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Effect of combining nisin with modified atmosphere packaging on inhibition of *Listeria monocytogenes* in ready-to-eat turkey bologna

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ABSTRACT The objective of this study was to evaluate the effect of nisin in combination with different types of packaging on the survival of *Listeria monocytogenes* in ready-to-eat low-fat turkey bologna. Bologna was inoculated with *L. monocytogenes* exposed to 1 of 6 treatments: 3 packaging treatments (100% CO₂, air, vacuum), each with and without nisin. Bologna was re-

frigerated and sampled 9 times over 42 d. Nisin reduced initial *L. monocytogenes* populations by 1.5 to 2 log cycles and 100% CO₂ packaging prevented outgrowth throughout 42 d of storage, whereas non-CO₂ packaging displayed a 2-log increase in population during storage. Nisin (500 IU/mL) combined with 100% CO₂ was effective in reducing *Listeria* and preventing outgrowth on bologna over 42 d of refrigerated storage.

Key words: *Listeria monocytogenes*, nisin, modified atmosphere packaging, carbon dioxide, ready to-eat meat

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INTRODUCTION

Postcooking contamination of ready-to-eat (RTE) meat and poultry products by *Listeria monocytogenes* is a major food safety problem as well as an economic hardship for the food industry. *Listeria monocytogenes* is an important foodborne pathogen that can cause life-threatening invasive infections in neonates, pregnant women, elderly, and immunocompromised individuals (Gerba et al., 1996; Slutsker and Schuchat, 1999). *Listeria* is a psychrotrophic organism capable of survival and growth at refrigeration temperatures. The majority of the food product recalls associated with *Listeria* involve RTE meat and poultry products. These food product recalls have serious economic and public perception effects on the food industry (Thomsen and McKenzie, 2001; Ivanek and Grohn, 2004; Marsh et al., 2004; Teratanavat and Hooker, 2004; Kramer et al., 2005). Use of the additional hurdles to control growth of *L. monocytogenes* in RTE meat would provide an increased margin of safety in RTE meats during refrigerated storage. Nisin and modified atmosphere packaging (MAP) are 2 hurdles used to retard microbial growth in foods.

Nisin is a polypeptide bacteriocin produced by *Lactococcus lactis* ssp. *lactis* during fermentation. Nisin is the most commonly used natural antimicrobial compound and was approved for use in food in 1969 and was awarded generally recognized as safe (GRAS) status in the United States in 1988 (US Food and Drug Administration, 1988). Nisin has shown synergistic antibacterial activity with several compounds and processes. Nisin is predominately active against gram-positive bacteria including sporeformers due to the adsorption of nisin on the cell membrane of sensitive strains but exhibits little or no activity against gram-negative bacteria, yeasts, and molds. Nisin destabilizes the cytoplasmic membrane causing cell death by release of cytoplasmic materials (Gao et al., 1991; Ray, 1992).

Postprocess operations during preparation of RTE meat products such as peeling, sorting, loading, and slicing are potential sources for recontamination of products with *L. monocytogenes* (Murphy et al., 2005). The organism can survive in food-processing facilities for long periods (Tompkin, 2002), and thus, processing equipment and other food contact surfaces can act as a source of this organism during postprocess operations.

In 2003, the USDA Food Safety and Inspection Service released an interim final rule to control *L. monocytogenes* for postlethality treatments used for RTE meat and poultry products (USDA, 2003). This ruling includes 3 alternative approaches that establishments can implement during processing of RTE meat and poultry products using a postlethality treatment,

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antimicrobial agent, sanitation control measures, or a combination of these interventions. McCormick et al. (2003) determined the D-values for *L. monocytogenes* at 61 and 65°C were 124 and 16.2 s, respectively, and reported that when nisin-impregnated films were combined with in-package pasteurization, *L. monocytogenes* growth was suppressed during 2 mo of refrigerated storage (McCormick et al., 2005).

