Nisin, rosemary, and ethylenediaminetetraacetic acid affect the growth of *Listeria monocytogenes* on ready-to-eat turkey ham stored at four degrees Celsius for sixty-three days

A. Ruiz,* S. K. Williams,*[1] N. Djeri,* A. Hinton Jr.,† and G. E. Rodrick‡

*Department of Animal Sciences, PO Box 110910, University of Florida, Gainesville 32611;
†USDA, Agricultural Research Service, Richard B. Russell Agricultural Research Center, Athens, GA 30605;
and ‡Food Science and Human Nutrition Department, University of Florida, Gainesville 32611

ABSTRACT The objectives of this study were to determine the anti-*Listeria* and general antimicrobial properties of nisin, rosemary, and EDTA alone and in combination on *Listeria monocytogenes* inoculated on ready-to-eat vacuum-packaged diced turkey ham and to ascertain the effects of the treatments on pH and objective color. The turkey hams were cut into 0.5-cm pieces, inoculated with a *L. monocytogenes* cocktail containing 5 strains of the bacterium, and treated with either no treatment and no inoculum (negative control), inoculum only (positive control), 0.5% nisin, 20 m*M* EDTA, 1% rosemary, 0.5% nisin + 20 m*M* EDTA, 0.5% nisin + 1% rosemary, 0.5% nisin + 20 m*M* EDTA + 1% rosemary, or 20 m*M* EDTA + 1% rosemary. All samples were vacuum-packaged, stored for 63 d at 4°C ± 1°C, and analyzed at 1-wk intervals for total aerobes, *L. monocytogenes*, lactic acid organisms, pH, and objective color. Nisin, nisin with rosemary, nisin with EDTA, and nisin with rosemary and EDTA treatments reduced ($P < 0.05$) *L. monocytogenes* counts by 4.42, 4.20, 3.73, and 4.11 log cfu/g when compared with the positive control, respectively, on d 0. *Listeria monocytogenes* counts remained less than 2.75 log cfu/g for all hams treated with nisin. The EDTA and rosemary treatments alone and in combination were ineffective in inhibiting growth of *L. monocytogenes*. Although none of the treatments completely eliminated *L. monocytogenes*, the results indicated that ready-to-eat turkey ham can have significantly decreased *L. monocytogenes* when treated with nisin alone or in combination with rosemary or EDTA, or both.

Key words: nisin, rosemary, ethylenediaminetetraacetic acid, *Listeria monocytogenes*, turkey ham

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INTRODUCTION

Listeria monocytogenes is a foodborne pathogen that is widely distributed in nature and whose control in food is made difficult by its ability to grow at temperatures ranging from 0 to 45°C (Barbosa et al., 1994), its high tolerance for salt (Farber and Peterkin, 1991), and its ability to initiate growth at a relatively low pH (Bell and Kyriakides, 2005). Numerous outbreaks have been linked to consumption of ready-to-eat (**RTE**) products contaminated with *L. monocytogenes* (Gombas et al., 2003). To date, RTE products are being recalled because of *L. monocytogenes* contamination (USDA FSIS, 2009). Contamination of RTE meat products may occur in processing plants or delicatessens. Although the heat treatment (cooking) that RTE meat and poultry products undergo eliminates the pathogen, recontamination may occur during postprocessing procedures such as peeling, slicing, and repackaging (Farber and Peterkin, 1999). Therefore, new postprocessing hurdle technologies that control or eliminate the incidence of foodborne pathogens are needed for the meat industry (Bernard and Scott, 1999).

The bacteriocin nisin has been used as an antimicrobial in foods since the 1960s (Montville et al., 1995). Nisin is generally recognized as safe for use as a biopreservative in food systems (US Food and Drug Administration, 2008). Nisin is approved for use in meat and poultry products at 250 mg/kg in the finished product, 6.30 mg/kg in the finished product when used in casings, 5.0 mg/kg on cooked meat and poultry products, and 550 mg/kg of a blend of encapsulated nisin preparation (90.9%), rosemary extract (8.2%), and salt (0.9%) for frankfurter and other similar cooked meat

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[1]Corresponding author: wsallyk@ufl.edu

and poultry sausages (USDA FSIS, 2006). Nisin is produced by the lactic acid bacteria (**LAB**) *Lactococcus lactis* (Altena et al., 2000). The mechanism of nisin activity has been shown to involve alteration of the cell membrane of sensitive organisms resulting in the leakage of low molecular weight cytoplasmic components (Garcera et al., 1993; Abee et al., 1994; Winkowski et al., 1994) and destruction of the proton motive force (Gao et al., 1991; Bruno and Montville, 1993).

Ethylenediaminetetraacetic acid has been used extensively in food systems for retardation of crystal formation, food preservation and stabilization, as an antioxidant, and as a chelating and sequestering agent (Winter, 1999). In general, chelators bind magnesium ions in the lipopolysaccharide layer of bacterial cell walls and increase susceptibility of the cells to nisin (Shelef and Seiter, 1993). Ethylenediaminetetraacetic acid has been reported to have an antimicrobial effect by limiting the availability of cations and functioning to destabilize the cell membrane of bacteria by complexing divalent cations that act as salt bridges between membrane macromolecules, such as lipopolysaccharides (Vaara, 1992; Shelef and Seiter, 1993). Hamm (2001) combined EDTA with lysozyme in the treatment of RTE pork hams to enhance antimicrobial properties of the lysozyme. Hamm reported that the EDTA was essential to obtain maximum anti-*Listeria* properties when lysozyme was used.

