# Biodegradable Polylactic Acid Polymer with Nisin for Use in Antimicrobial Food Packaging

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**ABSTRACT: Biodegradable polylactic acid (PLA) polymer was evaluated for its application as a material for antimicrobial food packaging. PLA films were incorporated with nisin to for control of foodborne pathogens. Antimicrobial activity of PLA/nisin films against *Listeria monocytogenes*, *Escherichia coli* O157:H7, and *Salmonella* Enteritidis were evaluated in culture media and liquid foods (orange juice and liquid egg white). Scanned electron micrograph and confocal laser microscopy revealed that nisin particles were evenly distributed in PLA polymer matrix on the surface and inside of the PLA/nisin films. PLA/nisin significantly inhibited growth of *L. monocytogenes* in culture medium and liquid egg white. The greatest inhibition occurred at 24 h when the cell counts of *L. monocytogenes* in the PLA/nisin samples were 4.5 log CFU/mL less than the controls. PLA/nisin reduced the cell population of *E. coli* O157:H7 in orange juice from 7.5 to 3.5 log at 72 h whereas the control remained at about 6 log CFU/mL. PLA/nisin treatment resulted in a 2 log reduction of *S.* Enteritidis in liquid egg white at 24 °C. After 21 d at 4 °C the *S.* Enteritidis population from PLA/nisin treated liquid egg white (3.5 log CFU/mL) was significantly less than the control (6.8 log CFU/mL). *E. coli* O157:H7 in orange juice was more sensitive to PLA/nisin treatments than in culture medium. The results of this research demonstrated the retention of nisin activity when incorporated into the PLA polymer and its antimicrobial effectiveness against foodborne pathogens. The combination of a biopolymer and natural bacteriocin has potential for use in antimicrobial food packaging.**

**Keywords: juice, liquid egg, nisin, packaging, pathogen, PLA**



## Introduction

Demand for safe, minimally processed, "fresh" food products presents major challenges to the food-packaging industry to develop packaging concepts for maintaining the safety and quality of packaged foods. Recent outbreaks of foodborne pathogens such as *E. coli* O157:H7, *Salmonella* spp., and *L. monocytogenes* continue to drive a search for innovative ways to inhibit microbial growth in foods while maintaining quality, freshness, and safety. As an additional hurdle to nonthermal processes, antimicrobial packaging can play an important role in reducing the risk of pathogen contamination as well as in extending the shelf life of minimally processed foods. Antimicrobial packaging systems incorporate antimicrobials into the packaging to prevent microbial growth on the surface of solid foods and to reduce the need for larger quantities of antimicrobials in liquid foods. Currently, food application of an antimicrobial packaging system is limited due to the availability of suitable antimicrobials, new polymer materials, regulatory concerns, and appropriate testing methods.

Polylactic acid (PLA) polymer is widely used in such medical applications as surgical implants (Jain 2000), tissue cultures (Mikos and others 1994), resorbable sutures (Taylor and others 1994), wound closures, and controlled release systems (Park and others 1992). PLA derived from renewable resources, for example, corn, wood residues, or other biomass, is of current interest not only because of the need to ultimately replace many petroleum-based polymers but also because of their potentially useful physical and mechanical characteristics. The use of biopolymers in food packaging has already received wide attention (Conn and others 1995; Sinclair 1996; Haugaard and others 2002; Frederiksen and others 2003). There have been developments in Europe and North America that have involved the use of PLA-based packaging for supermarket products. PLA containers have been used for packaging foods such as Biota™ PLA bottled water, Noble™ PLA bottled juices, and Dannon™ yogurts. The containers meet German and EU food grade requirements. The special characteristics of PLA, such as GRAS status, biodegradability, and being a bioresource, put PLA in a unique position for food applications. An antimicrobial packaging system based on PLA would be superior to other antimicrobial system due to its comparable cost, effective antimicrobial activity, few regulatory concerns, and environmental friendliness. Although its potential for antimicrobial packaging has not been explored yet, further research and development is needed.

Nisin is a heat-stable bacteriocin produced by certain strains of *Lactococcus lactis*. Nisin is primarily active against Gram-positive bacteria, including *Clostridium*, *Bacillus*, *Staphylococcus* (Ray and Daeschel 1994), and *Listeria* species (Ponce and others 1998; Schillinger and others 2001; Brewer and others 2002). Direct addition of nisin into foods results in an immediate reduction of bacterial populations but may not prevent the recovery of injured cells or the growth of cells that were not destroyed by direct addition if residues of the antimicrobial are rapidly depleted (Zhang and others 2004). Nisin, at various concentrations alone and with other antimicrobial agents incorporated into polyethylene or other edible polymer films, was effective against various microorganisms, including *L. plantarum*, *L. monocytogenes*, *E. coli*, and *Salmonella* spp. (Padgett and others 1998; Cutter and others 2001; Eswaranandam and others 2004). A variety of polymer films have been used to deliver nisin. Examples are sodium caseinate films (Kristo and others 2008), glucomanna-gellan gum blend films (Xu and others 2007), alginate films (Natrajan and Sheldon 2000a; Cha and others 2002; Millette and others 2007), glucomanna-chitosan films (Li and

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others 2006), methylcellulose/hydroxypropyl methylcellulose films (Franklin and others 2004), poly(vinyl chloride), linear low-density polyethylene and nylon films (Natrajan and Sheldon 2000b), corn zein films (Hoffman and others 2001; Janes and others 2002), and whey protein, soy protein, egg albumin, and wheat gluten films (Ko and others 2001). However, limited information is available for using PLA polymers as a carrier of nisin. Salmaso and others (2004) loaded nisin into PLA particles and evaluated the sustained antimicrobial activity of nisin from the nisn/PLA particles. However, neither a film form of nisin/PLA nor pathogens in a food system was investigated in their study.

