml) and then stomached for 2 min. The solution was decimally diluted with PBS and plated in duplicate onto total plate count agar plates. The plates were incubated at 37 °C for 24 h, and CFU/ml was determined.

Effect of different concentrations of nisin incorporated into edible films on *L. monocytogenes*

Fifteen µL of the bacterial suspension (10⁵ CFU/g) was placed on prepared WPI, SPI, EA, and WG film discs (8.0 mm in diameter) containing nisin (3,000, 30,000, 60,000, 90,000, and 120,000 IU/15 ml of film solution) and on discs without nisin as controls. The films prepared at pH 3.0 were selected for this experiment since this was the most effective pH value to reduce *L. monocytogenes* on edible films with nisin. The films were incubated for 60 min at ambient temperature. After incubation, the film discs (0.02 g) were placed into stomacher bags containing 0.98 ml of PBS, then stomached for 2 min. The solution was decimally diluted with PBS and plated in duplicate onto total plate count agar plates. The plates were incubated at 37 °C for 24 h and CFU/ml was determined.

Statistical analysis

Means were calculated from 3 or more separate experiments. Data were analyzed by Duncan’s multiple range test where *p* < 0.05 is significantly different.

Results and Discussion

Hydrophobicity/hydrophilicity determination

The hydrophobicity of WPI, SPI, EA, and WG film-forming solutions was measured to determine the effect of the hydrophobicity/hydrophilicity of films on the activity of nisin incorporated in edible films for inhibition of *L. monocytogenes*. The surface hydrophobicities of WPI, SPI, EA, and WG film solutions were 44, 282, 232, and 142, respectively. Higher *S₀* resulted from the exposure of hydrophobic groups inside the fold structure of native proteins. The WPI film had the highest hydrophobicity because it is water-insoluble due to formation of covalent disulfide bonds. Conversely, glutamic acid is present in greater amounts and cystine is present in very small amounts in wheat gluten, so there is a lower level of covalent disulfide bonds possible (Krochta and others 1994). This may account for relatively lower hydrophobicity of a wheat gluten film-forming solution compared to SPI film.

Hydrophobicity/hydrophilicity determination

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Effect of nisin on the thickness, mechanical properties and water vapor permeability

The effects of nisin addition on thickness, tensile strength (TS), puncture strength (PS), and water vapor permeability (WVP) of edible films are summarized in Tables 1a, b, c, and d. Film thickness is used to calculate mechanical and moisture barrier properties. TS represents the maximum stress developed in a film strip during tensile testing, and WVP is a measure of the ease with which a material can be penetrated by water vapor. WPI, SPI, and WG films with/without nisin at pH 3.0 and EA with/without nisin at pH 4.0 were selected to determine the effect of nisin on film thickness, mechanical properties and water vapor permeability because these films showed the highest antimicrobial activity (see Table 3).

Incorporation of nisin did not increase the thickness of WPI films (Table 1a), whereas the thicknesses of SPI or WG films were increased by addition of nisin (Table 1b, 1d). There was a significant difference in tensile strengths of WPI films with and without nisin (Table 1a). This result indicated that nisin addition significantly increased the tensile strength of WPI film. Incorporation of nisin into film-forming solutions may have caused rearrangement of disulfide and hydrophobic bonds or more protein-protein interactions, resulting in increased TS for WPI films. Protein film formation is a result of polymerization of heat-denatured proteins with disulfide, and hydrophobic bonds being the main forces maintaining the film network (Fukushima and Van Buren 1970). In addition, the electrostatic interaction between nisin molecules and protein molecules forming the film network may have contributed to the increase of TS.

However, the PS values for the WPI films to which nisin was added (Table 1a) did not show similar trends to those for TS. There was no significant difference between PS of WPI with and without nisin. Theoretically, addition of nisin would be expected to improve water barrier property of films by hydrophobic interaction with protein constituents because nisin itself is a hydrophobic protein (Klaenhammer 1993). However, nisin at the amounts added had no significant effect on the WVP values of WPI films.

When nisin was incorporated into SPI films, the thickness of SPI films with nisin was significantly greater than that of SPI films without nisin (\(p < 0.05\)) (Table 1b). Tensile strength values of SPI films with and without nisin were not significantly different (\(p < 0.05\)). SPI films had lower hydrophobicity values than WPI films. As a result of this, the lower number of potential hydrophobic bonds between nisin and protein molecules in SPI films compared to that of WPI films account for there being no significant differences in TS between SPI control films and SPI with nisin added. SPI films with nisin had a significantly higher PS value than SPI films without nisin (Table 1b). Water vapor permeability (WVP) for SPI film values were not significantly different (Table 1b). In summary, these results indicate that nisin addition into SPI films did not affect the tensile strength (TS) or WVP, whereas nisin addition had an effect on the film’s thickness and PS.