In addition to EDTA, natural essential oils such as rosemary are being used extensively in the food industry as natural antioxidants (Shelef, 1983). Rosemary (*Rosmarinus officinalis*) extract has shown antimicrobial properties against food spoilage and foodborne pathogenic microorganisms. Rosemary's antibacterial activity has been linked to α-pinene, bornyl acetate, camphor, and 1, 8-cineole (del Campo et al., 2000; Pintore et al., 2002).

The objectives of this study were to determine the anti-*Listeria* and general antimicrobial properties of nisin, rosemary, and EDTA alone and in combination on RTE vacuum-packaged diced turkey ham inoculated with *L. monocytogenes* and to ascertain the effects of the treatments on pH and objective color of the product.

MATERIALS AND METHODS

Inoculum Cultivation, Storage, and Preparation

Five reference strains of *L. monocytogenes* (1/2a, 1/2b, 4b, Scott A, and 19115) were obtained from ABC Research Corporation in Gainesville, Florida, and used as the inoculum in this study. Each strain was transferred to test tubes containing 10 mL of tryptic soy broth (**TSB**; DF 0369-17-6, Difco Laboratories, Detroit, MI) using a flame-sterilized 3-mm inoculation loop. The broth was incubated at 35°C for 24 h. After incubation, the cultures were transferred to sterile 15-mL centrifuge tubes and centrifuged (Sorvall RC-5B, Dupont Instruments, Newton, CT) at 5,000 × *g* for 10 min. The supernatant was discarded and the pellets were resuspended in 10 mL of sterile distilled water and recentrifuged. The supernatant was discarded and the pellets were resuspended in 1 mL of 3% TSB with 30% glycerol in a 2-mL cryovial (03-374-2, Corning Inc., Corning, NY), stored at −45°C, and used as the stock culture for the inoculation studies.

Twenty-four hours before conducting the study, 1 tube of each of the individual strains was removed from the freezer and allowed to thaw at room temperature for 10 min. A loopful of the cultures from each strain was transferred and mixed in test tubes containing 10 mL of 3% TSB, vortexed, and incubated at 35°C for 24 h. After incubation, each culture was centrifuged (5,000 × g for 10 min at 16°C), washed with sterile 0.1% buffered peptone water (**BPW**; DF O1897-17-4, Difco Laboratories), resuspended in 0.1% BPW (DF O1897-17-4, Difco Laboratories), mixed to form the 5-strain inoculum, and serially diluted with BPW to concentrations of 10^{-1} to 10^{-8}. Preliminary work was conducted to determine the concentration of inoculum needed to yield 4 to 5 log cfu/g on the ham samples.

Preparation of Nisin Solutions for Turkey Ham Samples

Nisaplin (Danisco, Copenhagen, Denmark) is a commercial nisin preparation that contains 2.5% nisin. Nisaplin (5.0%), 0.75% of 20 m*M* EDTA (59H03591, Sigma Chemical., St. Louis, MO), 1.0% rosemary (Herbalox Seasoning, 41-19-02, Kalsec, Kalamazoo, MI), 0.17% of 0.02 *N* HCl (7647-01-0, Fisher Scientific, Pittsburgh, PA), and 0.75% salt (S9625-500G, Sigma Chemical) were blended into 10.0% formula water and added to the turkey ham (90%). All additives were based on the final product weight. The HCl and salt were added to enhance solubility of the nisin.

Inoculation and Treatment

Commercially available turkey hams were purchased from a local supermarket and used in this study. All hams purchased had sell-by dates of at least 60 d. The turkey hams were transported to the research laboratory on ice packs and stored in a walk-in cooler at 4 ± 1°C for no longer than 24 h before using. The hams were aseptically transferred from the vacuum-packaged bag to presterilized trays and cut into approximately 0.5-cm pieces as is typical for ham used in salads and sandwiches. A final concentration of 5 log cfu/g was achieved by spraying the turkey ham pieces with an 8 log cfu/ml *L. monocytogenes* inoculum. A total volume of approximately 2 mL of the inoculum was sprayed onto the surface of the ham pieces. The volume of inoculum was determined by measuring the volume

of inoculum before and after treating the ham pieces and analyzing the ham to determine the concentration of inoculum. Inoculated samples were left to stand at room temperature for 20 min to allow for bacterial attachment to ensure a final concentration of 4 to 5 log cfu/g.

Predetermined aliquots of the inoculated chopped turkey ham were aseptically weighed and placed into labeled FoodSaver bags (FoodSaver Vacloc Roll, Tilia, San Francisco, CA). Nine treatments were prepared containing either water only (negative control, no inoculum), *L. monocytogenes* inoculum plus water (positive control), 0.5% nisin, 20 m*M* EDTA, 1% rosemary, 0.5% nisin + 20 m*M* EDTA, 0.5% nisin + 1% rosemary, 0.5% nisin + 20 m*M* EDTA + 1% rosemary, or 20 m*M* EDTA + 1% rosemary. The formulations consisted of diced turkey ham (90%) and water (10%). The actual amount of added water was adjusted based on the percentage of antimicrobials in the formulation. The treatment solutions were added directly to each bag and blended manually. This technique was chosen to ensure that each sample received the exact amount of the antimicrobial ingredients. All bags were vacuum-sealed (FoodSaver Bagvac, Tilia) and stored at 4 ± 1°C for 63 d. Duplicate samples per treatment were analyzed after 0, 7, 14, 21, 28, 35, 42, 49, 56, and 63 d for aerobic plate count (**APC**; d 0 only), *L. monocytogenes*, LAB, pH, and objective color. Aerobic plate counts were performed on d 0 only to monitor sanitation and ensure no cross contamination during sample preparation.

