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Inhibition of *Listeria monocytogenes* on the Surface of Individually Packaged Hot Dogs with a Packaging Film Coating Containing Nisin

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ABSTRACT

The objective of this study was to determine the effectiveness of packaging films coated with a methylcellulose/hydroxypropyl methylcellulose–based solution containing 10,000, 7,500, 2,500, or 156.3 IU/ml nisin for controlling *Listeria monocytogenes* on the surfaces of vacuum-packaged hot dogs. Barrier film coated with a methylcellulose/hydroxypropyl methylcellulose–based solution containing nisin or no nisin (control) was heat sealed to form individual pouches. Hot dogs were placed in control and nisin-containing pouches and inoculated with a five-strain *L. monocytogenes* cocktail (approximately 5 log CFU per package), vacuum sealed, and stored for intervals of 2 h and 7, 15, 21, 28, and 60 d at 4°C. After storage, hot dogs and packages were rinsed with 0.1% peptone water. Diluent was spiral plated on modified oxford agar and tryptic soy agar and incubated to obtain counts (CFU per package). *L. monocytogenes* counts on hot dogs packaged in films coated with 156.3 IU/ml nisin decreased slightly (~0.5-log reduction) through day 15 of refrigerated storage but was statistically the same ($P > 0.05$) as hot dogs packaged in films without nisin after 60 d of storage. Packaging films coated with a cellulose-based solution containing 10,000 and 7,500 IU/ml nisin significantly decreased ($P < 0.05$) *L. monocytogenes* populations on the surface of hot dogs by greater than 2 log CFU per package throughout the 60-d study. Similar results were observed for hot dogs packaged in films coated with 2,500 IU/ml nisin; however, *L. monocytogenes* populations were observed to be approximately 4 log CFU per package after 60 d of refrigerated storage from plate counts on tryptic soy and modified oxford agars.

Foodborne illnesses from pathogens are responsible for 76 million illnesses a year resulting in 325,000 hospitalizations and 5,000 deaths *(11)*. One food pathogen of particular concern to food producers is *Listeria monocytogenes,* which is responsible for approximately 2,500 illnesses a year resulting in approximately 500 deaths *(4)*.

L. monocytogenes is a gram-positive, non–spore-forming, facultatively anaerobic, rod-shaped organism. It can be isolated from soil, silage, and other environmental sources. In addition, *L. monocytogenes* is associated with poultry, dairy, and ready-to-eat (RTE) products. *L. monocytogenes* is of particular concern in RTE products such as luncheon deli meats and hot dogs. RTE products are defined as meat products that can be consumed without further heat processing after purchase.

Major foodborne outbreaks have occurred involving *L. monocytogenes*–contaminated RTE meat products. In 2000, Cargill Turkey Products in Waco, Tex., recalled approximately 16.9 million pounds of RTE turkey and chicken products because of potential *Listeria* contamination; 29 illnesses, 4 deaths, and 3 miscarriages or stillbirths were linked to the outbreak *(1)*. In 1999, a major outbreak linked to hot dogs and deli meats produced by Bil Mar Foods was responsible for 101 cases of illness and 21 deaths *(3)*; 35 million pounds of hot dogs and deli meat were recalled as a result *(7)*. Even though hot dogs are cooked, they can be contaminated with *L. monocytogenes* during the postcooking operations of casing removal and vacuum packaging.

Packaging films containing antimicrobial agents might provide an additional hurdle to combat food pathogens present on the surfaces of RTE meat products. Antimicrobial packaging acts to reduce, inhibit, or retard growth of microorganisms that might be present on the food surface *(2)*. Many studies involving antimicrobial packaging involve incorporating antimycotic agents in the packaging material *(5, 16, 17)*. Antimicrobial agents also can be incorporated into packaging for RTE meats.

Ming et al. *(12)* applied pediocin, a bacteriocin similar to nisin, to cellulosic casing to inhibit *L. monocytogenes* on meat. It was determined that pediocin (7.75 μg/cm^2 or 5 AU/cm^2) significantly decreased *L. monocytogenes* ATCC 19115 on vacuum-packaged meat for up to 12 weeks when coated onto cellulosic casings and stored at 4°C. Cutter and Siragusa *(6)* immobilized nisin in calcium alginate gel to inhibit *Brochothrix thermosphacta* on beef surfaces. Siragusa et al. *(14)* incorporated nisin into low-density polyethylene and determined the ability of the film to inhibit the growth of bacteria on the surface of vacuum-packaged meat.