Surface application of single antimicrobial agents as well as in combination are effective in controlling *L. monocytogenes* in RTE meats (Gill and Holley, 2000; Samelis et al., 2001). Lopez-Mendoza et al. (2007) studied the combined effects of nisin, lactic acid, and MAP for inhibiting *L. monocytogenes* on fresh, ground pork. These researchers did not directly compare MAP to air packaging but found that with MAP, adding nisin (500 ppm) only slightly improved the antilisterial effect of 2% lactic acid. Cosby et al. (1999) found that among combinations of nisin, EDTA, and packaging atmosphere [vacuum or MAP (80% O₂/20% CO₂)], vacuum packaging with both nisin and EDTA had the greatest inhibitory effect on the aerobic microorganism population on fresh chicken drumettes. Previous researchers have used nisin with vacuum packaging of RTE products such as frankfurters (Luchansky and Call, 2004), bologna and ham (Geornaras et al., 2005), and pork bologna (Samelis et al., 2001) to inhibit *L. monocytogenes*. There are few published data directly comparing the antilisterial effects of vacuum, air, and 100% CO₂ with and without nisin. Therefore, the objective of this study was to evaluate the surface application of nisin in combination with MAP, vacuum packaging, or air packaging of RTE low-fat turkey bologna to inactivate *L. monocytogenes*.

MATERIALS AND METHODS

Food Product Preparation

Ready-to-eat low fat turkey bologna averaging 14.3% fat, 10.7% protein, and 71.4% moisture with 2% salt was used for the experiment. Bologna samples were kept frozen at -70°C and thawed overnight at 4°C before experimentation. For inoculation and nisin treatment, bologna slices of experimentally 2.5-mm thickness were cut into 4 × 4 cm² pieces using a sterile cutting template, and each piece was used as an experimental unit and placed in a sterile Petri dish.

Inoculum Preparation

Listeria monocytogenes ATCC 15313 was preserved by freezing the culture at -70°C in vials containing BHI (Becton Dickinson, Sparks, MD) broth supplemented with 20% (vol/vol) glycerol (Sigma, St. Louis, MO). To propagate the culture, a frozen vial was thawed at room temperature, and 0.1 mL of the thawed culture was transferred to 9.9 mL of Enrichment *Lis-*

teria broth (Difco, Sparks, MD) in screw-capped tubes and incubated aerobically for 16 and 18 h at 37°C with agitation (Thermolyne Maxi-Mix III type 65800, Barnstead/Thermolyne, Dubuque, IA). The inoculum was prepared from a second transfer of this culture (0.1 mL) to another 9.9-mL tube of Enrichment *Listeria* broth (Difco), and incubated aerobically for 16 to 18 h at 37°C with agitation. After overnight incubation, the cells were harvested by centrifugation at 3,000 × *g* at 22°C for 10 min (IEC HN-SII centrifuge, International Equipment Co. Inc., Needham Heights, MA), the pellet resuspended in 0.1% sterile peptone water (Bacto peptone, Becton Dickinson) to obtain a population of approximately 8 to 9 log cfu/mL. One milliliter of the suspension was transferred into 99 mL (10⁻²) of 0.1% sterile peptone water to obtain a population of approximately 5 to 6 log cfu/mL. Initial cell populations were verified by enumeration of the cells following pour-plating in BHI agar and incubating at 37°C for 48 h.

Antimicrobial Preparation

Nisaplin, a commercial nisin product (2.5% nisin, 10⁶ IU/g), was provided by Danisco (Danisco USA Inc., New Century, KS). Solution of required nisin concentration (500 IU/mL) was prepared on the day of the experiment by dissolving the appropriate amount of nisin (0.5 mg) in sterile distilled water. The activity of nisin was determined by critical dilution assay (Pucci et al., 1988). Serial 2-fold dilutions of the antimicrobial agents were tested against *L. monocytogenes* ATCC 15313. Ten microliters of each dilution was spotted on the surface of the BHI agar medium seeded uniformly with a suspension of *L. monocytogenes*. After incubation (48 h at 37°C), the plates were checked for zones of inhibition. Titer of nisin in arbitrary units (AU) per milliliter was expressed as the reciprocal of the highest dilution showing the zone of inhibition. Activity of the nisin was expressed in AU per milligram based on the weight of the antimicrobial compounds used in serial dilution and converted into AU per milliliter based on the weight (mg) of the antimicrobial compound used in the application solution.