Microbiological and pH Analyses

Twenty-five grams of chopped turkey ham was transferred aseptically from the vacuum bag to a sterile stomacher bag (01-002-44, Fisher Scientific) that contained 225 mL of sterile 0.1% BPW (DF 01897-17-4, Difco Laboratories) and agitated for approximately 60 s. The appropriate serial dilutions were prepared by transferring 1.0 mL of the sample homogenate to 9 mL of sterile 0.1% BPW. One microliter of the dilutions was pipetted onto duplicate 3M Petrifilm for APC (6404, St. Paul, MN), modified Oxford agar (DF0225-17-0) with Oxford media supplement (DF0214-60-9) for *L. monocytogenes* and All Purpose medium with Tween 80 (DF0654-17-0) for LAB. Plates were incubated 48 h at 35 ± 1°C for APC, modified oxford agar, and All Purpose medium with Tween 80 agar plates. After incubation, colony-forming units from each plate were counted, recorded, averaged, and reported as colony-forming units per gram.

Immediately after the microbiological analyses were completed, pH values were recorded for each sample homogenate using an Accumet AB15 pH meter (Cole-Parmer, Vernon Hills, IL). The pH probe was placed into the sample homogenate and allowed to equilibrate for 1 min before the reading was taken. All pH readings were performed in duplicate followed by recording an average of the results.

Color Analysis

A portable Minolta Chroma Meter (CR310, Minolta, Ramsey, NJ) was utilized to obtain objective data for color of the RTE chopped turkey ham which employs the L*a*b* color spectra. This spectra include L* (lightness), which is a measure of total light reflected on a scale ranging from 0 = black to 100 = white (MacDougall, 2002). The a* (red-green) value is a measure of the degree of redness in the sample (MacDougall, 2002). In meat and poultry, as the value of a* increases, the sample has an increase in redness and the value decreases with decreased redness. The a* value has been used as an indicator of color stability in meat and meat products (García-Esteban et al., 2003). The b* (blue-yellow) value is a measure of the yellowness (positive values) and blue (negative values) colors of a sample (MacDougall, 2002). In meat and poultry, as the value of b* increases, the sample takes on a more yellow coloration. Decreasing b*values for meat samples usually denote that a brown color is formed.

The colorimeter was calibrated as described in the user's manual on each sampling day. To account for the packaging material, a single sheet of the film was placed over the calibration plate before the color measurements were conducted. After calibration, 2 measurements per package were taken and averaged.

Data Analysis

A complete randomized block design was employed. A total of 360 samples were analyzed (i.e., duplicate samples at 9 treatments at 10 storage days at 2 trials). The GLM program (PROC GLM) of SAS (SAS Institute, 2001) was employed to determine differences between trials, among treatments and storage days, and treatment × day interaction.

RESULTS AND DISCUSSION

Microbiological Analysis

APC. No aerobic bacteria were detected in the negative control, which indicated that the APC were less than 10 cfu/g (Table 1). All samples treated with nisin alone or nisin in combination with EDTA or rosemary had lower ($P < 0.05$) APC when compared with the positive control and hams treated with EDTA and rosemary alone or in combination. All treatments containing nisin achieved a reduction of approximately 3 log cfu/g, when compared with the positive control. Aerobic plate counts were similar ($P > 0.05$) for the positive control and hams treated with only rosemary, EDTA, and combinations of rosemary and EDTA. This observation suggested that EDTA and rosemary had no immedi-

Table 1. Mean aerobic plate counts for ready-to-eat turkey ham inoculated with *Listeria monocytogenes* and analyzed before storage

Treatment	Day 0 (log cfu/g)
Negative control	0.00[c]
Positive control	5.20[a]
0.5% Nisin	1.09[b]
20 m*M* EDTA	5.09[a]
1% Rosemary	4.79[a]
0.5% Nisin + 20 m*M* EDTA	1.71[b]
0.5% Nisin + 1% rosemary	1.55[b]
0.5% Nisin + 20 m*M* EDTA + 1% rosemary	1.21[b]
20 m*M* EDTA + 1% rosemary	4.91[a]

[a–c]Means within a column lacking a common superscript differ ($P < 0.05$).

ate antimicrobial effect against the aerobic organisms present on the turkey ham on d 0, and the microbial reductions observed could be attributed primarily to the inhibitory activity of nisin rather than EDTA or rosemary. Aerobic counts were conducted only on d 0 before vacuum-packaging because the predominant microflora in vacuum-packaged meat is anaerobic bacteria and facultative anaerobes. The initial aerobic counts were conducted to determine the initial total microbial populations of the meat.

***Listeria monocytogenes* Analysis.** The hams treated with nisin alone, nisin in combination with rosemary, and nisin in combination with rosemary and EDTA experienced an initial 4 log cfu/g reduction in *L. monocytogenes* on d 0 when compared with the positive control (Table 2). The 4 log cfu/g reduction continued for the hams treated with nisin alone through d 14. An initial 3 log cfu/g reduction in *L. monocytogenes* was observed for hams treated with a combination of nisin and EDTA on d 0 when compared with the positive control. All hams treated with nisin alone or in combination with rosemary and EDTA resulted in lower ($P < 0.05$) *L. monocytogenes* counts through 49 d of storage when compared with hams treated with EDTA or rosemary alone or in combination. Anti-*Listeria* effects of nisin treatments continued through 63 d. These findings revealed that the use of nisin alone or in combination with EDTA or rosemary, or both, had a bactericidal effect against *L. monocytogenes* populations.