A polymer structure coated with a cellulose-based solution containing nisin could reduce *L. monocytogenes* on the surface of hot dogs. Vojdani and Torres *(15)* used meth-

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ylcellulose (MC) and hydroxypropyl methylcellulose (HPMC) coatings to control the diffusion of potassium sorbate. A MC/HPMC-based coating might have a similar effect for the release of nisin.

The objective of this study was to determine the ability of a packaging film coated with a MC/HPMC solution containing different levels of nisin to inhibit *L. monocytogenes* on the surface of hot dogs stored at 4°C. This study is an adaptation of a study by Porto et al. *(13)* that determined the effectiveness of sodium lactate formulation for reducing *L. monocytogenes* populations on the surface of hot dogs.

MATERIALS AND METHODS

The experiment was performed in two phases. Phase I tested films that were coated with MC/HPMC solutions containing 10,000 and 156.3 IU/ml nisin against *L. monocytogenes* grown on nutrient agar. These levels were studied because 10,000 IU/ml nisin is the maximum level of nisin allowed by FDA in pasteurized process cheese products and 156.3 IU/ml nisin was found to be the MIC of *L. monocytogenes (9).* Phase I results showed 10,000 IU/ml nisin was too high a concentration and 156.3 IU/ml was too low a concentration for inhibition of *L. monocytogenes* in vacuum-packaged hot dogs. As a result, phase II of the research tested films coated with MC/HPMC solutions containing 2,500 and 7,500 IU/ml nisin against *L. monocytogenes* to determine a reasonable level of nisin. Qualitative comparisons between the four nisin levels can be made because all test parameters remained the same.

Coating preparation: phase I. The packaging film coating was prepared by first dissolving the nisin in 1.25 ml of 0.02 M acetic acid (pH 2) and adding 25 ml of distilled water. Nisin film coating solutions were prepared containing 10,000 (2.5 g) or 156.3 IU/ml (0.039 g) nisin. Methylcellulose (0.875 g) and hydroxypropyl methylcellulose (0.375 g) were then added to the nisin solution and homogenized at 7,000 rpm for approximately 2 min by a Vertis Vertishear with a 20-mm shaft (The Vertis Company, Gardner, N.Y.). Ethanol (25 ml) and polyethylene glycol (PEG) 400 (0.75 ml) were added to the solution and homogenized at 7,000 rpm for approximately 2 min more. The film coating solution was then allowed to degas for approximately 5 min. Total biopolymer concentration in final solution was 70/30% MC/HPMC on a dry weight basis. A control also was prepared without nisin. All coating ingredients were obtained from Sigma (St. Louis, Mo.).

Coating preparation: phase II. The packaging film coating was prepared as defined in phase I; however, the nisin concentrations were 7,500 (1.875 g), 2,500 IU/ml (0.625 g) or no nisin (control).

Coating method: phases I and II. Barrier film with an oxygen transmission rate of 6 cm^3/m^2/day at 40°C was supplied by Cryovac (Cryovac Division, Sealed Air Corporation, Duncan, S.C.) as preformed bags. The bags were cut to yield flat film squares and taped on glass plates (20 by 20 cm); lab tape was used to mask the areas of the film that needed to be heat sealed. Solutions (50 ml) containing nisin (10,000, 7,500, 2,500, or 156.3 IU/ml) or no nisin (control) were cast onto the film with a thin layer chromatography plate coater (CAMAG, Muttenz, Switzerland) with a fixed gate set at 500 μm. After drying at ambient conditions (22°C, 28% relative humidity), all film samples were cut and heat sealed (Midwest Pacific Impulse heat sealer, Taiwan) to produce individual hot dog pouches with the approximate dimensions of 6½ by 2 in. Prior to testing, nisin-treated film and control pouches were sealed in plastic bags and treated under UV light (Zeta 7400, Loctite Corporation, Newington, Conn.) for 5 min to sterilize any contaminates on the film introduced during production.

Bacterial culture. Five strains of *L. monocytogenes* (ATCC 43256, ATCC 51414, ATCC 49594, ATCC 7647, and ATCC 13932) were obtained from Steris Food Labs, Inc. (Manhattan, Kans.). Each strain was stored in brain heart infusion (BHI) broth with 20% glycerol at −70°C until needed. The cultures were thawed to 25°C, and 100 μl of each strain was individually transferred to 9 ml of BHI broth and grown at 37°C for 24 h. A loopful of each broth was transferred onto separate BHI agar slants and incubated at 37°C for 24 h. The cultures were then maintained on BHI agar slants at 4°C until needed. All microbiological work was performed in a Labconco Purifier class II safety cabinet (Labconco Corporation, Kansas City, Mo.). All microbiological media was purchased from Difco (Difco Laboratories, Sparks, Md.).