Antimicrobial Treatment and Inoculation

Six different treatment combinations were used: no nisin + vacuum packaging (VAC), nisin + vacuum packaging (VAC + nisin), no nisin + CO₂ tray pack (CO₂), nisin + CO₂ tray pack (CO₂ + nisin), no nisin + air tray pack (air), nisin + air tray pack (air + nisin). Frozen bologna samples were thawed overnight at 4 ± 1°C. On d 0, bologna samples were removed from the refrigerator and aseptically transferred to a sterile surface under a Germ-free Bioflow chamber (Ormond Beach, FL). Each bologna slice was cut into 4 × 4 cm² pieces using the sterile cutting template as previously described and placed in a sterile Petri dish, and

then divided into 2 groups (nisin-treated and control samples). For a treated group, 0.1 mL of nisin solution (500 IU/slice) was spread on one side of the slice by even distribution, and then allowed to dry for 15 to 20 min. On the same side, control and treated bologna squares were uniformly surface-treated with 0.1 mL of the inoculum culture 5 to 6 log₁₀ cfu/mL, yielding 4 to 5 log₁₀ cfu per bologna square.

Hurdle Treatment

After inoculation, treated and control samples were transferred and divided into 3 groups aseptically. The first group was placed into plastic bags (CN530, Sealed Air Corporation, Duncan, SC) for the vacuum packaging machine (Koch Supplies Inc., Kansas City, MO); the second and third groups were transferred to a foam tray for packaging under 100% CO₂ and air (Ross Junior preformed tray packaging machine, Canton, MA). All samples kept at 4°C for periodical sampling. For samples that were incubated under gas treatment (CO₂ and air) were checked for gas composition changing throughout the storage period.

Microbiological Analysis

Treated bologna samples were stored at 4°C for 42 d. Sampling was on 0, 2, 4, 7, 14, 21, 28, 35, and 42 d of storage for *Listeria monocytogenes* viable count. Day 0 samples were held for 2 h after packaging. Samples were aseptically removed from the bags or foam trays and homogenized with 20 mL of 0.1% peptone water. Homogenates were then serially diluted, and appropriate serial dilutions were surface plated on Enrichment *Listeria* agar (Difco, Becton Dickinson and Co., Sparks, MD) duplicate plates. Plates were incubated at 37°C for 48 h before enumerating colonies on dilution plates having between 25 and 250 colonies. The limit of sensitivity for bacterial numbers was about 10² cfu/mL. The cfu counts were converted to log cfu/cm² of the sample before analysis of the data.

pH Determination

The pH of bologna samples were taken on 0, 2, 4, 7, 14, 21, 28, 35, and 42 d of refrigerated storage. Ten g of meat was blended with 100 mL of deionized water for 30 s. The pH was measured in duplicate using a probe and digital meter (model 420A, Orion Research Inc., Boston, MA).

Statistical Analysis

The experiment was replicated 3 times. The data were analyzed to determine the inhibitory effect of packaging treatments without nisin and the inhibitory effect of the nisin-packaging combinations. A 2 × 2 factorial design was used to statistically analyze the ini-

tial inhibitory effect of nisin at 0 h. Two levels of nisin (present, not present) and the nisin × packaging interaction were included in the model. Data were analyzed by ANOVA using the GLM procedure of SAS (2008). Least square difference multiple comparison procedure was used to evaluate significant differences (*P* < 0.05) among means.