The central idea behind the project was to develop a biodegradable PLA-based packaging system that would provide an extended shelf life for foods and improve food safety. An important requirement was the need to incorporate a natural bacteriocin in the packaging that would prevent microbial growth on foods during storage. In our previous study (Liu and others 2007), nisin was coated directly onto the PLA film surface and limited antibacterial activity was observed due to the hydrophobic and smooth surface characteristics of PLA film which limit the nisin embedding capacity. In the present study, we utilized a different method of incorporating nisin into the PLA polymer to improve nisin embedding capacity. The objective of this study was to evaluate PLA for its suitability as a polymer material for antimicrobial food packaging with the incorporation of nisin. This study evaluated films developed from the PLA/nisin complex, which could be used to coat the surface of bottles, sheets or films for different applications.



## Materials and Methods

### PLA/nisin film preparation

PLA polymer films were prepared by a solvent casting method. Nisin (Sigma Chemical Co., St. Louis, Mo., U.S.A.) was reported to be 2.5% pure with the remaining components listed being sodium chloride and denatured milk solids. One gram of PLA resin (Natureworks, Minnetonka, Minn., U.S.A.) and 0.25 g of nisin were accurately weighted and dispersed in 15 mL of methylene chloride (Fisher Scientific, Fairlawn, N.J., U.S.A.). This mixture was stirred by a magnetic bar until the polymer was totally dissolved. The solution was distributed to Teflon Petri dishes 10 cm in diameter and 1.5-cm deep (Performance Plastics, Wayne, N.J., U.S.A.). The methylene chloride was allowed to evaporate at room temperature under a chemical hood. Each dish was stored over a desiccant for 24 h, and then films were peeled from the dishes and placed in a sealed container until time of use. Each PLA film had 157 $cm^2$ total surface area with 0.15 mm average thickness. The amount of pure nisin in the PLA/nisin film was calculated to be 6.25 mg/g of film or 0.04 mg/$cm^2$ surface area of film.

### Bacterial inhibition testing

*L. monocytogenes* Scott A 724, *E. coli* O157:H7 Oklahoma, and *S.* Enteritidis (ATCC 13076) were obtained from the culture collection of the U.S. Dept. of Agriculture, Agricultural Research Service, Eastern Regional Research Center. Prior to inoculum preparation, *E. coli* O157:H7 and *S.* Enteritidis were grown in tryptic soy broth (TSB: Remel Inc., Lenexa, Kans., U.S.A.), and *L. monocytogenes* was grown aerobically at 37 °C for 16 to 18 h in brain heart infusion broth (BHIB: Difco Laboratory, Detroit, Mich., U.S.A.).

Bacterial inhibition by antimicrobial films was evaluated using an agar diffusion method and a liquid incubation method as described by Appendini and Hotchkiss (2002).

In the agar diffusion test, each film sample (1.4 × 1.4 cm) was placed on the surface of a BHI agar plate overlaid with the seeded semi-soft BHI agar (0.5% [w/v] agar). The seed density of overlay was approximately $10^6$ CFU/mL of *L. monocytogenes*. The agar plates were incubated at 37 °C for 24 h. Diameters of inhibition zone around film specimen were used to determine antimicrobial activity of each film sample.

For the liquid incubation test, 6 pieces of films (total surface area of approximate 24 $cm^2$) were placed in a glass bottle with 50 mL liquid medium (BHIB, orange juice, or liquid egg white), creating a ratio of 2.08 mL of liquid/$cm^2$ of exposed polymer surface. Orange juice and pasteurized liquid egg white (LEW) were purchased from a local store; these contained no preservatives as stated on their labels. The orange juice was autoclaved (121 °C for 25 min) before use. The pH of liquid egg white was 8.46 and the pH of orange juice was 3.78. The medium in the bottle was inoculated with 1 mL of an overnight culture of selected strains and shaken at 24 or 4 °C at 150 rpm. The final cell populations were approximately $10^7$ CFU/mL for orange juice and $10^4$ CFU/mL for all other tests. One milliliter of the inoculated medium was sampled at each sampling time. Specimens were serially diluted with sterile phosphate buffer (Hardy Diagnostics, Santa Maria, Calif., U.S.A.), then pour plated onto BHI agar. Plates were incubated at 37 °C for 24 h. Inoculated medium without a film served as a control. The nisin diffusing from films to liquid media during incubation was equivalent to 200 IU/mL of liquid medium.