Hot dog inoculation. One loopful of each *L. monocytogenes* strain was transferred into 9-ml tubes of BHI broth and incubated at 37°C for 24 ± 2 h. An isolation streak of each strain was performed on tryptic soy agar (TSA) and incubated at 37°C for 24 ± 2 h.

A single colony of each strain was transferred into 50 ml of BHI broth and incubated at 37°C for 24 h. A 100-μl volume of each strain was transferred to individual Nephelo culture flasks containing 50 ml of BHI broth and incubated at 37°C for 18 h. The optical density (OD) of each broth was confirmed to be in the 0.50 to 0.60 range (Spectronic 20, ThermoSpectronic, Rochester, N.Y.) to ensure that each culture flask contained approximately 9 log CFU/ml. Twenty milliliters of each strain was transferred to a sterile bottle and mixed well to yield a five-strain *L. monocytogenes* cocktail. Serial dilutions (1 ml into 9 ml 0.1% peptone water) were prepared from the *L. monocytogenes* cocktail to obtain a population of approximately 5 log CFU/ml.

Inoculation and vacuum packaging of hot dogs. All-beef, skinless hot dogs (Carolina Pride, Greenwood Packing Plant, Greenwood, S.C.) were purchased from a local grocer. All hot dog packages had Sell By dates approximately 1 month from the start date of the research and were from the same lot. Hot dog packages were wiped with 70% ethanol and aseptically opened with sterile scissors. A single hot dog was transferred by sterile tongs to individual hot dog pouches coated with MC/HPMC solutions containing nisin concentrations of 10,000, 7,500, 2,500, or 156.3 IU/ml or no nisin. One milliliter of the *L. monocytogenes* cocktail was added to achieve a target population of approximately 5 log CFU/ml. The dilution used to inoculate the hot dog was spiral plated (Autoplate 4000, Spiral Biotech, Bethesda, Md.) to obtain a plate count to confirm the target population and to determine *L. monocytogenes* recovery efficiency. *L. monocytogenes* recovery efficiency by the package rinse method from hot dogs packaged with no nisin films (control) was approximately 96%. Each package was massaged by hand for approximately 2 min to coat the surface of the hot dog with the inoculum. All microbiological work was performed in a Labconco Purifier class II safety cabinet.

Inoculated packages were vacuum sealed to a dial reading of 711 mm Hg with an Ultravac 250 (Koch, Kansas City, Mo.). The packages were stored at 4°C, and *L. monocytogenes* populations were observed at hour 2 and days 7, 15, 21, 28, and 60. Populations were observed after 2 h as a baseline reading; this was the time period necessary for packaging all hot dogs on day 0. Two