In general, *L. monocytogenes* counts were similar ($P > 0.05$) for the positive control and ham treated with EDTA or rosemary alone, or with EDTA combined with rosemary during 63 d of storage. Neither EDTA nor rosemary inhibited *L. monocytogenes* during 63 d of storage. The use of rosemary or EDTA did not result in increased antimicrobial activity when compared with the use of nisin alone. In contrast, researchers have reported an increase in the antimicrobial activity of nisin against gram-positive (Kalachyanand et al., 1992) and gram-negative bacteria (Stevens et al., 1992; Cutter and Siragusa, 1995) when used in combination with membrane-disrupting agents such as EDTA.

The lack of antimicrobial activity by EDTA may be attributed largely to membrane structure differences between gram-positive and gram-negative bacteria. In gram-negative organisms, it has been shown that EDTA can act to destabilize the cell membrane of bacteria by complexing divalent cations, such as Ca^{2+} and Mg^{2+}, which act as salt bridges between membrane macromolecules, such as lipopolysaccharide (Shelef and Seiter, 1993). Gram-negative bacteria have an outer membrane that covers a thin layer of peptidoglycan. The primary target of EDTA is the lipids and lipid proteins present in the outer membrane of gram-negative bacteria (Doyle et al., 2001). In contrast, gram-positive bacteria have a thick layer of peptidoglycan (a sugar-protein shell) that confers resistance to physical disruption (Farber and Peterkin, 1999), which might have attributed significantly to the lack of anti-*Listeria* properties of EDTA against *L. monocytogenes* in this study. Similar findings have been reported by Parente et al. (1998) and Shanshan and Mustapha (1999) wherein EDTA exhibited no significant inhibition against gram-positive bacteria, and when combined with nisin, EDTA reduced the antimicrobial effect of nisin.

In this study, rosemary extract alone was not effective in controlling *L. monocytogenes* growth. Del Campo et

Table 2. *Listeria monocytogenes* counts for ready-to-eat turkey ham inoculated with *L. monocytogenes* and stored at 4 ± 1°C for 63 d

Treatment	Storage day									
	0	7	14	21	28	35	42	49	56	63
	(log cfu/g)									
Negative control	0.00[c,v]	0.00[d,v]	0.00[f,v]	0.00[d,v]	0.00[e,v]	0.00[c,v]	0.00[d,v]	0.00[e,v]	0.00[e,v]	0.00[d,v]
Positive control	5.33[a,v]	4.78[a,vw]	4.87[a,wx]	4.33[a,yz]	4.33[a,yz]	4.14[a,yz]	3.76[a,z]	4.44[a,xy]	4.26[a,yz]	3.91[a,yz]
Nisin	0.91[bc,xy]	0.13[d,y]	0.73[ef,xy]	0.76[cd,xy]	2.42[d,v]	1.68[b,xy]	1.00[cd,xy]	2.36[c,vw]	2.04[d,wx]	2.39[bc,v]
EDTA	4.98[a,v]	4.38[a,w]	4.00[bc,wx]	4.14[a,w]	3.93[ab,wx]	3.63[a,xy]	3.18[ab,y]	3.41[b,y]	3.18[bc,y]	3.43[ab,y]
Rosemary	5.18[a,v]	4.48[a,wx]	4.60[ab,w]	4.06[a,xy]	3.92[ab,yz]	3.70[a,yz]	3.52[a,z]	3.87[ab,yz]	3.54[ab,z]	3.89[a,yz]
Nisin + EDTA	1.60[b,wx]	0.87[cd,wx]	0.45[f,x]	0.37[d,x]	2.72[c,wx]	1.71[b,wx]	0.77[d,wx]	1.65[d,wx]	2.71[cd,v]	1.99[c,vw]
Nisin + rosemary	1.13[b,w]	1.12[c,w]	1.83[d,vw]	2.10[b,vw]	2.04[d,vw]	1.99[b,vw]	1.94[c,vw]	2.31[c,vw]	2.55[cd,v]	2.66[abc,v]
Nisin + EDTA + rosemary	1.22[b,xy]	1.16[c,y]	1.43[de,xy]	1.60[bc,xy]	2.39[d,vw]	2.01[b,xy]	2.02[bc,xy]	2.07[cd,xy]	2.30[d,wx]	2.59[abc,v]
EDTA + rosemary	4.90[a,v]	3.13[b,wx]	3.66[c,w]	3.69[a,w]	3.28[b,wx]	3.66[a,w]	3.24[a,wx]	3.41[b,wx]	3.52[ab,w]	3.22[abc,x]

[a–f]Means within a column lacking a common superscript differ ($P < 0.05$).

[v–z]Means within a row lacking a common superscript differ ($P < 0.05$).

