INTRODUCTION

Listeria monocytogenes has emerged into a highly problematic and fatal food-borne pathogen throughout the world, including the United States. In 1999, the Centers for Disease Control and Prevention reported an estimated 2,490 cases of listeriosis in the United States with a mortality rate of ca. 25% (Mead et al., 1999). Further, L. monocytogenes is of tremendous economic significance, causing an estimated monetary loss of $2.3 billion annually in the United States (Economic Research Service, 2001). L. monocytogenes is widespread in nature, occurring in soil, vegetation, and untreated water. Humans and a wide variety of farm animals, including cattle, sheep, goat, pig, and poultry are known sources of the pathogen (Brackett, 1998). L. monocytogenes is also frequently isolated from food processing equipment, air ducts, workers’ shoes, floor drains, stagnant water pools, and floors in food processing plants.
(Cox et al., 1989; Taormina and Beuchat, 2002). *L. monocytogenes* can survive in biofilms attached to a variety of processing plant surfaces such as stainless steel, glass, and rubber (Jeong and Frank, 1994). Although a wide spectrum of foods, including milk, cheese, beef, pork, chicken, seafoods, fruits, and vegetables, has been identified as vehicles of *L. monocytogenes*, ready-to-eat (RTE) foods, especially frankfurters have been regarded as potentially high-risk foods due to the opportunities for post-processing contamination (CDC, 1999; Meng and Doyle, 1997; Schwartz et al., 1988).

*L. monocytogenes* possesses several characteristics which enable it to successfully contaminate, survive and grow in foods, thereby resulting in outbreaks. These traits include an ability to grow at refrigeration temperature and in a medium with minimal nutrients, the ability to survive in acidic conditions (e.g., pH 4.2), the ability to tolerate up to 10% sodium chloride, ability to survive incomplete cooking or subminimal pasteurization treatments, and the ability to survive in biofilms on equipment in food processing plants and resist superficial cleaning and disinfection treatments (Nickelson, 1999). United States Federal Regulatory Agencies have established a “zero tolerance” policy for *L. monocytogenes* on ready-to-eat foods (Crawford, 1989; Klima and Montville, 1995; FDA, 1999). Therefore it is critical to include pre- and post-processing hurdles to inactivate or inhibit *L. monocytogenes* on frankfurters. Effective methods for reducing *L. monocytogenes* in foods would reduce the likelihood of food-borne outbreaks of listeriosis, and decrease economic losses to the meat industry.

In June 2003, the Food Safety and Inspection Service (FSIS) of the United States Department of Agriculture (USDA) issued a directive to all meat processors who produce RTE meat products such as frankfurters, requiring that these products be treated to eliminate *L. monocytogenes* (www.fsis.usda.gov/OPHS/lmrisk/DraftLm.22603). One of the methods
suggested by the FSIS is the application of a post-lethality treatment or an antimicrobial agent or process. To achieve this, a variety of approaches including application of GRAS (generally regarded as safe) chemicals such as lactates (Blom et al., 1997; Shelef, 1994) and acetates (Shelef and Addala, 1994; Weaver and Shelef, 1993) as ingredients in the frankfurter emulsion; or sodium benzoate, sodium propionate, and potassium sorbate (Islam et al., 2002) as dips on frankfurter surfaces have been previously investigated with varying degrees of success. In addition, natural antimicrobials of microbial origin (Bredholt et al., 1999; Degnan et al., 1992), antimicrobial-impregnated packaging materials (Padgett et al., 1998), thermal pasteurization (Cygnarowicz-Provost et al., 1994; Roering et al., 1998), irradiation (Sommers et al., 2000), and high pressure (Lucore et al., 2000; Murano et al., 1999) have been investigated for controlling \( L. \) monocytogenes on RTE meat products.

Fatty acids and their monoglycerides are potential antimicrobials that can be used in food systems (Sun et al., 2002). They have broad spectrum antimicrobial activity in culture media against enveloped viruses, Chlamydia, and Gram positive/negative bacteria (Kabara et al., 1972; Kabara 1979; Isaacs et al., 1995; Petschow et al., 1996; Bergsson et al., 1998; 1999). 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 (Oh and Marshall, 1992; Wang and Johnson, 1992; Petschow et al., 1996), while only a minimal inhibitory effect was observed in food (Wang and Johson, 1992). However, Mohan Nair et al. (2004) recently showed that caprylic acids and monocaprylin effectively killed \( E. \) 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 (Jensen et al., 2000), and coconut oil (Sprong et al., 2001), and has been approved as GRAS by the FDA (CFR 184.1025).
Monocaprylin (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 (Isaacs and Lampe, 2000).