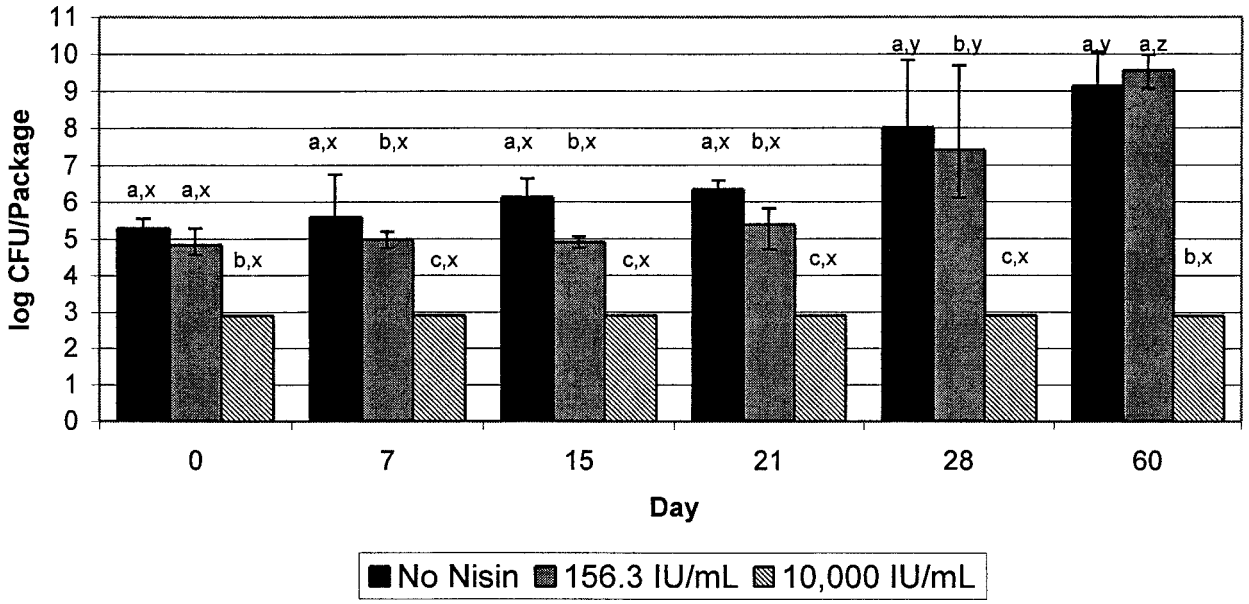

FIGURE 1. Listeria monocytogenes *(five-strain cocktail) populations on the surface of hot dogs packaged in film coated with methyl cellulose/hydroxypropyl methyl cellulose solutions containing 10,000 IU/ml, 156.3 IU/ml, or no nisin (control) when enumerated on tryptic soy agar (TSA). Populations on hot dogs packaged in film coated with 10,000 IU/ml were below the detectable limit (<2.9 log CFU per package) throughout the study. Letters indicate significant differences (*$P < 0.05$*) between nisin treatments (no nisin, 156.3 IU/ml, and 10,000 IU/ml) for each day of storage (a through c) and between storage days for each treatment (x and y).*

hot dogs were analyzed per sampling interval for each treatment. The experiment was performed in triplicate.

Microbiological analyses. Each package was aseptically opened and 9 ml of sterile 0.1% peptone water was added. The packages were massaged by hand for 2 min, and the resulting fluid was transferred to a 5-ml spiral disposable polystyrene beaker. Serial dilutions were prepared from the rinsate. *L. monocytogenes* populations were enumerated by spiral plating 100 μl of rinsate dilutions of hot dogs with expected high populations (no nisin and 156.3 IU/ml films). *L. monocytogenes* populations were enumerated by spiral plating 250 μl of the rinsate of hot dogs with expected low populations (2,500, 7,500, and 10,000 IU/ml films). All were plated in duplicate on MOX agar and TSA and incubated at 37°C for 48 ± 2 h. Colonies typical of *L. monocytogenes* were confirmed by Listeria Kit (api Listeria, BioMerieux, Marcy l'Etoile, France). Bacterial counts were expressed as CFU per package. All microbiological media was purchased from Difco.

Statistical analysis. For both phases (I and II), the experiment was a completely random design with a three by two by six factorial arrangement between treatment, media, and day. Data were analyzed by analysis of variance with SAS (SAS Inc., Cary, N.C.). Means were examined with pairwise comparisons ($P < 0.05$). Both phases were each performed in triplicate.

RESULTS AND DISCUSSION

Phase I. Counts for hot dogs inoculated with a 5-log *L. monocytogenes* cocktail and packaged with films coated with a cellulose-based solution containing 10,000 IU/ml nisin were below the detectable limit of 2.9 log CFU per package for all time periods. *L. monocytogenes* population on the surface of hot dogs packaged with these films were significantly decreased ($P < 0.05$) by greater than 2 log CFU per package throughout the 60-day study when enumerated on both TSA and MOX agar compared to *L. monocytogenes* counts on hot dogs packaged with no nisin films. Grower *(9)* determined that a solution containing 10,000 IU/ml nisin was effective for inhibiting *L. monocytogenes* on nutrient agar by drop assay. Franklin *(8)* determined that the packaging film coating containing 10,000 IU/ml used in an agar diffusion test effectively inhibited *L. monocytogenes* on MOX and TSA agar when grown at 4 and 37°C.

L. monocytogenes populations from hot dogs packaged in films coated with no nisin and 156.3 IU/ml nisin were statistically equal ($P < 0.05$) on day 0 (hour 2) when enumerated on TSA (Fig. 1); however, populations of *L. monocytogenes* on hot dogs packaged with no nisin (day 0) were higher ($P < 0.05$) than on hot dogs packaged in films coated with 156.3 IU/ml nisin when enumerated on MOX agar (Fig. 2). This difference was attributed to the selectivity of MOX. Cells injured from the nisin treatment were unable to recover on MOX because of the presence of lithium chloride and antimicrobial supplements moxalactan and colistin sulfate in the medium formulation, whereas cells were able to recover on TSA. *L. monocytogenes* counts (TSA and MOX agar) on hot dogs packaged with films containing no nisin increased from 5.29 (2 h) to approximately 8 log CFU per package (day 28).