Table 3. Lactic acid bacteria counts for ready-to-eat turkey ham inoculated with *Listeria monocytogenes* and stored at 4 ± 1°C for 63 d

Treatment	Storage day									
	0	7	14	21	28	35	42	49	56	63
	(log cfu/g)									
Negative control	$2.58^{b,y}$	$5.14^{a,x}$	$5.38^{ab,wx}$	$5.31^{a,wx}$	$6.54^{a,v}$	$6.13^{a,vw}$	$5.77^{a,wx}$	$5.27^{a,wx}$	$5.77^{ab,wx}$	$6.04^{a,vw}$
Positive control	$5.32^{a,v}$	$5.64^{a,v}$	$5.93^{a,v}$	$5.67^{a,v}$	$5.74^{ab,v}$	$5.60^{a,v}$	$5.75^{a,v}$	$5.62^{a,v}$	$5.64^{ab,v}$	$5.36^{a,v}$
Nisin	$0.25^{d,z}$	$1.13^{c,z}$	$2.29^{c,yz}$	$3.08^{cd,xy}$	$5.08^{bc,vw}$	$5.43^{a,vw}$	$4.32^{b,wx}$	$5.46^{a,vw}$	$6.02^{a,v}$	$4.96^{a,vw}$
EDTA	$4.92^{a,v}$	$4.46^{a,vw}$	$4.00^{b,wx}$	$4.19^{b,wx}$	$4.51^{c,vw}$	$3.75^{b,xy}$	$3.56^{b,y}$	$3.49^{b,y}$	$3.49^{c,y}$	$3.68^{b,xy}$
Rosemary	$5.06^{a,w}$	$5.57^{a,vw}$	$5.44^{ab,vw}$	$5.16^{a,w}$	$5.52^{b,vw}$	$5.46^{a,vw}$	$5.53^{a,vw}$	$5.78^{a,v}$	$4.96^{b,w}$	$5.53^{a,vw}$
Nisin + EDTA	$1.26^{c,w}$	$0.80^{c,w}$	$0.86^{c,w}$	$2.04^{e,vw}$	$1.81^{e,vw}$	$2.07^{c,vw}$	$1.95^{c,vw}$	$1.68^{c,vw}$	$2.94^{cd,v}$	$2.15^{d,vw}$
Nisin + rosemary	$1.61^{bc,y}$	$3.04^{b,x}$	$4.08^{b,wx}$	$3.83^{bc,x}$	$5.73^{ab,v}$	$5.72^{a,v}$	$5.38^{a,v}$	$5.10^{a,vw}$	$5.08^{ab,vw}$	$5.14^{a,vw}$
Nisin + EDTA + rosemary	$0.97^{cd,w}$	$1.87^{bc,vw}$	$2.28^{c,v}$	$2.40^{de,v}$	$2.58^{e,v}$	$2.54^{c,v}$	$2.34^{c,v}$	$2.32^{c,v}$	$2.30^{d,v}$	$2.31^{cd,v}$
EDTA + Rosemary	$4.87^{a,v}$	$4.47^{a,vw}$	$4.20^{b,xy}$	$4.31^{b,wx}$	$3.51^{d,z}$	$4.40^{b,vw}$	$3.67^{b,yz}$	$3.70^{b,yz}$	$3.72^{c,yz}$	$3.37^{bc,z}$

[a–e]Means within a column lacking a common superscript differ ($P < 0.05$).

[v–z]Means within a row lacking a common superscript differ ($P < 0.05$).

al. (2000) found that gram-positive bacteria were the most sensitive to rosemary extracts when incorporated into foods with low fat and low protein content. The product used in this study was rich in protein, which may have contributed to the limited anti-*Listeria* effect observed. Pandit and Shelef (1994) reported that the use of encapsulated rosemary oil was much more effective than standard rosemary essential oil extract against *L. monocytogenes* in pork liver sausage. This suggested that the method of direct application of rosemary to the product may have interfered with its antimicrobial properties in this study.

LAB Analysis. Ham treated with nisin alone had lower ($P < 0.05$) LAB during 0 through 28 d when compared with the negative control (Table 3). The EDTA treatment alone and in combination with rosemary resulted in lower ($P < 0.05$) LAB for hams on d 21 and through 63 d. The most pronounced antimicrobial effect was observed on d 0 through 63 for hams treated with nisin in combination with EDTA, and nisin in combination with EDTA and rosemary where LAB counts remained lower ($P < 0.05$) than the negative control throughout the 63-d storage period. Limited and minimal antimicrobial effect was observed for hams treated with rosemary only and nisin in combination with rosemary. These observations revealed that EDTA alone and in combination with rosemary or nisin, or both (i.e., rosemary and nisin), exhibited significant antimicrobial properties against LAB.

pH Analysis

Turkey ham treated with EDTA alone or in combination with nisin or rosemary, or both, had higher ($P < 0.05$) pH values than all other treatments by d 63 (Table 4), which is indicative of the ability of EDTA to function as a food preservative and pH stabilizer (Winter, 1999). In general, the pH was similar for turkey ham treated with nisin, rosemary, nisin combined with rosemary, and the positive and negative controls during storage.

Over time, there was a slight decrease in the pH values for treatments that did not contain EDTA. This may be attributed to the production of various compounds such as acidic metabolites and carbonic acids by spoilage bacteria (Doyle et al., 2001). It was revealed that EDTA, under the conditions in this study, had an effect on the pH value due to its buffering capacity. The data demonstrated that changes in pH were consistent with changes in LAB population, which suggested that the pH effect of EDTA could be considered a key factor for LAB population reduction observed in this study.