Incorporation of nisin did not significantly increase the thickness of EA films. There was no significant difference of tensile strength between EA films with nisin and EA films without nisin \((p < 0.05)\). This result may be due to the same reasons as in SPI films. The addition of nisin into EA films did not significantly affect PS. Nisin addition caused no significant effect on the WVP of EA films. This result may be due to the low concentration of nisin incorporated into the film-forming solutions.

The thickness of WG films with nisin was significantly greater than that of WG films without nisin \((p < 0.05)\) (Table 1-d). There was no significant difference of tensile strength between WG films with nisin and WG films without nisin \((p < 0.05)\). This result may be for the same reasons as with in SPI and EA films. Puncture strength of WG films with nisin were significantly lower than those of WG films without nisin (Table 1-d), similar to the trend for EA films \((p < 0.05)\). There was no significant difference in WVP values between WG films with or without nisin.

Ability of nisin incorporated into soy protein film to reduce \(L.\ monocytogenes\) counts

Figure 1 shows the effect of nisin (120 IU/film disk (0.02 g)) added to SPI films on inhibition of \(L.\ monocytogenes\) that was directly inoculated onto the film discs for 30, 60, 90, and 120 min at ambient temperature. The maximum reduction in counts of \(L.\ monocytogenes\) by nisin in soy protein films occurred by 60 min. There was a log reduction from 5.24 to 3.0 after 60 min of incubation. There was no further reduction in bacterial counts from 60 min to 120 min of incubation.

Effect of different nisin concentrations incorporated into edible films on \(L.\ monocytogenes\) counts

Varying amounts (3,000, 30,000, 60,000, 90,000, and 120,000 IU/15 ml of film solution) of nisin were incorporated into the WPI, SPI, EA, or WG films to determine the appropriate amount of nisin concentration for film formation and its antimicrobial activity. The antimicrobial activities of nisin incorporated into edible films (SPI, WPI, EA, or WG) against \(L.\ monocytogenes\) are shown in Table 2. As the nisin concentration increased from 4.0 IU to 160 IU/film disk (0.02 g), the amount of inhibition progressively increased in all tested

Table 2—Effects of different levels of nisin added into edible films against \(L.\ monocytogenes\)

<table>
<thead>
<tr>
<th>Edible films</th>
<th>Control</th>
<th>4 IU</th>
<th>12 IU</th>
<th>60 IU</th>
<th>120 IU</th>
</tr>
</thead>
<tbody>
<tr>
<td>WPI</td>
<td>3.96 ± 0.07a</td>
<td>3.32 ± 0.01b</td>
<td>2.99 ± 0.09b</td>
<td>2.77 ± 0.06c</td>
<td>2.76 ± 0.03d</td>
</tr>
<tr>
<td>SPI</td>
<td>4.05 ± 0.09b</td>
<td>3.50 ± 0.16b</td>
<td>2.87 ± 0.17b</td>
<td>2.75 ± 0.15c</td>
<td>2.71 ± 0.12b</td>
</tr>
<tr>
<td>EA</td>
<td>4.83 ± 0.1b</td>
<td>4.33 ± 0.16b</td>
<td>4.09 ± 0.11b</td>
<td>3.90 ± 0.13b</td>
<td>3.92 ± 0.13b</td>
</tr>
<tr>
<td>WG</td>
<td>4.90 ± 0.09b</td>
<td>4.48 ± 0.11b</td>
<td>4.28 ± 0.1b</td>
<td>4.12 ± 0.08b</td>
<td>4.08 ± 0.1b</td>
</tr>
</tbody>
</table>

Significance level: a, b, c, d, or e means of three separate experiments within same horizontal row followed by the same superscripts are not significantly different (Duncan’s multiple range test, \(P < 0.05\)).
films. There was a log reduction for all four films with nisin at 120 IU/film disk after one hour of exposure. There was no difference between 120 IU to 160 IU/film disk (0.02 g) in the amount of inhibition, so 120 IU/film disk of nisin concentration was selected for subsequent experiments. The maximum concentration of nisin that can be incorporated into edible films was 160 IU/film disk. The higher amount of nisin caused the films to be brittle. This may be due to an increase in intermolecular interaction between adjacent polymer chains in film networks. The amount of plasticizer may need to be increased at high nisin concentrations to make more flexible films.

WPI films containing nisin were the most effective in reducing L. monocytogenes counts, whereas WG films with nisin showed the lowest antimicrobial activity against L. monocytogenes. Collectively, these results indicate that nisin was more active for inhibition of L. monocytogenes in a hydrophobic film such as the WPI films than in a lower hydrophobic film such as WG films.