Populations of *L. monocytogenes* were significantly reduced ($P < 0.05$) on hot dogs packaged in films coated with 156.3 IU/ml nisin and stored from day 7 to day 28 (TSA and MOX agar) compared to hot dogs packaged with films containing no nisin (Fig. 1).

L. monocytogenes counts on hot dogs packaged in films coated with 156.3 IU/ml nisin decreased through day 15 of refrigerated storage. Populations of *L. monocytogenes* on hot dogs packaged in films coated with 156.3 IU/ml nisin significantly ($P < 0.05$) increased from 4.42 (day 15)

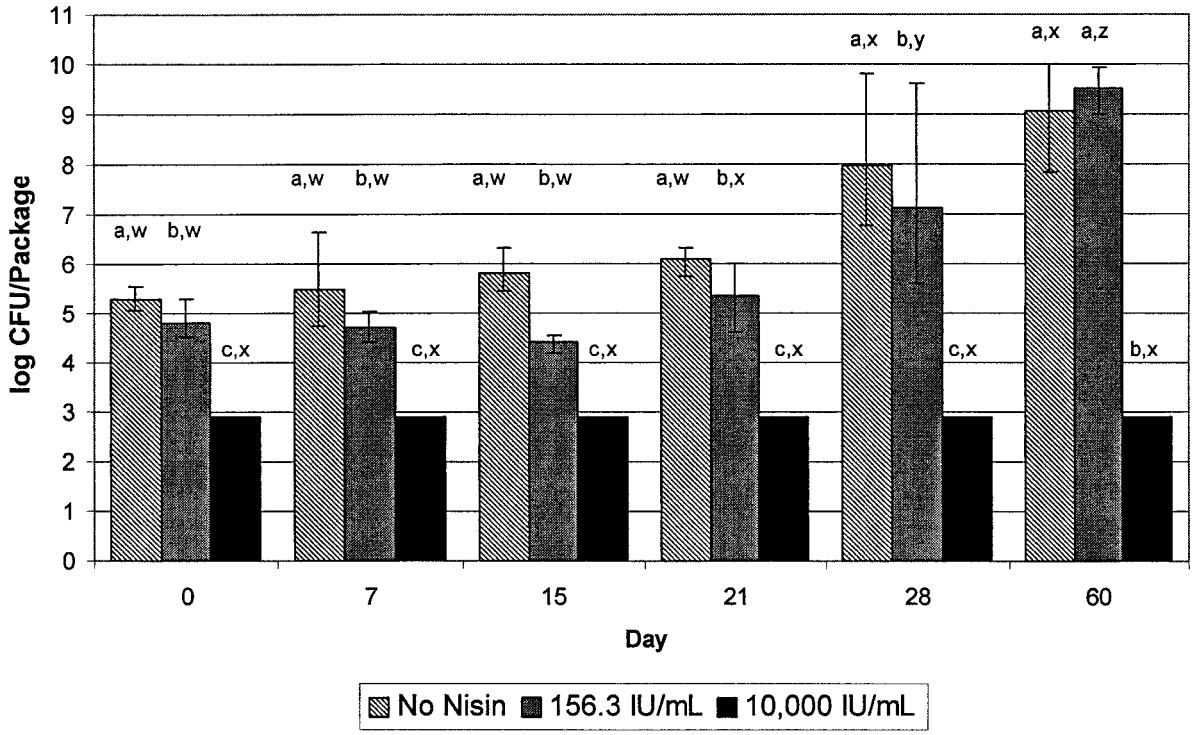

FIGURE 2. Listeria monocytogenes *(five-strain cocktail) populations on the surface of hot dogs packaged in film coated with methyl cellulose/hydroxypropyl methyl cellulose solutions containing 10,000 IU/ml, 156.3 IU/ml, or no nisin (control) when enumerated on modified oxford (MOX) agar. Populations on hot dogs packaged in film coated with 10,000 IU/ml were below the detectable limit (<2.9 log CFU per package) of the plating procedure throughout the study. Letters indicate significant differences (*$P < 0.05$*) between nisin treatments (no nisin, 156.3 IU/ml, and 10,000 IU/ml) for each day of storage (a through c) and between storage days for each treatment (x and y).*

to 7.12 log CFU per package (day 28) on MOX (Fig. 2); similar results were observed on TSA. These results indicate ineffectiveness of packaging films with 156.3 IU/ml nisin for inhibiting *L. monocytogenes* on the surface of hot dogs after 15 days of storage at 4°C. After 60 days of storage, there is no significant difference ($P < 0.05$) between *L. monocytogenes* counts on hot dogs packaged in films coated with 156.3 IU/ml nisin and in films with no nisin.