Table 4. pH measurements for ready-to-eat turkey ham inoculated with *Listeria monocytogenes* and stored at 4 ± 1°C for 63 d

Treatment	Storage day									
	0	7	14	21	28	35	42	49	56	63
Negative control	$5.72^{bc,vw}$	$5.34^{d,wxy}$	$5.00^{c,y}$	$5.28^{c,xy}$	$5.42^{c,vwx}$	$5.74^{bc,v}$	$5.51^{bc,vwx}$	$5.16^{d,xy}$	$5.08^{e,xy}$	$5.29^{d,wxy}$
Positive control	$5.79^{b,v}$	$5.45^{cd,vw}$	$5.25^{bc,vw}$	$5.42^{c,vw}$	$5.16^{c,w}$	$5.65^{cd,vw}$	$5.36^{bc,vw}$	$5.18^{cd,w}$	$5.62^{bc,vw}$	$5.48^{cd,vw}$
Nisin	$5.86^{ab,v}$	$5.82^{a,v}$	$5.69^{a,v}$	$5.82^{ab,v}$	$5.97^{ab,v}$	$5.87^{ab,v}$	$5.72^{ab,v}$	$5.59^{bc,v}$	$5.57^{cd,v}$	$5.74^{bc,v}$
EDTA	$5.48^{d,y}$	$5.66^{bc,xy}$	$5.73^{a,xy}$	$5.85^{ab,wx}$	$6.03^{ab,vw}$	$6.11^{ab,v}$	$5.97^{a,vwx}$	$5.99^{ab,vw}$	$5.99^{ab,vw}$	$6.10^{ab,v}$
Rosemary	$5.95^{a,v}$	$5.54^{bc,vw}$	$5.39^{ab,w}$	$5.24^{c,w}$	$5.31^{c,w}$	$5.48^{d,w}$	$5.31^{c,w}$	$5.22^{cd,w}$	$5.43^{de,w}$	$5.50^{cd,vw}$
Nisin + EDTA	$5.49^{d,z}$	$1.59^{ab,xy}$	$5.73^{a,y}$	$5.83^{ab,wx}$	$6.09^{a,v}$	$6.17^{a,v}$	$5.97^{a,wx}$	$5.99^{ab,vw}$	$6.09^{a,v}$	$6.11^{ab,v}$
Nisin + rosemary	$5.78^{b,vw}$	$5.75^{a,v}$	$5.57^{ab,wx}$	$5.52^{bc,wx}$	$5.60^{bc,vwx}$	$5.59^{cd,wx}$	$5.31^{c,xy}$	$5.37^{cd,xy}$	$5.14^{e,y}$	$5.56^{cd,wx}$
Nisin + EDTA + rosemary	$5.46^{d,z}$	$5.73^{ab,z}$	$5.80^{a,yz}$	$5.89^{a,xyz}$	$6.03^{ab,vwx}$	$6.12^{ab,vw}$	$6.07^{a,vwx}$	$6.01^{a,wxy}$	$6.11^{a,vwx}$	$6.26^{a,v}$
EDTA + rosemary	$5.58^{cd,y}$	$5.74^{ab,xy}$	$5.71^{a,xy}$	$5.78^{ab,wx}$	$6.07^{a,v}$	$6.07^{ab,v}$	$6.02^{a,v}$	$6.04^{a,v}$	$5.94^{ab,vw}$	$6.12^{ab,v}$

[a–e]Means within a column lacking a common superscript differ ($P < 0.05$).

[v–z]Means within a row lacking a common superscript differ ($P < 0.05$).

Table 5. L* color values for ready-to-eat turkey ham inoculated with *Listeria monocytogenes* and stored at 4 ± 1°C for 63 d

	Storage day									
Treatment	0	7	14	21	28	35	42	49	56	63
Negative control	62.07^{ab,wx}	58.72^{bc,x}	62.20^{bc,wx}	63.59^{a,w}	62.94^{a,w}	61.43^{ab,wx}	60.98^{a,wx}	60.68^{ab,wx}	62.98^{bc,w}	61.78^{ab,wx}
Positive control	61.56^{ab,x}	61.44^{a,x}	64.65^{a,w}	63.32^{a,wx}	63.38^{a,wx}	62.66^{ab,wx}	62.92^{a,wx}	61.96^{a,wx}	61.88^{d,wx}	63.31^{ab,wx}
Nisin	61.44^{ab,w}	60.12^{ab,w}	61.53^{c,w}	61.31^{ab,w}	61.24^{a,w}	61.11^{ab,w}	61.13^{a,w}	60.05^{ab,w}	61.76^{d,w}	61.34^{b,w}
EDTA	60.11^{b,xy}	58.61^{bc,y}	60.31^{c,xy}	60.42^{ab,xy}	61.92^{a,x}	64.43^{a,w}	61.59^{a,x}	61.95^{a,x}	61.34^{d,x}	61.33^{ab,x}
Rosemary	61.44^{ab,w}	61.25^{a,w}	61.02^{c,w}	61.07^{ab,w}	63.61^{a,w}	62.66^{ab,w}	60.39^{a,w}	61.36^{ab,w}	64.06^{a,w}	61.03^{b,w}
Nisin + EDTA	61.08^{ab,wx}	59.10^{b,x}	61.31^{c,wx}	60.56^{ab,wx}	61.42^{a,wx}	60.96^{ab,wx}	60.62^{a,wx}	61.02^{ab,wx}	58.59^{e,x}	63.20^{ab,w}
Nisin + rosemary	61.46^{ab,xyz}	60.28^{ab,xyz}	62.50^{ab,wx}	62.46^{ab,wx}	61.83^{a,xyz}	59.42^{b,z}	62.09^{a,wxy}	59.71^{b,z}	63.93^{ab,w}	62.85^{ab,wx}
Nisin + EDTA + rosemary	60.48^{b,x}	59.73^{ab,x}	64.07^{ab,w}	59.53^{b,x}	61.84^{a,wx}	61.32^{ab,wx}	61.40^{a,wx}	60.69^{ab,x}	62.38^{cd,wx}	62.43^{ab,wx}
EDTA + rosemary	63.11^{a,wx}	57.10^{c,z}	61.36^{c,wxy}	61.64^{ab,wxy}	62.69^{a,wx}	61.24^{ab,wxy}	60.22^{a,xy}	59.28^{b,yz}	61.40^{d,wxy}	64.69^{a,w}

