Inactivation of *Listeria monocytogenes* on Frankfurters by Monocaprylin Alone or in Combination with Acetic Acid

MARILYN GARCIA, MARY ANNE ROSHNI AMALARADJOU, MANOJ KUMAR MOHAN NAIR, THIRUNAVUKKARASU ANNAMALAI, SUMAN SURENDRANATH, SEOK LEE, THOMAS HOAGLAND, DAVID DZUREC, CAMERON FAUSTMAN, AND KUMAR VENKITANARAYAN

Department of Animal Science, Unit-4040, University of Connecticut, Storrs, Connecticut 06269, USA

MS 06-547: Received 20 October 2006/Accepted 10 February 2007

**ABSTRACT**

The antilisterial activity of monocaprylin (MC) and its combination with acetic acid (AA) on frankfurters was investigated. Each frankfurter was surface inoculated with a three-strain mixture of *Listeria monocytogenes* to obtain an inoculation level of 4.0 log CFU per frankfurter, and then dipped for 35 s in sterile deionized water (45 or 50°C) containing 1% ethanol (control), 50 mM MC plus 1% ethanol, 1% AA plus 1% ethanol, or 50 mM MC plus 1% AA plus 1% ethanol. Samples were vacuum packaged, stored at 4°C for 77 days, and analyzed for *L. monocytogenes*. Sensory odor and color of frankfurters were evaluated using a 9-point hedonic scale. Color was also objectively measured using the Minolta Chroma Meter. From day 0 to day 77, population counts of *L. monocytogenes* on frankfurters dipped in antimicrobial solutions at 50°C were consistently lower than the control counts. Similar results were observed for samples treated at 45°C. However, *L. monocytogenes* grew readily on control samples at both temperatures. Dipping of frankfurters in antimicrobial solutions (45 or 50°C) significantly reduced (*P* < 0.05) the populations of *L. monocytogenes*. After 70 days of storage, *L. monocytogenes* was completely killed in samples dipped in MC+AA solution at 50°C. The antimicrobial treatments did not affect the odor or color of the samples (*P* > 0.05). Overall, results indicated that dipping of frankfurters with MC reduced *L. monocytogenes*, and inclusion of AA further enhanced MC antilisterial activity, without any negative effect on odor or color.

---

*Listeria monocytogenes* is a pathogen of great concern in ready-to-eat (RTE) meat and poultry products. Several outbreaks of listeriosis have been associated with the consumption of contaminated hot dogs and turkey (31). Several methods are applied during meat processing to inhibit pathogen growth including the addition of sodium chloride, sodium nitrite, smoking, cooking, and packaging under modified atmosphere or vacuum conditions. The ubiquitous nature of *L. monocytogenes* and its ability to form biofilms in food-processing facilities provide the potential for cooked products to be recontaminated during peeling and packaging steps. Once *L. monocytogenes* has contaminated a product postprocessing, it can survive and grow in the presence or absence of oxygen (8), in the presence of NaCl (17, 29) and sodium nitrite (15, 36), and at refrigerated temperatures (7, 13, 22).

The U.S. Food Safety and Inspection Service (FSIS) has reported that listeriosis is rare (<0.1% of total foodborne illness) when compared with infections caused by other pathogens such as *Campylobacter* and *Salmonella*, but *L. monocytogenes* can cause a more severe illness. It has the highest rate of hospitalization among foodborne pathogens and a high fatality rate (31). The federal government has taken several actions to safeguard public health, which include implementation of a “zero tolerance policy” in RTE meats and poultry products. It is also a requirement for all meat plants producing RTE meat and poultry products under FSIS alternatives 1 and 2 of the *Listeria monocytogenes* regulations to include additional post-lethality treatments of the products, which may include use of antimicrobial agents (33).

Several generally recognized as safe (GRAS) compounds, including organic acids, have demonstrated differential effectiveness against *L. monocytogenes* in RTE meats. Food-grade organic acids are applied in RTE meats either as active ingredients in dipping solutions (22) or are included in the product formulation (10). Studies have shown that combinations of organic acids or their salts and other antimicrobials were more bactericidal and bacteriostatic than when these preservatives were used alone (22).