Phase II. *L. monocytogenes* populations on hot dogs packaged with films containing no nisin increased throughout the study. The results for hot dogs packaged in films coated with 7,500 IU/ml nisin (Figs. 3 and 4) were similar to those of hot dogs packaged in films coated with 10,000 IU/ml nisin in phase I. The populations of *L. monocytogenes* decreased below the detectable limit throughout the 60-day study for hot dogs packaged in film coatings containing 7,500 IU/ml nisin. *L. monocytogenes* populations on hot dogs packaged in films coated with 2,500 IU/ml nisin decreased below the detectable limit (2.9 log CFU/ml) up to day 28; however, *L. monocytogenes* populations were observed to be approximately 4 log CFU per package after 60 days of refrigerated storage (TSA and MOX), which is significantly higher ($P < 0.05$) than *L. monocytogenes* populations on the surface of hot dogs packaged in films coated with 7,500 IU/ml nisin. This suggests that films coated with 2,500 IU/ml nisin lose their effectiveness between 28 and 60 days.

Day 0 measurements were taken within 2 h of inoculation and vacuum packaging; during this time, populations on hot dogs packaged in films coated with 10,000, 7,500, and 2,500 IU/ml nisin were reduced below the minimum detectable limit (2.9 log CFU/ml) of the plating procedure. This is supported by previous research. Grower *(9)* measured the release of nisin into solution from MC/HPMC coatings at various time periods over 8 days. The study determined that low-density polyethylene film coated with a MC/HPMC solution containing 10,000, 7,500, and 2,500 IU/ml nisin released enough nisin in solution after 1 min to inhibit *L. monocytogenes* ATCC 15313.

The results of this study agree with previous research regarding antimicrobial films containing nisin. Immobilization of nisin by a calcium alginate gel was effective for inhibiting *B. thermosphacta* on beef surfaces *(6).* Nisin (100 $\mu g\ ml^{-1}$) immobilized in calcium alginate gel suppressed *B. thermosphacta* by greater than 2.42 log CFU cm^{-2} on beef surfaces compared with untreated beef samples after 7 days of storage at 4°C. Siragusa et al. *(14)* incorporated nisin into low-density polyethylene and determined the ability of the film to inhibit the growth of bacteria on the surface of vacuum-packaged meat. Low-density polyethylene with a nisin content of 0.1% by weight initially reduced *B. thermosphacta* by 2 log CFU cm^{-2} on beef carcass tissue samples within the first 2 days of storage at 4°C. After 20 days of refrigerated storage, *B. thermosphacta* populations from beef samples packaged with nisin-containing film was significantly less compared with beef samples packaged with film containing no nisin.