$^{a–e}$Means within a column lacking a common superscript differ ($P < 0.05$).

$^{w–z}$Means within a row lacking a common superscript differ ($P < 0.05$).

Objective Color Measurement for L*a*b* values

L* Values. No significant treatment × day interaction was revealed for L* color values. The significant day effect was due to differences in L* values among some of the treatments on all days except d 28 and 42 (Table 5). The L* values oscillated between a range of 58.0 to 64.0 for all treatments. Typical L* values for cured sausage and ham range from 50.0 to 64.0 (Zhang et al., 2007; Paxton et al., 2009), which accounted for all values in Table 5. However, Sheridan et al. (2007) cautioned of the difficulty in determining typical color values for muscle food products because of color variability within muscles.

a* Values. Except for d 7, ham treated with rosemary had higher ($P < 0.05$) a* values through 63 d when compared with a* values for d 0 (Table 6). The data demonstrated a similar effect for hams treated with nisin plus rosemary where, except for d 21, hams treated with nisin plus rosemary had higher ($P < 0.05$) a* values through 63 d when compared with a* values for d 0. These observations suggested that the lack of color change might have been due to stabilization of the cured myoglobin nitrosohemochrome pigment in the cured chopped ham product by rosemary. However, to confirm this statement, it would be necessary to analyze the pigment states in the cured ham during storage. The a* values were similar ($P > 0.05$) for the positive and negative controls and hams treated with EDTA alone, and EDTA plus rosemary. The a* values for hams treated with nisin alone increased ($P < 0.05$) after 28 d and through 49 d when compared with a* values on d 0. The a* values for hams treated with a combination of nisin, EDTA and rosemary, and nisin plus EDTA decreased ($P < 0.05$) on d 56 and 63, respectively. Except for this observation, the a* values for all hams remained similar ($P > 0.05$) through 63 d of storage when compared with d 0.

b* Values. No significant treatment × day interaction was revealed. The b* values ranged from 6.25 to 9.68 (Table 7). The significant day effect was due primarily to differences ($P < 0.05$) revealed between b* values for the negative control and ham treatments on d 0, 7, 21, and 56. The b* values for hams treated with rosemary, nisin plus rosemary, and nisin plus EDTA plus rosemary were higher ($P < 0.05$) when compared with the negative control. Except for hams treated with nisin, EDTA, and rosemary and the positive control on d 7, all treatments resulted in higher ($P < 0.05$) b* values, and except for hams treated with EDTA and nisin plus EDTA, all treatments resulted in higher ($P < 0.05$) b* values when compared with the negative control. Hams treated with the nisin, EDTA, plus rosemary had higher b* values when compared with all other treatments and the negative control. The b*

Table 6. a* color values for ready-to-eat turkey ham inoculated with *Listeria monocytogenes* and stored at 4 ± 1°C for 63 d

	Storage day									
Treatment	0	7	14	21	28	35	42	49	56	63
Negative control	17.46^{a,yz}	17.10^{a,z}	17.96^{ab,yz}	17.85^{a,yz}	18.08^{a,xyz}	18.59^{a,wxy}	19.56^{a,w}	17.59^{a,yz}	18.55^{abc,wxy}	19.34^{a,wx}
Positive control	16.36^{ab,wx}	16.79^{a,wx}	18.79^{a,w}	17.88^{a,wx}	18.13^{a,wx}	19.03^{a,w}	18.29^{a,wx}	17.91^{a,wx}	19.22^{a,w}	13.84^{abc,x}
Nisin	16.59^{ab,yz}	17.12^{a,y}	17.00^{ab,y}	17.57^{a,wxy}	18.83^{a,w}	18.52^{ab,wx}	18.52^{a,wx}	15.53^{b,z}	17.73^{cd,wxy}	17.51^{ab,xy}
EDTA	15.82^{b,wx}	14.05^{b,x}	17.01^{ab,wx}	15.64^{a,wx}	13.47^{c,x}	15.28^{c,wx}	14.51^{b,x}	16.66^{ab,wx}	18.67^{ab,w}	16.03^{ab,wx}
Rosemary	15.51^{b,z}	16.51^{a,yz}	18.41^{ab,w}	17.68^{a,wx}	17.62^{a,wx}	17.25^{abc,xy}	18.61^{a,w}	17.56^{a,wxy}	16.81^{e,xy}	18.43^{a,w}
Nisin + EDTA	16.62^{ab,wx}	16.65^{a,wx}	14.34^{c,wx}	15.94^{a,wx}	14.78^{bc,wx}	16.08^{bc,wx}	13.62^{b,wxy}	12.52^{c,xy}	17.11^{de,w}	9.06^{c,y}
Nisin + rosemary	16.65^{ab,y}	16.50^{a,y}	17.75^{ab,wx}	17.17^{a,xy}	18.15^{a,wx}	18.53^{a,w}	18.29^{a,w}	17.83^{a,wx}	18.51^{abc,w}	18.30^{a,w}
Nisin + EDTA + rosemary	15.91^{b,w}	15.85^{a,w}	16.66^{b,w}	17.18^{a,w}	16.94^{ab,w}	17.55^{abc,w}	16.50^{ab,w}	17.59^{a,w}	13.11^{f,x}	11.88^{bc,x}
EDTA + rosemary	16.56^{ab,wx}	16.99^{a,wx}	18.49^{ab,w}	14.70^{a,x}	18.06^{a,w}	18.20^{ab,w}	18.10^{a,w}	16.92^{ab,wx}	18.30^{bc,w}	14.05^{abc,x}