Fatty acids and their monoglycerides are potential antimicrobials that can be used in food systems (27). They have broad-spectrum antimicrobial activity in the culture media against enveloped viruses, *Chlamydia*, and gram-positive and -negative bacteria (3, 4, 8, 13, 22, 23, 33). Their antibacterial efficacies are highly dependent on the nature and composition of the growth medium. They were found to be highly inhibitory when used in synthetic, laboratory media (3, 20, 23, 37), whereas only a minimal inhibitory effect was observed in food (37). However, a recent study from our laboratory (18) has shown that caprylic acid and monocaprylin were effective in killing *Escherichia coli* O157:H7 and *L. monocytogenes* in fluid milk at different storage temperatures. Caprylic acid is an eight-carbon fatty acid present in breast milk, bovine milk (14), and...
coconut oil (26), and has been approved as GRAS by the U.S. Food and Drug Administration (CFR 184.1025). Monorylin (MC) is a monoglyceride ester of caprylic acid. Since the fatty acid carboxyl group is esterified directly to the glycerol backbone, MC maintains its antimicrobial activity across a wide pH range (12).

In this study, we examined the efficacy of MC alone or its combination with acetic acid (AA) as an antimicrobial dip for killing L. monocytogenes on pork-beef frankfurters that were subsequently vacuum packaged and kept at 4°C for 77 days. Sensory evaluation was also conducted to determine the effect of MC on color and odor of the treated frankfurters.

**MATERIALS AND METHODS**

**Bacterial culture.** Three strains of L. monocytogenes, including Scott A (human), LM 101 (salami), and ATCC 19115 (human), were used in the study. All the strains were obtained from Dr. Michael P. Doyle at the Center for Food Safety, University of Georgia, Griffin. The three strains of L. monocytogenes were cultured individually in 100 ml of tryptic soy broth (TSB; Difco, Becton Dickinson, Sparks, Md.) at 37°C for 24 h. The cultures were sedimented by centrifugation (3,600 × g for 15 min), washed twice, and resuspended in 10 ml of sterile phosphate-buffered saline (PBS, pH 7.2). Equal portions of the three strains were combined, diluted appropriately, and the resulting suspension was used as inoculum. The bacterial count of the three-strain mixture of L. monocytogenes was confirmed by plating 0.1 ml portions of appropriate dilutions on tryptic soy agar (TSA; Difco, Becton Dickinson) plates with incubation at 37°C for 24 h.

**Frankfurters.** Fresh pork-beef skinless frankfurters (20% fat) were purchased from a local meat processor. The frankfurters contained water, salt, dextrose, corn syrup, flavorings, spice, and chemical preservatives, including potassium lactate and sodium diacetate. Prior to inoculation, representative samples were placed in a sterile sampling bag (one piece per sampling bag) containing distilled water, and the pH was measured.

**Inoculation and treatments.** The skinless frankfurters were inoculated with the three-strain mixture of L. monocytogenes (1). Each peeled frankfurter was placed aseptically in a sterile sampling bag and surface inoculated with 500 μl of the three-strain mixture of L. monocytogenes (7.0 log CFU/ml) to obtain an inoculation level of 4.0 log CFU per frankfurter. The inoculum was spread uniformly over the entire surface of the frankfurter by swirling the sample by hand for 30 s. After inoculation, the frankfurters were placed in a sterile, dry container to allow for bacterial attachment (15 min at 5°C), and were then immersed for 35 s in 500 ml of different dipping solutions held at 45 or 50°C. MC (Nu-Check Prep, Inc., Elysian, Minn.) was dissolved in 1% ethanol prior to use. The dipping solutions included control (sterile deionized water plus 1% ethanol), MC (deionized water plus 50 mM MC plus 1% ethanol), AA (deionized water plus 1% AA plus 1% ethanol), and MC+AA (deionized water plus 50 mM MC plus 1% AA plus 1% ethanol). After immersion, the samples were drained (<30 s), vacuum packaged (Supervac, Smith Equipment Co., Clifton, N.J.; 3-ml bags, Koch Bag & Supply Co., Kansas City, Mo.), and stored at 4°C. L. monocytogenes counts on each frankfurter were determined on days 0, 1, 3, 5, and 7 of storage and thereafter every week through 77 days.