### Nisin released from films

To determine the amount of nisin released from PLA/nisin films, a standard curve was prepared by a seeded lawn overlay spot method (Boziaris and others 1998; Siragusa and others 1999) with some modifications. BHI agar plates were overlaid with 8 mL of semi soft BHI agar (0.5% [w/v] agar) seeded with 10 $\mu$L of an overnight culture of *L. monocytogenes*. The seed density was approximately 1 × $10^6$ colony forming units (CFU)/mL of overlay. Nisin standard solutions (100 to 10000 IU/mL) were freshly prepared by diluting nisin stock solution in BHI broth. Twenty microliters of nisin standard solution were drop-dotted on the top agar. The plates were incubated at 37 °C for 24 h and examined for zone of inhibition. The size of the zone is proportional to the amount of nisin in it. A regression line describing the relationship between zone size and known nisin concentration serves as a standard curve. To obtain maximal release of nisin from PLA/nisin films, a PLA/nisin film sample was placed in a glass tube with 10 mL BHI broth, heated in a beaker with boiling water (100 °C) for 5 min, cooled, and stirred at room temperature for 144 h. The concentration of nisin in the extract was determined using the seeded lawn overlay spot method previously described. In this study, *L. monocytogenes* was used as an indicator bacterium and correspondingly BHI agar and stock solution of BHI broth were used. In addition, the pH of the stock solution was not adjusted by HCl to avoid a possible interference of HCl/pH on microbial inactivation. Because the bacterial inhibition tests of films in this study were performed with *L. monocytogenes* in BHI broth/BHI agar, the use of the same bacterium as indicator and target pathogen and same growth media in the same conditions would provide more consistent and comparable results.

### Scanning electron microscopy

PLA and PLA/nisin films were cut with surgical scissors into 3 × 5 mm pieces and mounted directly on specimen stubs with 2-sided adhesive tabs of carbon (Electron Microscopy Sciences, Hatfield, Pa., U.S.A.). Mounted film strips were sputter coated with a thin layer of gold using a Scancoat Six Sputter Coater (BOC Edwards, Wilmington, Mass., U.S.A.). Digital images of topographical

features of the film strips were collected using a Quanta 200 FEG environmental scanning electron microscope (FEI Co. Inc., Hillsboro, Oreg., U.S.A.) operated in the high vacuum/secondary electron imaging mode at an accelerating voltage of 10 kV.

### Confocal laser microscopy

Film specimens were glued to a 1 × 3 cm microscope slide and placed on an IRBE optical microscope with a 10× lens integrated with a model TCS-SP laser scanning confocal microscope (Leica Microsystems, Exton, Pa., U.S.A.). Images were made at 633 nm for confocal reflection and at 425/475 nm (ex./em.) for autofluorescence at 2 channels.

### Statistical analysis

Each antimicrobial experiment was performed in duplicate on different days, with 2 film specimens used in each replication (n = 4). Each data point was expressed as the mean ± SD. All data were analyzed by analysis of variance using SAS version 9.1 software (SAS Inst. Inc., Cary, N.C., U.S.A.). Duncan's multiple range tests were used to determine the significant difference of mean values. Unless otherwise stated, significance is expressed at 5% level.

## Results

### Nisin particles distribution in PLA polymer

The surface and internal microstructure of the films were evaluated using scanning electron microscopy (SEM) and confocal laser microscopy (CLM). Figure 1 shows scanning electron micrographs of the outer surface of the PLA film (left) and the outer surface of the PLA/nisin film (right) at a magnification of 5000×. The surface structure of the PLA/nisin film was compact with a smaller uniform polymer network than those in the PLA film, and the PLA film had a smoother surface. The SEM microphotographs of PLA/nisin films showed bright marbling on the film surface, which was uniformly distributed. These white areas could represent the deposits of nisin particles in the PLA matrix. To further identify these white areas in the PLA/nisin film, confocal laser microscopy was employed. Figure 2 shows the outer surface structure of PLA/nisin film (left) and a cross-sectional view of PLA/nisin film (right) by confocal laser microscopy. As shown in Figure 2, the red area correlated with PLA reflection; the green area correlated with peptides and proteins. The images indicate a well-mixed integrated structure, showing an even distribution of the nisin compound in the PLA matrices.

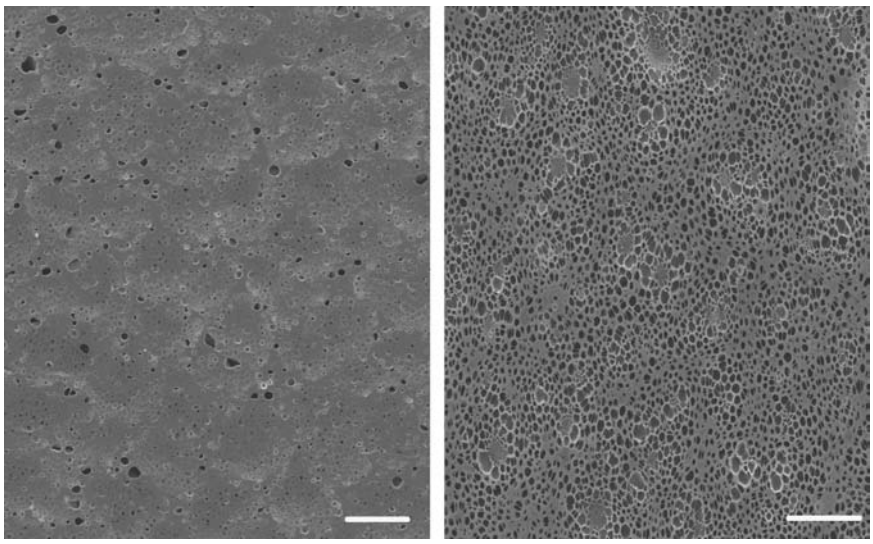

**Figure 1—SEM microphotographs of surface structure of PLA film (left) and PLA/nisin film (right). White bars represent 50 μm.**