RESULTS AND DISCUSSION

Antimicrobial Activity Assay

Nisin activity as determined by critical dilution assay was 1.0 × 10³ AU/mg. Nisaplin (0.5 mg) had an activity of 500 AU/mg, and thus in the solution (0.5 mg/mL) used for the experiment, the activity was 500 AU/mL. Nisin has been used on meat products at 6.25 µg/g in vacuum-packed bologna-style sausages (Davies et al., 1999) and was also effective at 1.875 µg/g with 40 ppm of nitrite in cured meats against *Clostridium perfringens* (Rayman et al., 1981). Bell and DeLacy (1987) used 12.5% µg/g of nisin in combination with 0.125% sorbic acid and 0.25 to 5.0% monolaurin to successfully inhibit *Bacillus licheniformis* in pasteurized cured meat. These same researchers used 12.5% nisin to inhibit *B. licheniformis* growth on cooked luncheon meat held at 20°C for 10 d (Bell and DeLacy, 1986). These concentrations were based on pure nisin, whereas the nisin used in the present study was a commercial preparation containing 2.5% nisin. Calculating 2.5% of the 0.5 mg/mL used in the present study is equal to 12.5 µg/g of pure nisin and thus falls in the range of nisin used by previous researchers for inhibiting bacteria on cooked meat products.

pH of Bologna in Different MAP

The pH did not fluctuate more than 0.05 units for bologna held under vacuum or air packaging treatments over 42 d. The pH decreased by about 0.2 units from the initial 6.5 level for bologna packaged in air and vacuum but was 6.30 when first measured when packed in 100% CO₂. This decrease was detected at the 2-d measurement and remained fairly constant throughout the 42 d of storage. Fluctuation of pH values among all packaging treatments was only about 0.24 pH units from 6.53 to 6.29.

L. monocytogenes Populations on Bologna

When the population of *L. monocytogenes* on bologna was pooled over all 9 sampling times, the 3 treatments that did not include nisin revealed that bologna stored in 100% CO₂ MAP had a lower number of cells compared with bologna stored in either vacuum packaging or in tray packs with an air headspace (Table 1). Pooled mean populations of *L. monocytogenes* were 1 to 2 log cfu/cm² lower for each treatment with nisin, and

Table 1. Pooled *Listeria monocytogenes* counts (cfu/cm²) over 42 d of storage at 4°C for bologna stored using different packaging treatments with and without nisin¹

Storage treatment	Log cfu/cm ²
Air	5.6 ^a
Air + nisin	3.8 ^c
CO ₂	4.6 ^b
CO ₂ + nisin	2.6 ^d
VAC	5.4 ^a
VAC + nisin	4.1 ^{bc}

^{a-d}Means with different superscripts are significantly different ($P \leq 0.05$).

¹Air = no nisin + air tray pack; air + nisin = nisin + air tray pack; CO₂ = no nisin + CO₂ tray pack; CO₂ + nisin = nisin + CO₂ tray pack; VAC = no nisin + vacuum packaging; VAC + nisin = nisin + vacuum packaging. Standard error = 1.98.

for all nisin treatments MAP bologna had 1 to 1.5 log cfu/cm² lower populations than vacuum- or air-stored meat. By comparing the differences in cfu counts between no-nisin and nisin treatments given in Table 1, *L. monocytogenes* reduction was highest in CO₂ packaging (~2 log), followed by air packaging (~1.8 log) and then vacuum packaging (~1.3 log).

Survival of *L. monocytogenes* on Bologna During Refrigerated Storage

The initial population of *L. monocytogenes* on untreated bologna was 4.5 log cfu/cm² (Figure 1), and there was an initial 1.5- to 2-log reduction in nisin-treated bologna. These differences are reflected in the d 0 populations for each treatment. Thus, bologna treated with nisin and 100% CO₂ reduced *L. monocytogenes* by 2 log in 2 h, whereas nisin with vacuum or air reduced *L. monocytogenes* by 1.5 log. Treatments initially fell into 2 population groups on d 0: non-nisin treatments (open marks, 4 to 4.5 log cfu/cm²) and nisin treatments (filled marks, 2.5 to 3 cfu/cm²; Figure 1). *Listeria monocytogenes* populations sampled over 42 d of refrigerated storage were reduced by 2 log cycles by MAP + nisin on d 0 and more importantly prevented any increase in *L. monocytogenes* populations throughout the 42 d of storage. In contrast, bologna stored in air without nisin was initially 1 to 2 log cycles higher than “with nisin” treatments but increased to 7 log cfu/cm² by d 42. One hundred percent CO₂ prevented *L. monocytogenes* outgrowth on bologna with and without nisin, whereas neither vacuum packaging nor air packaging inhibited outgrowth of *L. monocytogenes* during refrigerated storage. Interestingly, after 28 d through 42 d of storage, 100% CO₂ and nisin-treated bologna packaged in air did not differ in reducing *L. monocytogenes*. Thus, CO₂ and nisin each individually inhibited *L. monocytogenes* but also acted synergistically to further reduce populations when used in combination. Whereas this further reduction in cell numbers from combining nisin and CO₂ was additive, the slight reduction in meat pH by CO₂ MAP may have enhanced nisin activity. Thomas and