**Enumeration of L. monocytogenes.** On each sampling day, a frankfurter from each vacuum package was transferred aseptically to a sterile sampling bag containing 50 ml of sterile PBS and homogenized in a stomacher for 1 min. A volume of 100 μl of meat homogenate was plated directly or after serial dilution (1:10 in PBS) on duplicate Oxford agar plates. The plates were incubated at 37°C for 48 h before counting the colonies. Enrichment was performed by transferring 1 ml of the meat homogenate to 100 ml of TSB and incubating at 37°C for 24 h. The culture was then streaked on Oxford agar, incubated at 37°C for 48 h, and observed for black colonies. Representative colonies on Oxford agar were confirmed for L. monocytogenes with the API-Listeria test kit (bioMérieux, Inc., Hazelwood, Mo.). In addition, samples of the different treatment solutions after dipping the frankfurters were enriched in 100 ml of TSB at 37°C for 24 h for detection of L. monocytogenes.

**pH Determination.** The pH of control and treated frankfurters was determined using a pH meter (model 720, Orion Research, Inc., Boston, Mass.) standardized against pH 4.0 and pH 7.0 buffers. Thirty grams of sample was blended with 90 ml of distilled water, and the pH was measured.

**Color evaluation.** Consumer acceptability testing of treated frankfurters was conducted at the University of Connecticut Dairy Store. A total of 25 untrained panelists were asked to rate their relative liking of randomly coded frankfurter samples based on color and odor. A 9-point hedonic rating scale was used for evaluation, with 9 = like extremely and 1 = dislike extremely.

**Objective analysis of color.** Color was determined using the measurement values of Commission Internationale de l’Eclairage, L*, a*, and b* (6), using a Minolta Chroma Meter CR 200 (Osaka, Japan) calibrated to standard white plate. The illuminant used was C (6,774 K), and the measuring area was 8 mm. The reported values were the average from three different surface locations on each frankfurter.

**Statistical analysis.** The design used was a completely randomized design (16 factorial). Factors included four treatments and 16 sampling points (days 0, 1, 3, 5, 7, 12, 21, 28, 35, 42, 49, 56, 63, 70, and 77). Three frankfurters (n = 3) were included at each time point for each treatment and control, and the entire study was duplicated. Data were analyzed using analysis of variance and mean separation procedures of the Statistical Analysis Software (SAS Institute, Inc., Cary, N.C.). The model statement accounted for variation due to different factors and interactions. Differences among means were detected at the 5% level with the least significant difference test.

**RESULTS AND DISCUSSION**

Selective enumeration media such as Oxford agar can potentially inhibit the full recovery of heat- and acid-stressed cells of L. monocytogenes. Therefore, preliminary experiments were conducted to compare the populations of surviving L. monocytogenes on frankfurters after exposure to MC and AA at 50°C on Oxford agar and TSA plates. The counts of L. monocytogenes recovered on TSA did not differ significantly (P > 0.05) when compared with counts obtained on Oxford agar (data not shown). Therefore, Oxford agar was used as the enumeration medium of L. monocytogenes on frankfurters.
TABLE 1. pH values of dipping solutions and treated frankfurters

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dipping solutions</th>
<th>Frankfurters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.44 ± 0.03 A</td>
<td>6.35 ± 0.09 A</td>
</tr>
<tr>
<td>MC</td>
<td>5.28 ± 0.03 b</td>
<td>6.34 ± 0.09 A</td>
</tr>
<tr>
<td>MC+AA</td>
<td>3.46 ± 0.03 c</td>
<td>5.82 ± 0.09 b</td>
</tr>
<tr>
<td>AA</td>
<td>3.51 ± 0.03 c</td>
<td>5.83 ± 0.09 b</td>
</tr>
</tbody>
</table>

a Values are means ± standard errors. Means within a column with different letters are significantly different (P < 0.05). MC, monocaprylin; AA, acetic acid.

Prior to inoculation, representative samples of commercially purchased frankfurters were analyzed for the presence of L. monocytogenes. Results showed that the frankfurters were not contaminated with L. monocytogenes. Aqueous ethanol (1%) was used as the MC carrier to improve its solubility. Ethanol at a concentration of up to 2.0% did not inhibit the growth of L. monocytogenes (18). Furthermore, increased antilisterial activity of fatty acids was related to increased solubility (37). Inhibitory fatty acids must be sufficiently soluble to reach an effective concentration in aqueous solution and yet sufficiently hydrophobic to interact with proteins and/or lipids on the bacterial cell surface (37).

The pH of dipping solutions was affected (P < 0.05) by the added antimicrobials (Table 1). However, MC had minimal effect (P > 0.05) on the pH of the samples. The pH values of the frankfurters dipped in the control solution and of those dipped in MC were 6.35 and 6.34, respectively. AA and MC+AA decreased the pH values of frankfurters, compared with control samples (P < 0.05) (Table 1).