$^{a–f}$Means within a column lacking a common superscript differ ($P < 0.05$).

$^{w–z}$Means within a row lacking a common superscript differ ($P < 0.05$).

Table 7. b* color values for ready-to-eat turkey ham inoculated with *Listeria monocytogenes* and stored at 4 ± 1°C for 63 d

	Storage day									
Treatment	0	7	14	21	28	35	42	49	56	63
Negative control	$7.05^{b,wx}$	$6.25^{c,x}$	$7.97^{ab,wx}$	$7.16^{d,wx}$	$7.96^{ab,wx}$	$7.64^{a,wx}$	$8.42^{ab,w}$	$8.35^{abc,w}$	$7.89^{bc,wx}$	$8.77^{a,w}$
Positive control	$7.63^{b,xyz}$	$6.73^{bc,z}$	$7.77^{ab,xy}$	$8.97^{abc,w}$	$6.85^{b,yz}$	$6.97^{a,yz}$	$7.53^{b,xyz}$	$6.99^{c,yz}$	$7.58^{bc,xyz}$	$8.41^{a,wx}$
Nisin	$7.73^{b,wx}$	$6.98^{b,wx}$	$6.65^{b,x}$	$8.10^{abc,w}$	$7.81^{ab,wx}$	$7.08^{a,wx}$	$7.62^{b,wx}$	$8.18^{abc,w}$	$7.46^{c,wx}$	$7.82^{a,wx}$
EDTA	$7.59^{b,y}$	$7.20^{ab,y}$	$8.25^{a,wxy}$	$7.73^{cd,xy}$	$9.15^{a,w}$	$7.96^{a,wxy}$	$9.00^{ab,wx}$	$8.61^{bc,y}$	$8.01^{bc,wxy}$	$9.21^{a,w}$
Rosemary	$9.21^{a,w}$	$7.87^{a,w}$	$8.02^{ab,w}$	$9.21^{abc,w}$	$7.89^{ab,w}$	$8.61^{a,w}$	$9.55^{a,w}$	$8.16^{abc,w}$	$8.41^{ab,w}$	$9.37^{a,w}$
Nisin + EDTA	$7.38^{b,x}$	$7.33^{ab,x}$	$8.50^{a,wx}$	$7.96^{bcd,wx}$	$8.68^{a,wx}$	$8.78^{a,wx}$	$9.27^{a,w}$	$8.94^{ab,wx}$	$8.05^{bc,wx}$	$9.68^{a,w}$
Nisin + rosemary	$9.54^{a,w}$	$7.30^{ab,y}$	$8.55^{a,wxy}$	$8.23^{abc,wxy}$	$7.52^{ab,xy}$	$8.86^{a,wx}$	$7.42^{b,xy}$	$7.57^{bc,xy}$	$8.12^{bc,wxy}$	$8.16^{a,wxy}$
Nisin + EDTA + rosemary	$9.50^{a,wx}$	$6.85^{bc,z}$	$7.21^{ab,yz}$	$9.55^{a,wx}$	$8.62^{a,wx}$	$8.57^{a,wxy}$	$8.19^{ab,xyz}$	$8.53^{abc,wxy}$	$9.02^{a,wx}$	$9.82^{a,w}$
EDTA + rosemary	$8.20^{ab,wxy}$	$7.05^{b,y}$	$7.26^{ab,xy}$	$9.30^{ab,wx}$	$7.63^{ab,wxy}$	$7.35^{a,wxy}$	$7.51^{b,wxy}$	$9.56^{a,w}$	$7.26^{c,xy}$	$8.68^{a,wxy}$

[a–d]Means within a column lacking a common superscript differ ($P < 0.05$).

[w–z]Means within a row lacking a common superscript differ ($P < 0.05$).

values reported in this study were not indicative of off-color or discoloration in the samples.

In conclusion, this study revealed that *L. monocytogenes* growth was retarded when the chopped ham was treated with nisin alone and nisin in combination with either EDTA or rosemary or both (i.e., EDTA plus rosemary). No synergistic effect was demonstrated when nisin was combined with EDTA or rosemary. The treatment of ham with EDTA alone or in combination with rosemary had limited or no effect on reducing *L. monocytogenes.* The EDTA treatment alone and in combination with nisin and rosemary, and the nisin alone treatments, retarded ($P < 0.05$) the growth of LAB. Rosemary alone had limited effect on the LAB.

Comparison of microbial counts for APC and LAB revealed similar counts for all treatments on d 0. This observation suggested that the initial microflora on the hams on d 0 was composed primarily of aerobic and facultative anaerobic bacteria (which included LAB and *L. monocytogenes*).

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