Wimpenny (1996) reported that using 100 IU/mL of nisin at pH values between 4.1 and 5.6, populations of less than 2-log from an initial 9-log population after 18 h incubation at 25°C were found. However, when the pH was 6.2 and 6.5 the populations increased to 2.7 and 5.6, respectively. The pH of bologna was found in this critical range (Table 1) with the CO₂ MAP-treated meat having a pH of about 6.3, whereas the meat packaged in air and under vacuum was around 6.5.

Szabo and Cahill (1998) found similar results for a cocktail of 7 *L. monocytogenes* using 400 IU/mL of nisin with 100% CO₂ compared with nisin with 100% N₂ and 40%CO₂/60%N₂ in buffered tryptone soy broth (pH 6.0). At 4°C, 100% CO₂ prevented outgrowth, whereas the other atmospheres showed a 2-log increase in *L. monocytogenes* with 400 IU/mL of nisin. This same effect was not observed at an incubation temperature of 12°C where all atmospheres with 400 IU/mL of nisin showed a 5-log increase over 7 d. These researchers also found that increasing the nisin concentration to 1,250 IU/mL in the broth prevented *L. monocytogenes* outgrowth under all atmospheres. High CO₂ atmospheres can inhibit aerobic bacterial growth by exclusion of O₂, but additional bactericidal and bacteriostatic activity are believed to be due to the penetration of the gas into the bacterial cell membrane, causing an intracellular pH change beyond the buffering capability of the organism. Zeitoun and Debevere (1993) stated that CO₂ could inhibit cytoplasmic enzymes that in turn affect the rate of cellular reactions that affect reproduction and growth. In the current study, nisin and 100% CO₂ appear to act synergistically to inhibit *L. monocytogenes* because the combined treatments have a greater impact on population both initially and throughout refrigerated storage than when either treatment is used singly. Lopez-Mendoza et al. (2007) found a similar synergism for refrigerated raw pork. These researchers reported that adding nisin to pork packed in 30% CO₂/70% O₂ had a greater inhibitory effect than when pork with nisin was packaged in air. Cosby et al. (1999) found MAP was not superior to vacuum when combined with nisin and EDTA for inhibiting spoilage organisms on refrigerated raw chicken; however, this study did not examine *Listeria* and used 80% O₂/20% CO₂.

Nisin is approved in the United States for use in cooked sausage at 5 to 25 mg/kg to target lactic acid spoilage as well as *L. monocytogenes*. In light of Federal Food Safety regulations to reduce *L. monocytogenes* in RTE meats and the mortality rate of listeriosis, the combined effects of nisin and 100% CO₂ packaging are viable options to reduce risk of illness and death due to this pathogen.