The survival curves for L. monocytogenes on frankfurters dipped in different solutions at 50°C, vacuum packaged, and kept at 4°C for 77 days are presented in Figure 1. In control samples, the initial population (day 0) of L. monocytogenes was 3.9 log CFU per frankfurter, and after 14 days of storage, the pathogen population increased by approximately 1.0 log CFU per frankfurter (Fig. 1). The final count of L. monocytogenes on frankfurters at 77 days of storage was 3.0 log CFU per frankfurter, which was significantly lower than the initial population was at day 0 (P < 0.05). This decrease in L. monocytogenes counts on control frankfurters at the end of the study period could be attributed to the antilisterial activity of potassium lactate and sodium diacetate present in the frankfurters.

Dipping of inoculated frankfurters in MC and MC+AA solutions at 50°C resulted in immediate reductions (day 0) in L. monocytogenes counts (between 0.9 and 1.0 log CFU per frankfurter). During the entire storage period, the antimicrobial treatments significantly reduced (P < 0.05) L. monocytogenes counts on frankfurters when compared with controls. The greatest reduction in counts occurred on frankfurters dipped in MC+AA solutions, and the least reduction was observed for frankfurters dipped in AA only. At the 70 days of storage, L. monocytogenes was completely killed (enrichment negative) on frankfurters dipped in MC+AA, whereas samples treated with MC alone had a final count of 1.1 log CFU per frankfurter, which was significantly lower (P < 0.05) than the counts of L. monocytogenes on AA-treated frankfurters (2.1 log CFU per frankfurter) (Fig. 1).

Frankfurters dipped in the control solution at 45°C had initial L. monocytogenes counts of 3.5 log CFU per frankfurter, which increased by approximately 0.5 log CFU per frankfurter after 21 days of storage. However, thereafter the pathogen population gradually decreased, reaching a final count of 2.8 log CFU per frankfurter at 77 days (P < 0.05). Frankfurters dipped in MC, AA, and MC+AA solutions at 45°C had lower L. monocytogenes counts throughout the storage period when compared with those on control frankfurters (P < 0.05). Among the three antimicrobial treat-

![FIGURE 1. Inactivation of Listeria monocytogenes on frankfurters by monocaprylin alone or in combination with acetic acid at 50°C. The error bars represent the standard error (SE). ▲, Control; ▲, 50 mM of monocaprylin; ▽, 50 mM of monocaprylin and acetic acid; ■, acetic acid.](image-url)
ments, MC + AA was the most effective in reducing *L. monocytogenes* on frankfurters. After 56 days of storage, frankfurters treated with MC + AA had <1.0 log CFU per frankfurter *L. monocytogenes* (enrichment positive but no growth on plates) (Fig. 2).

The antimicrobial mechanism of caprylic acid and its monoglyceride is not known, but there are some hypotheses that have been postulated for free fatty acids in general. For example, altering the bacterial plasma membrane permeability and acidification of the intracellular environment are two commonly cited possibilities. Based on electron microscopic studies, monoglycerides have been proposed to act as nonionic surfactants that may penetrate and become incorporated into the bacterial lipid membrane, thereby altering its permeability (2, 11). In another electron microscopic study, Noseda et al. (19) provided further evidence for cell membrane damage induced by antimicrobial lipids. The lipids destabilized cell membranes, inducing morphological damage including formation of blebs, formation of holes, and increased porosity. Short- and medium-chain fatty acids have also been proposed to cause intracellular acidification and inactivation of intracellular enzymes, and/or inhibition of amino acid transport (9, 28, 35).

Compared with the controls, AA or MC alone significantly reduced (*P* < 0.05) *L. monocytogenes* populations on frankfurters (Figs. 1 and 2), and the combination of AA and MC consistently demonstrated an enhanced inhibitory effect on *L. monocytogenes* as compared with each antimicrobial used singly. The enhanced inhibitory activity of MC + AA against *L. monocytogenes* was a result of the complementary effect of MC and AA. Previously, while comparing the antibacterial activity of monolaurin (glycerol ester of lauric acid) and monolaurin in combination with lactic acid on *E. coli* O157:H7, Venkitanarayanan et al. (34) reported a significantly higher (*P* < 0.05) antibacterial effect when monolaurin was combined with lactic acid. Similarly, it was also found that the antilisterial activity of monolaurin was enhanced by combining it with an organic acid (e.g., lactic acid, acetic acid, or citric acid) (21). The antimicrobial activity of monolaurin is pH dependent and can be increased by decreasing the pH of the medium, as the uptake of monolaurin by bacterial cells under low pH is increased (20).