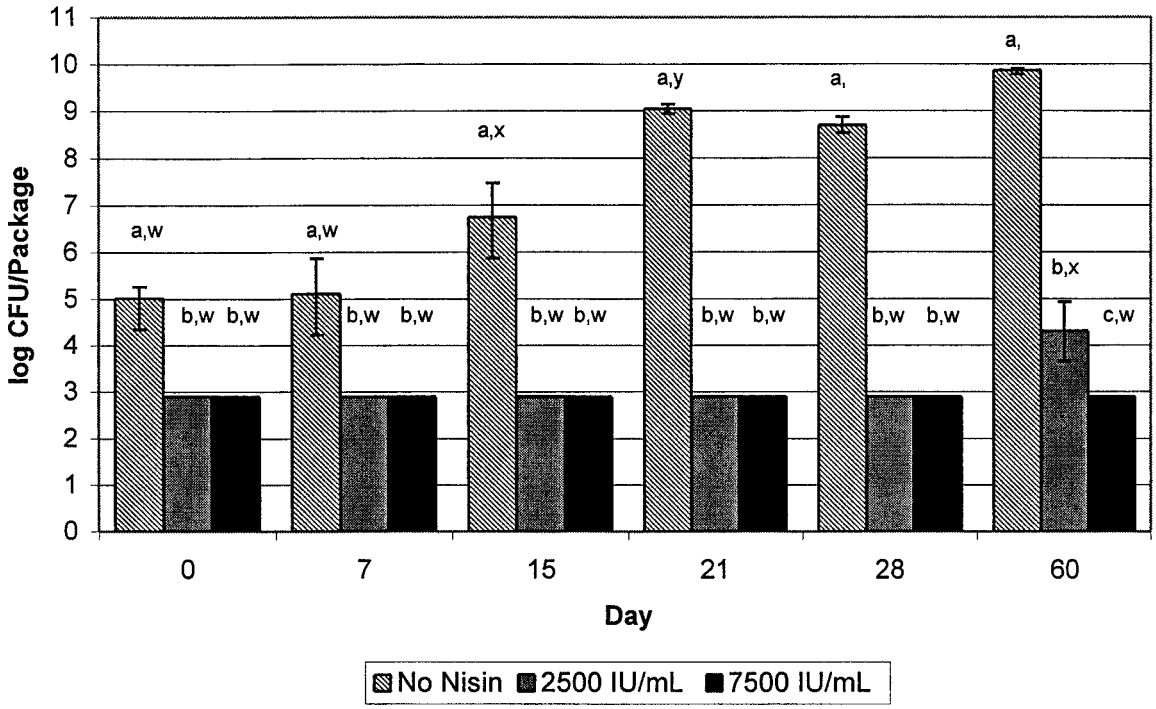

FIGURE 3. Listeria monocytogenes *(five-strain cocktail) populations on the surface of hot dogs packaged in film coated with methyl cellulose/hydroxypropyl methyl cellulose solutions containing 7,500 IU/ml, 2,500 IU/ml, or no nisin (control) when enumerated on tryptic soy agar (TSA). Populations on hot dogs packaged in film coated with 7,500 IU/ml were below the detectable limit (<2.9 log CFU per package) throughout the study. Populations on hot dogs packaged in film coated with 2,500 IU/ml were below the detectable limit until day 60. Letters indicate significant differences (*$P < 0.05$*) between nisin treatments (no nisin, 2,500 IU/ml, and 7,500 IU/ml) for each day of storage (a through c) and between storage days for each treatment (x and y).*