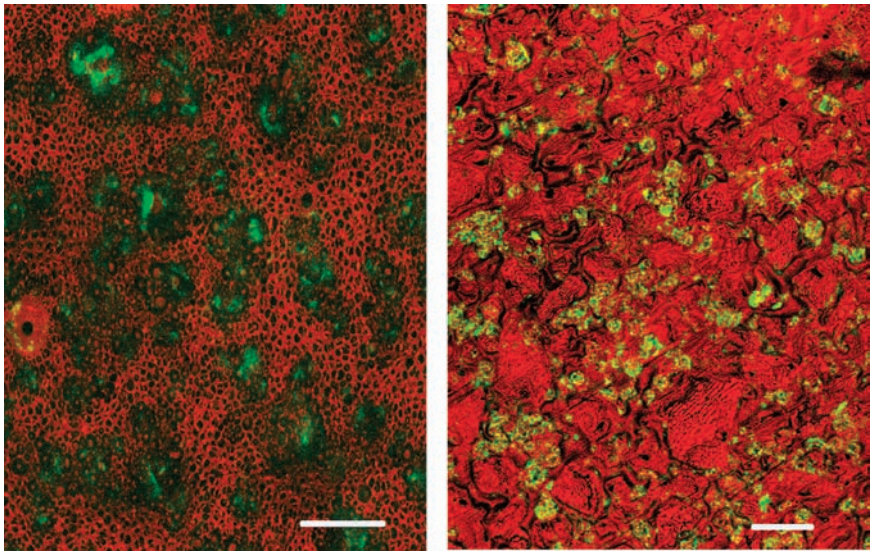

**Figure 2—CLM microphotographs of structure of PLA/nisin film. Left: surface section; right: cross-section. White bars represent 50 μm.**

Figure 2 also clearly shows that the sizes of particles were from 5 to 50 $\mu$m, which can be attributed to nisin peptide, milk proteins, and sodium chloride since the 2.5% purity nisin was used in this study. Wan and others (1997) studied the incorporation of nisin in calcium alginate particles and they observed from SEM images that Nisaplin (2.5% nisin) comprised particles smaller than 10 $\mu$m and sodium chloride crystals of up to 30 $\mu$m.

## Antimicrobial activity

Growth curves of *L. monocytogenes* in BHIB and *E. coli* O157:H7 in TSB, with and without addition of PLA or PLA/nisin film, were determined. Inhibition tests of *L. monocytogenes*, *E. coli* O157:H7, and *S.* Enteritidis were also performed in liquid egg white and orange juice.

Figure 3 shows the inhibitory activity of PLA/nisin against the growth of *L. monocytogenes* in BHIB. There was no significant difference between the control and PLA film throughout the 72-h incubation. The cell counts of *L. monocytogenes* in the PLA/nisin sample were 1.2, 2.1, 4.1, 6.4, and 6.5 log CFU/mL at 4, 8, 24, 48, and 72 h, respectively. The maximum reduction of *L. monocytogenes* was observed at 4 h. The population of *Listeria* cells treated by PLA/nisin remained significantly lower than the control and PLA film treatment through 72 h. These data indicated that the nisin incorporated into the PLA polymer was responsible for the antibacterial activity.

Figure 4 shows the effect of PLA/nisin on growth of *E. coli* O157:H7 in TSB. The PLA film without nisin did not show any antibacterial activity against *E. coli* O157:H7 in TSB. There was less than 0.5 log difference between the PLA/nisin sample and the control sample at 4 and 8 h. After 24 h of incubation, the growth of *E. coli* O157:H7 in TSB with PLA/nisin was approximately 1 log unit less than the control sample. The difference in growth