### Table 3—Effect of nisin incorporated into edible films at varying pH values on inhibition of L. monocytogenes directly inoculated onto film surfaces

<table>
<thead>
<tr>
<th>Edible films</th>
<th>2.0</th>
<th>3.0</th>
<th>4.0</th>
<th>5.0</th>
<th>6.0</th>
<th>7.0</th>
<th>8.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>WPI</td>
<td>4.99 ± 0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.73 ± 0.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>ND&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ND&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ND&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.62 ± 0.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.07 ± 0.07&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>WPI + Nisin</td>
<td>4.15 ± 0.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.31 ± 0.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>ND&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ND&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ND&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.43 ± 0.23&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.39 ± 0.14&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>SPI</td>
<td>5.57 ± 0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.37 ± 0.27&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ND&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.51 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.07 ± 0.07&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.60 ± 0.10&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>SPI + Nisin</td>
<td>4.48 ± 0.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.27 ± 0.15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>ND&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ND&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.91 ± 0.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.05 ± 0.17&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.61 ± 0.17&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>EA</td>
<td>5.29 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.38 ± 0.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.63 ± 0.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.67 ± 0.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.41 ± 0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.50 ± 0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.54 ± 0.11&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>EA + Nisin</td>
<td>4.56 ± 0.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.26 ± 0.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.31 ± 0.22&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.68 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.74 ± 0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.80 ± 0.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.98 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>WG</td>
<td>5.09 ± 0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.91 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ND&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ND&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ND&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ND&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>WG + Nisin</td>
<td>4.58 ± 0.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.95 ± 0.07&lt;sup&gt;b&lt;/sup&gt;</td>
<td>ND&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ND&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ND&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ND&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ND&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Not Determined—Films were not produced because they were not stable. Values are the mean values of three separate experiments.

<sup>b</sup>Means of three separate experiments within each horizontal row followed by the same superscripts are not significantly different (Duncan’s multiple range test, P < 0.05).

<sup>c</sup>Means of three separate experiments of each film with/without nisin within each vertical column followed by the same superscripts are not significantly different (t-test, P < 0.05).

<sup>d</sup>WPI, whey protein isolate; SPI, soy protein isolate; EA, egg albumen; WG, wheat gluten.

**Effect of nisin incorporated into edible films at various pH values on inhibition of L. monocytogenes [SHORTEN THIS??]***

Table 3 shows the effect of nisin added into several films (SPI, WPI, EA, and WG) at varying pH values (2.0 to 8.0) on the reduction of L. monocytogenes counts. WPI films with and without nisin were prepared at pH 2.0, 3.0, 7.0, and 8.0, but not at pH 4.0, 5.0 and 6.0. At pH values of 4.0 and 5.0, the isoelectric range of whey protein fractions in film-forming solution was reached, negatively affecting film formation due to protein coagulation. The most effective pH value to reduce L. monocytogenes in WPI film with nisin was 3.0, resulting in a 2.42 log cycle reduction compared to WPI control film without nisin at the same pH value, whereas, at a pH of 2.0, 7.0 or 8.0, there was only a 0.84, 1.19 or 1.68 log reduction, respectively, compared to WPI film without nisin.

In SPI film containing nisin, the maximum destruction of 2.1 logs of L. monocytogenes occurred at pH 3.0. SPI films were not formed at pH 4.0 and 5.0 since the proteins precipitated around their isoelectric points at which their net charges are zero, resulting in the formation of precipitates rather than films.

In EA film, the greatest reduction of L. monocytogenes counts with nisin addition occurred at a pH of 4.0, causing a 1.32 log cycle lower count than in the control film. EA films were produced at pH range of 2.0 to 8.0. WG films with nisin at a pH of 3.0 had the best ability to inhibit L. monocytogenes, showing a 0.96 log cycle reduction in numbers of L. monocytogenes. WG films were only prepared at pH 2.0 and 3.0 (Table 3).

All films tested with nisin added (WPI, SPI, EA, and WG films) exhibited greatest inhibitory effects against L. monocytogenes under acidic condition. These results are due to nisin being more active against L. monocytogenes at acidic and hydrophobic conditions (Klaenhammer 1993).

### Conclusion

Surface hydrophobicities of SPI, WPI, WG, and EA film solutions were measured to determine the effects of the hydrophobicity/hydrophilicity of films on the activity of nisin incorporated in edible films for inhibition of L. monocytogenes. The mechanical properties and water vapor permeabilities varied for each film tested when nisin was added to the films. These results suggest that nisin has different inter-
actions with the proteins of the different films.

Incorporation of nisin into edible films was effective in inhibiting \textit{L. monocytogenes}. Edible films with higher hydrophobicities and added nisin in an acidic environment exerted a greater inhibitory effect against \textit{L. monocytogenes}. The findings of this study indicate that edible films can act as suitable carriers for delivering effective antimicrobials to the surface of food products. Further practical tests of these edible films are needed to determine the ability of such films to deliver antimicrobial compounds in pharmaceutical, fruit, vegetable, and meat product applications.

\textbf{References}


Hoffman, KL, Dawson, PL, Acton, JC, Han, JX, and Ogale, AA 1998. Film formation effects on nisin activity in corn zein and polyethylene films. Research and Development associates for military food and packaging systems. 49/50:238-244.


We are grateful to the USDA/Food Safety Consortium for providing funding.

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