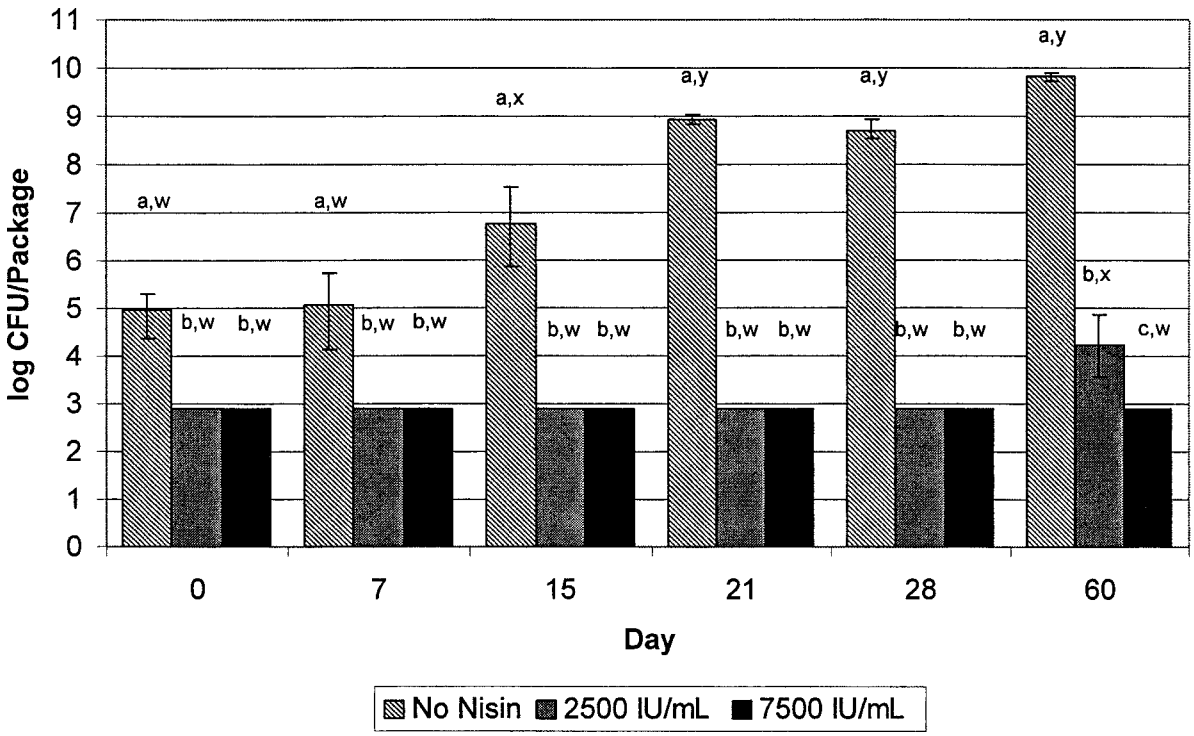

FIGURE 4. Listeria monocytogenes *(five-strain cocktail) populations on the surface of hot dogs packaged in film coated with methyl cellulose/hydroxypropyl methyl cellulose solutions containing 7,500 IU/ml, 2,500 IU/ml, or no nisin (control) when enumerated on modified oxford (MOX) agar. Populations on hot dogs packaged in film coated with 7,500 IU/ml were below the detectable limit (<2.9 log CFU per package) throughout the study. Populations on hot dogs packaged in film coated with 2,500 IU/ml were below the detectable limit until day 60. Letters indicate significant differences (*$P < 0.05$*) between nisin treatments (no nisin, 2,500 IU/ml, and 7,500 IU/ml) for each day of storage (a through c) and between storage days for each treatment (x and y).*

TABLE 1. *Estimated coating materials cost (not including barrier film) for methyl cellulose/hydroxypropyl methyl cellulose–based packaging coatings containing no nisin and different levels of nisin; the cost estimation is for the hot dog pouch area (83.87 cm^2) used to vacuum package an individual hot dog and was calculated on the basis of cost of film and coating materials used (g) per area (cm^2)*

Nisin concentration (IU/ml)	Cost/package
No nisin	$0.034
156.3	$0.049
2,500	$0.285
7,500	$0.730
10,000	$0.963

L. monocytogenes recovery efficiency with the package rinse method from individual hot dogs packaged with no nisin films (control) was approximately 96%. This efficiency agrees with a study performed by Luchansky et al. *(10)* that compared methods for *L. monocytogenes* recovery from vacuum-sealed packages of hot dogs. The study compared the USDA Food Safety and Inspection Service (FSIS) product composite enrichment method, the U.S. Department of Agriculture (USDA) Agriculture Research Service (ARS) product composite rinse method, and the USDA-ARS package rinse method. *L. monocytogenes* was recovered from hot dog packages inoculated with 20,133 CFU per package at efficiencies of 20 and 95% with the USDA-ARS product composite rinse method and the USDA-ARS package rinse method, respectively.

Packaging films containing antimicrobials such as nisin are proving to be an effective means of inhibiting bacteria on the surface of meat products; however, factors such as cost, legal (liability) issues, and processing limitations are slowing their acceptability in the industry. Table 1 estimates coating materials cost. Packaging films coated with solutions containing 10,000 IU/ml nisin would be too expensive for practical use. Films coated with solutions containing less nisin at a lower cost might be equally effective as those coated with 10,000 IU/ml nisin. For example, the lowest level of nisin (2,500 IU/ml) that was effective for 28 days of storage costs about $0.29 per pouch, whereas 60 days of inhibition provided by films containing 7,500 IU/ml would cost $0.73 per pouch. Cost issues will continue to decrease as development continues. Processing limitations include the inability of MC/HPMC-based coating to heat seal. A patterned gravure coating method that leaves the heat seal areas uncoated would need to be developed to make the film more commercially feasible.

Nisin was effective for inhibiting *L. monocytogenes* when used as a coating for packaging films. *L. monocytogenes* counts on hot dogs packaged with 156.3 IU/ml nisin-level films decreased through day 15 of refrigerated storage. Packaging films coated with a cellulose-based solution containing 10,000 and 7,500 IU/ml nisin significantly decreased *L. monocytogenes* populations on the surface of hot dogs by greater than 2 log CFU per package throughout the 60-day study. Similar results were observed for hot dogs packaged in films coated with 2,500 IU/ml nisin; however, nisin lost its effectiveness against *L. monocytogenes* between 28 and 60 days of refrigerated storage. Further studies need to be done to establish a nisin concentration between 156.3 and 2,500 IU/ml nisin that can be effective against *L. monocytogenes.*

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