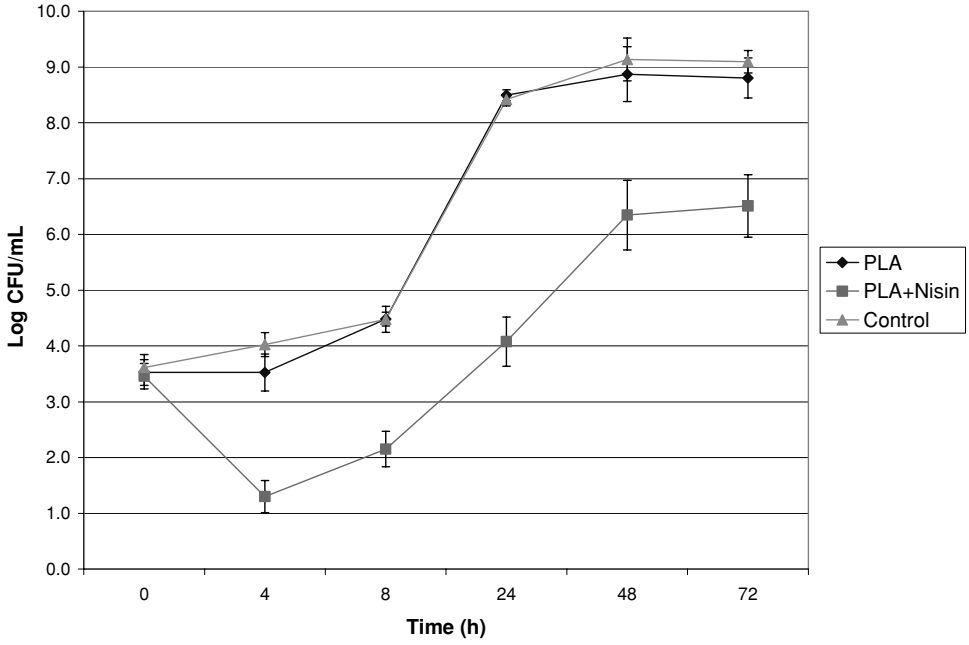


**Figure 3 — Effect of PLA/nisin against *L. monocytogenes* in brain heat infusion broth at 24 °C. Concentration of nisin from PLA/nisin films was equivalent to 200 IU/mL of BHI broth. Error bars represent the standard deviation of the mean.**

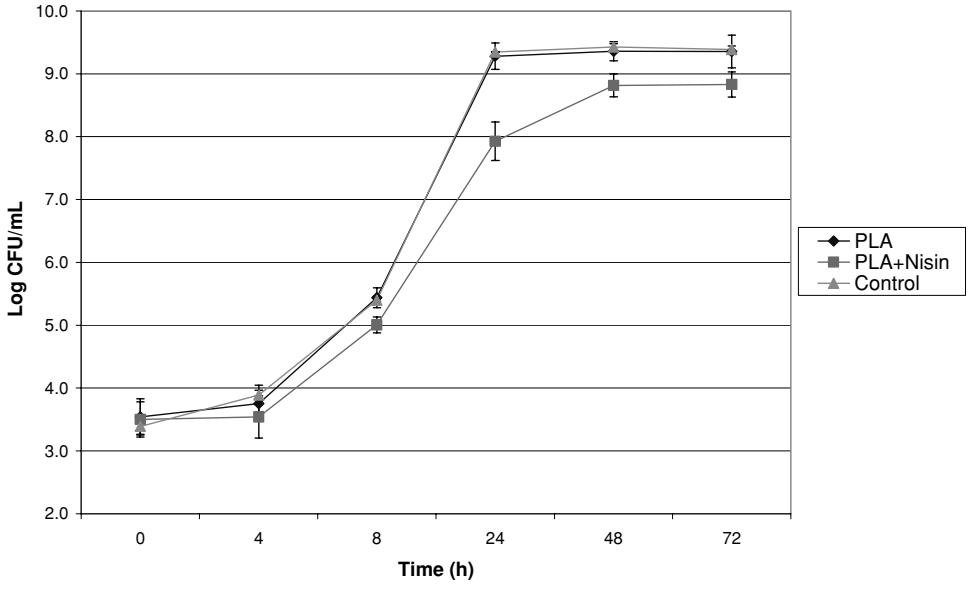


**Figure 4 — Effect of PLA/nisin against *E. coli* O157:H7 in tryptic soy broth at 24 °C. Concentration of nisin from PLA/nisin films was equivalent to 200 IU/mL of tryptic soy broth. Error bars represent the standard deviation of the mean.**

between treatments declined to about 0.5 log during further incubation, which was still statistically significant; however, the difference was not significant from a practical standpoint.

When 7.5 log cells of *E. coli* O157:H7 were inoculated in orange juice, there was no significant difference between PLA/nisin and control samples during the first 24 h; after 24 h, PLA/nisin reduced the population to 5.2 log units at 48 h and to 3.5 log units at 72 h whereas the control had declined to about 6 log units, as shown in Figure 5. The difference of cell counts between the PLA/nisn and the control was 2.5 log units/mL. This suggests that nisin incorporated into PLA films was an effective inhibitor of *E. coli* O157:H7 in orange juice at a ratio of 2.08 mL of liquid/cm$^2$ of exposed polymer surface.

In a response similar to that found in a culture medium (Figure 3), *L. monocytogenes* in LEW was reduced by 3 log units by PLA/nisin, compared to PLA and the control, during the first 8 h, and then remained 3 log units lower than the latter treatments through 72 h (Figure 6).

Figure 7 presents the growth of *S.* Enteritidis in LEW at 24 °C (A) and 4 °C (B). PLA/nisin significantly reduced the growth of *S.* Enteritidis in LEW at both temperatures. However, when stored at 4 °C, *S.* Enteritidis grew more slowly than at 24 °C. At 24 °C (Figure 7A), PLA/nisin treatment resulted in a 2 log reduction compared to PLA treatment from 24 to 72 h. At 4 °C (Figure 7B), the population of *Salmonella* for all treatments remained almost constant at 3 to 3.5 log during 7 d storage. Beginning at day 7, the population in the control and PLA treatments increased sharply to 6.8 log at 21 d, whereas the PLA/nisin treatment showed little change. In addition, Figure 7B suggests that growth of *S.* Enteritidis in liquid egg could be inhibited for at least for 21 d by the