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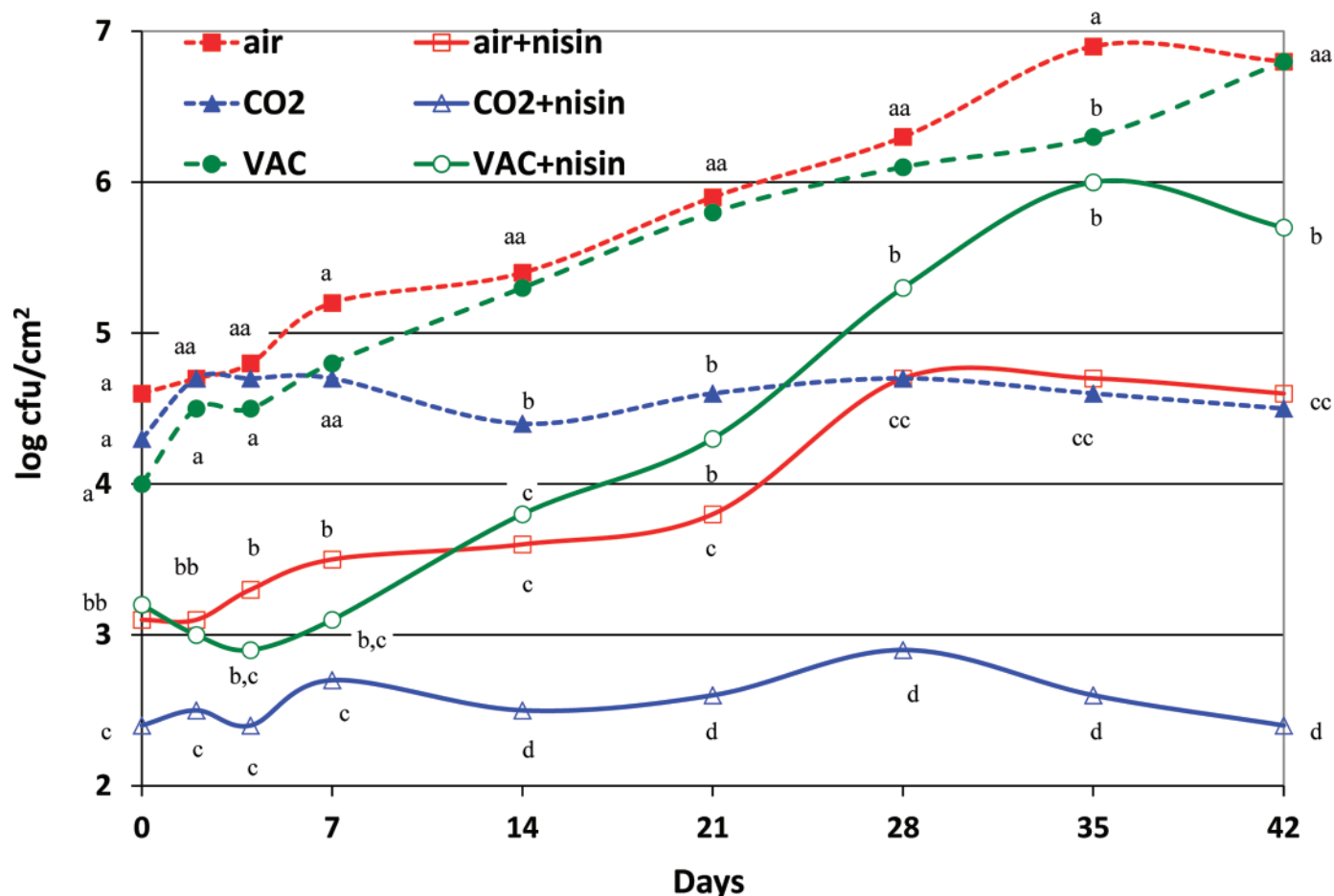


Figure 1. Population of *Listeria monocytogenes* on bologna stored using different packaging treatments with and without nisin under refrigeration for 42 d. Data points having different letters (a–d) are significantly different ($P \leq 0.05$). The 0-d samples were taken at 2 h after treatment with nisin and packaging. Air = no nisin + air tray pack; air + nisin = nisin + air tray pack; CO₂ = no nisin + CO₂ tray pack; CO₂ + nisin = nisin + CO₂ tray pack; VAC = no nisin + vacuum packaging; VAC + nisin = nisin + vacuum packaging. Standard error for each day was as follows: d 0, 0.11; d 7, 0.06; d 14, 0.22; d 21, 0.54; d 28, 0.73; d 35, 1.06; d 48, 0.82.

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