The antimicrobial activity of food preservatives can be increased or potentiated when combined with organic acids. In general, preservatives have optimal inhibitory activity at low pH because this favors the protonated state (generally hydrophobic) of molecules that permits them to cross the bacterial plasma membrane more rapidly and enter the cell. Once inside the cell, the protonated molecules will encounter the higher-pH cell cytoplasm, causing them to dissociate and resulting in the release of charged anions and protons, which cannot exit back across the plasma membrane (5). Accumulation of the released anions becomes toxic to bacterial cells (7), and the released protons stress the intracellular pH homeostasis (24).

**Sensory evaluation.** The application of antimicrobial treatments on frankfurters did not affect (*P* > 0.05) the odor of samples as evaluated by panelists using a 9-point hedonic rating scale (Table 2). Mean scores for control, MC, MC + AA, and AA were approximately 6.4, 6.0, 6.4, and 6.0, respectively. Treatments did affect (*P* < 0.05) the color of frankfurters. Mean scores for AA were lower (*P* < 0.05) than those for MC samples (5.9 vs. 6.4). Control

### Table 2. Panel scores for color and odor of frankfurters

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Color</th>
<th>Odor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.76 ± 0.31 A</td>
<td>6.40 ± 0.34 A</td>
</tr>
<tr>
<td>MC</td>
<td>6.36 ± 0.31 B</td>
<td>5.96 ± 0.34 A</td>
</tr>
<tr>
<td>MC + AA</td>
<td>7.04 ± 0.30 A</td>
<td>6.44 ± 0.30 A</td>
</tr>
<tr>
<td>AA</td>
<td>5.88 ± 0.26 C</td>
<td>6.00 ± 0.34 A</td>
</tr>
</tbody>
</table>

*a Values are means ± standard errors. Means within a column with different letters are significantly different (*P* < 0.05). Hedonic score descriptor: 9 = like extremely, 1 = dislike extremely. MC, monocaprylin; AA, acetic acid.
TABLE 3. Objective color measurements (L*, a*, and b*) ± standard error of frankfurters after different treatments

<table>
<thead>
<tr>
<th>Treatment</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>64.0 ± 1.8</td>
<td>18.4 ± 0.6</td>
<td>23.9 ± 0.5</td>
</tr>
<tr>
<td>MC</td>
<td>65.5 ± 0.5</td>
<td>18.9 ± 0.4</td>
<td>24.1 ± 0.6</td>
</tr>
<tr>
<td>MC+AA</td>
<td>64.8 ± 0.6</td>
<td>18.9 ± 0.4</td>
<td>23.8 ± 0.4</td>
</tr>
<tr>
<td>AA</td>
<td>64.7 ± 0.6</td>
<td>18.7 ± 0.5</td>
<td>24.0 ± 0.7</td>
</tr>
</tbody>
</table>

* Treatments are not significantly different (P > 0.05). MC, monocaprylin; AA, acetic acid.

and MC+AA samples had almost same (P > 0.05) mean color scores (6.8 and 7.0), which were significantly (P < 0.05) greater than those of AA or MC samples. Objective color measurements revealed that treatments, treatment interactions, and duration of storage did not significantly affect (P > 0.05) the color of samples. L*, a*, and b* values of frankfurters subjected to the different treatments were similar (P > 0.05) (Table 3).

The ability of L. monocytogenes to multiply in vacuum-packed RTE meats during refrigerated storage necessitates the postprocessing use of antimicrobials (33). Our study revealed that 50 mM MC and 1% AA exerted anti-listerial activity in vacuum-packaged pork-beef frankfurters kept at 4°C, and that the combination of these two antimicrobials used at 45 or 50°C resulted in a highly significant growth inhibition of L. monocytogenes. MC+AA has a great potential for postprocessing treatment of frankfurters that could be used by meat processors in compliance with the U.S. Department of Agriculture-FSIS directive issued in June 2003 (32). Further, L. monocytogenes was not detected in the dipping solutions containing MC or AA.

ACKNOWLEDGMENT

The study was supported by a grant from the American Meat Institute Foundation, Washington, D.C.

REFERENCES