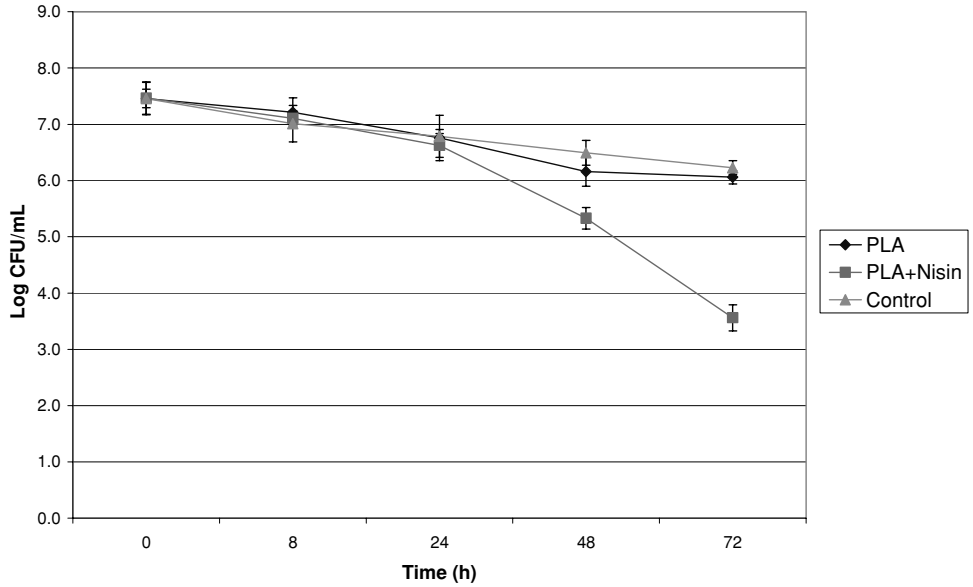


**Figure 5—Effect of PLA/nisin against *E. coli* O157:H7 in orange juice at 24 °C. Concentration of nisin from PLA/nisin films was equivalent to 200 IU/mL of orange juice. Error bars represent the standard deviation of the mean.**

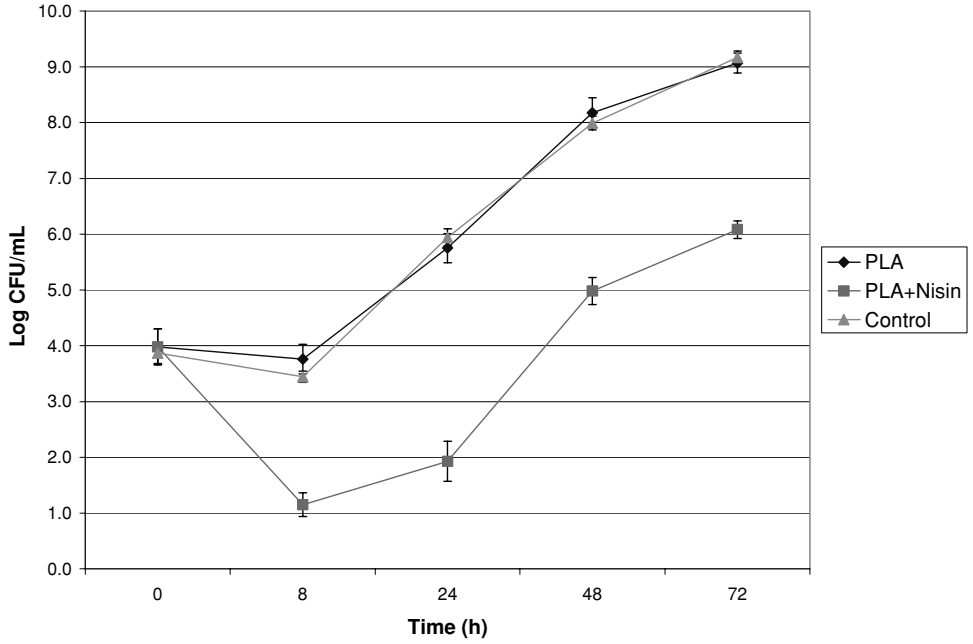


**Figure 6—Effect of PLA/nisin against *L. monocytogenes* in liquid egg white at 24 °C. Concentration of nisin from PLA/nisin films was equivalent to 200 IU/mL of liquid egg white. Error bars represent the standard deviation of the mean.**

nisin-incorporated polymer films when samples are held at 4 °C. The differences between PLA and PLA/nisin were significant; however, no significant difference of cell counts between the PLA and the control was observed at both test temperatures. It could be concluded that PLA itself did not contribute any antimicrobial activity against all 3 test pathogens.

The agar diffusion test was designed to test an antimicrobial film for solid foods packaging. Antimicrobial activity of the film was expressed in terms of inhibition zone. Figure 8 shows the presence of a zone of inhibition around PLA/nisin film (location A), which indicates that nisin molecules were released from the PLA polymer and diffused from the film surface into the solid phase.

## Discussion

Antimicrobials can be added to food formulations directly or through slow release from packaging materials. The application of antimicrobial films allows for the migration of the antimicrobial to the film surface and provides a continuous antimicrobial effect on the food during extended exposure. In this study, we have demonstrated that the retention of nisin activity occurred when nisin was incorporated into a PLA polymer. The PLA/nisin polymer exhibits effective antibacterial activity against foodborne *L. monocytogenes*, *E. coli* O157:H7, and *S.* Enteritidis. Our previous study showed that nisin in pectin/PLA film retained more activity against *L. monocytogenes* over 48 h than by direct addition of the compound (Jin and others 2007). Similarly, Salmaso and others (2004) observed that nisin-loaded PLA particles prolonged nisin activity up to 40 d while free nisin samples displayed antibacterial activity only for 7 d. Use of polymers as nisin carriers not only controls nisin release but also prevents dramatic reductions in nisin activity due to its affinity for food particles and its inactivation by proteolytic enzymes in foods (Cutter and Siragusa 1996; Wan and others 1997).

From SEM and CLM images, it was noticed that the nisin particles along with milk proteins and sodium chloride particles were

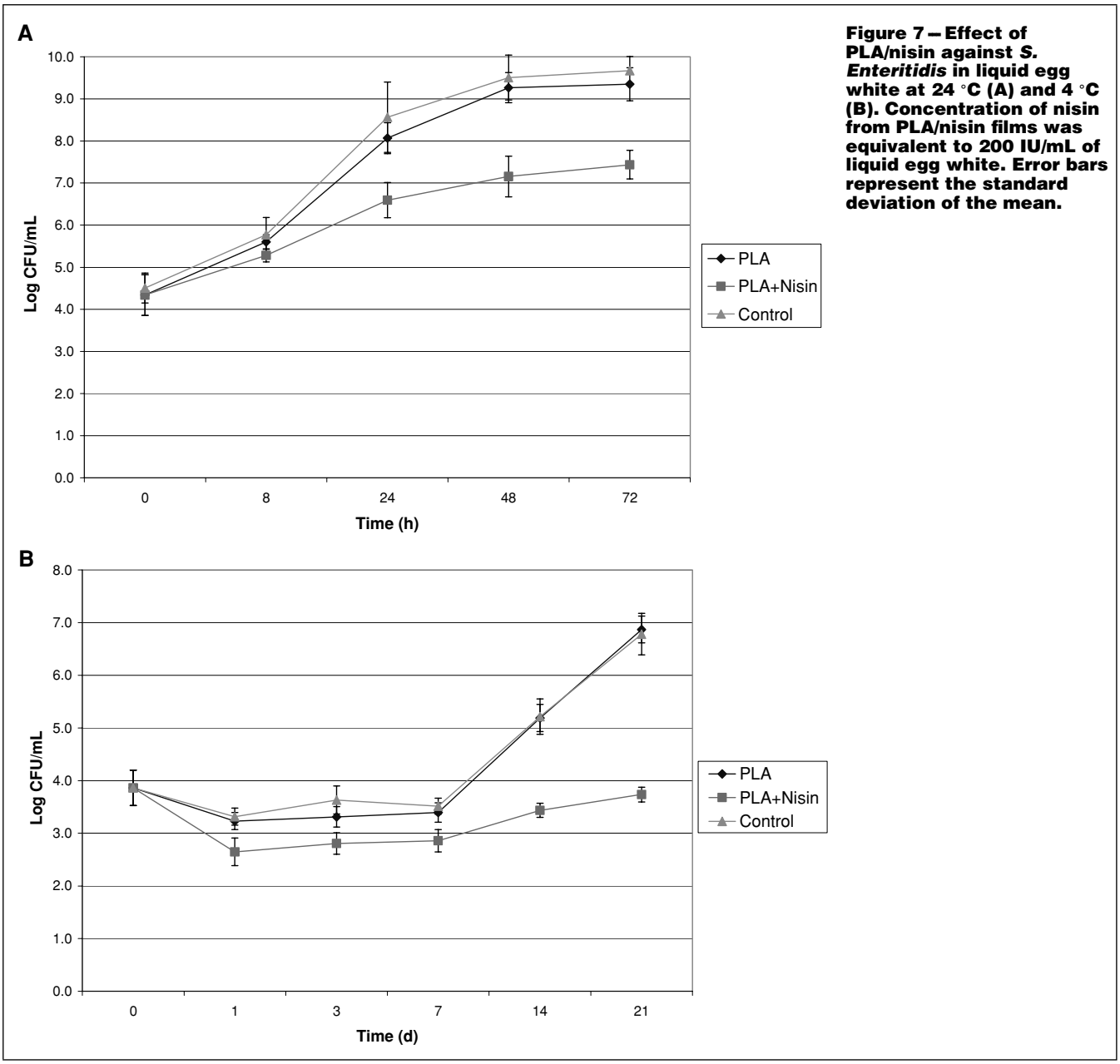


**Figure 7—Effect of PLA/nisin against *S. Enteritidis* in liquid egg white at 24 °C (A) and 4 °C (B). Concentration of nisin from PLA/nisin films was equivalent to 200 IU/mL of liquid egg white. Error bars represent the standard deviation of the mean.**

distributed throughout the polymer. The nisin particles on the surface provided initial release of nisin and the distribution of nisin particles inside the PLA polymer made it possible for slow release of nisin into media. Although the release kinetics of nisin from the PLA/nisin matrix need further investigation, other studies demonstrated that the kinetics of nisin diffusion in protein films followed a Fickian diffusion model (Teerakarn and others 2002). Salmaso and others (2004) found that only 1% to 3% of the total nisin was released from nisin/PLA particles into buffers after 30 min incubation and up to 35% of total loaded nisin was released after 1000 h of incubation.

Our results revealed that PLA/nisin possesses stronger antimicrobial activity against Gram-positive *L. monocytogenes* than Gram-negative *E. coli* O157:H7 or *S.* Enteritidis, which agrees with results from other researchers (Hauben and others 1996; Moll and others 1997). The cell wall membrane of *E. coli* or *Salmonella*, which consists of lipids, proteins, and lipopolysaccharides (LPS), provides effective protection against biocides such as nisin. However, nisin showed more effectiveness against Gram-negative *E. coli* O157:H7 in orange juice (Figure 5) than in TSB (Figure 4). This may be explained by the effect of the combination of nisin with low pH of orange juice. Lowering pH has been shown to increase inhibitory activity of nisin against Gram-negative *Salmonella gaminara and E. coli* O157:H7 (Eswaranandam and others 2004), as well as Gram-positive bacteria such as *L. monocytogenes*, *Staphylococcus aureus* (Thomas and Wimpenny 1996), and *Bacillus licheniformis* (Mansour and others 1998). The significant reduction of *E. coli* O157:H7 in orange juice by PLA/nisin could enhance microbiological safety and shelf life of juice products.

Salmonellosis is a leading cause of foodborne illness in the United States; in particular, the risk of illness increases when egg is used as an ingredient in prepared meals for the general public (Todd 2001). The high thermal sensitivity of liquid egg components prevents the application of more intense heat treatments to inactivate pathogens; therefore, it would be desirable to have an additional hurdle for improving the safety of liquid egg products. Our results show that the PLA/nisin treatments effectively inhibited the growth of *S.* Enteritidis in LEW at both temperatures. In particular, when stored at 4 °C (a storage temperature for commercial liquid egg products), the cell counts of *S.* Enteritidis treated by PLA/nisin films remained lower than the initial inoculated level for 21 d, in which the control reached to approximately 7 log CFU/mL, 3.5 log CFU/mL higher than the PLA/nisin treatment. The present study demonstrated that PLA/nisin treatment is an effective approach to reduce pathogens to a reasonably acceptable level and prolongs the shelf life of the egg products during refrigerated storage.

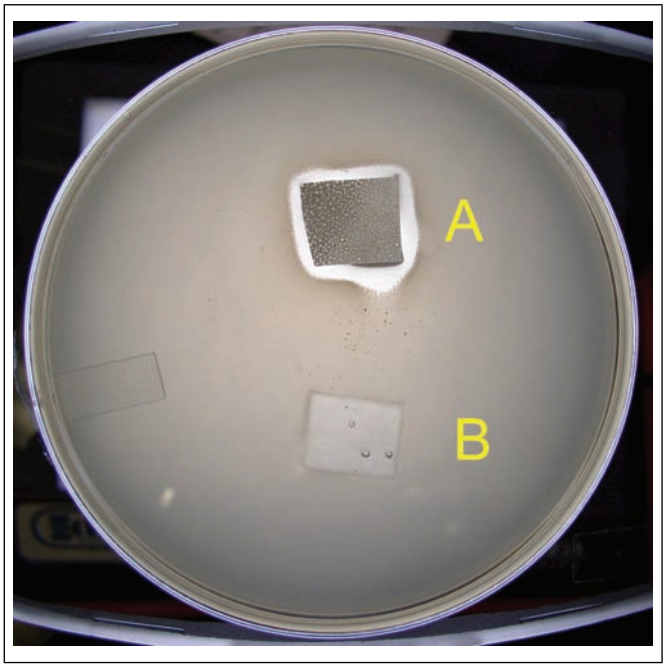


**Figure 8—Agar diffusion test of PLA/nisin film against *L. monocytogenes*. A: PLA/nisin film; B: PLA film.**

Lysozyme, a natural component in egg white, could have enhanced the effectiveness of PLA/nisin treatment against *L. monocytogenes* and *S.* Enteritidis in LEW, because lysozyme could increase the permeability of the outer membrane and thus allow nisin access to the cytoplasmatic membrane. Durance (1994) reported that lysozyme remained stable after 30 min at 71 °C, while liquid egg white is pasteurization processed at 56.6 °C for a minimum 3.5 min. However, the synergistic effect of PLA/nisin with lysozyme in LEW needs to be further investigated.

## Conclusions

The results of this study suggest that the incorporation of bacteriocins into PLA polymer could provide a possible delivery system for improving the efficacy of bacteriocins in food applications. In this study, only PLA/nisin films were evaluated. However, the PLA/nisin polymer can be used to make bottles or coated on the bottle surface for use in liquid food packaging, as well as being made into films or coated on the surface of films for use in solid food packaging.

This is a preliminary study, which provides a starting point to determine whether PLA/nisin has potential for antimicrobial packaging. Further research will be required to establish the parameters for optimal antimicrobial efficiency. Such parameters as levels of bacteriocin and bacteriocin purity will be the focus of further study. Application methods such as PLA/nisin coating and release will also be evaluated.



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