In this study, we examined the efficacy of MC and its combination with acetic acid (AA) as an antimicrobial dip for killing *L. monocytogenes* in pork-beef frankfurters that were subsequently vacuum packaged and kept at 4°C for 12 weeks. Sensory evaluation was also conducted to determine the effect of MC on color and odor of the treated frankfurters. The effect of monocaprylin as an ingredient in turkey breast slices on *L. monocytogenes* was also investigated.

**Experiment 1: Inactivation of *Listeria monocytogenes* on frankfurter by monocaprylin alone or in combination with acetic acid**

**MATERIALS AND METHODS**

**Bacterial culture:** Three strains of *L. monocytogenes* were cultured individually in 100 ml of tryptic soy broth (TSB, Difco) at 37°C for 24 h. The cultures were sedimented by centrifugation (3600 X g for 15 min), washed twice, and resuspended in 10 ml sterile phosphate buffered saline (PBS, pH 7.2). Equal portions of the three strains were combined, diluted appropriately and the resulting suspension used as inoculum. The bacterial count of the 3-strain mixture of *L. monocytogenes* was confirmed by plating 0.1-ml portions of appropriate dilutions on tryptic soy agar (TSA) plates with incubation at 37°C for 24 h.

**Frankfurters.** Pork-beef skinless frankfurters (20% fat) were purchased from a local meat processor. Prior to inoculation, representative samples were placed in a sterile sampling bag (one piece per sampling bag) with PBS, homogenized in the stomacher, and streaked on Oxford agar
(Difco, Detr, Mich.). This was done to determine if the purchased frankfurters were contaminated with \textit{L. monocytogenes}.

**Inoculation and Treatments.** The skinless frankfurters were inoculated with the 3-strain mixture of \textit{L. monocytogenes} as per Bedie et al. (2001). Each peeled frankfurter was placed aseptically in a sterile sampling bag and surface-inoculated with 500μL of the 3-strain mixture of \textit{L. monocytogenes} to obtain an inoculation level of 10^3 (low load) or 10^5 (high load) CFU/g. The inoculum was spread uniformly over the entire surface by swirling the sample by hand for 30 sec. 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 sec in different treatment solutions. MC (Nu-Check Prep, Inc., Elysian, MN) was dissolved in 1% ethanol prior to use. The antimicrobial treatments used were as follows: Control (water containing 1% ethanol), MC (water containing 50 mM MC + 1% ethanol), AA (water containing 1% Acetic Acid + 1% ethanol), MC + AA (water containing 50 mM MC + 1% AA + 1% ethanol). After immersion, the samples were drained (< 30 sec), vacuum-packaged (Supervac, Smith Equipment Co., Clifton, NJ 07012; Koch Industry bags, 3 mil, code 01-46-09, Kansas City) and stored at 4°C. \textit{L. monocytogenes} counts on each frankfurter were determined on days 0, 1, 3, 5, and 7 of storage and thereafter every week through 12 weeks.

**Enumeration of \textit{L. monocytogenes}.** On each sampling day, a frankfurter from each vacuum package was transferred aseptically to a sterile sampling bag containing 50 ml 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 aerobically at 37°C for 48 hr before counting the colonies. Enrichment was performed by transferring 1 ml of the meat homogenate to 100 ml Tryptic Soy Broth (TSB, Difco) and
incubating at 37°C for 24 hr. The culture was then streaked on Oxford agar, incubated at 37°C for 48 hr and observed for black colonies.

**pH Determination.** The pH of control and treated frankfurters was determined using a pH meter (model 720, Orion Research, MA) standardized against pH 4 and 7 buffers. Thirty grams sample was blended with 90 ml of distilled water and the pH was measured.

**Sensory Evaluation.** Consumer acceptability of control and treated MC-frankfurters was conducted at the UCONN Dairy Store. Although it is GRAS, monocaprylin supplied by both manufacturers in the United States (Sigma Co., and Nu-Check Prep. Inc) is not recommended for human consumption. Therefore sensory analysis of the treated and control patties included rating of the samples based on color and odor (not taste).

A total of 25 untrained panelists were asked to rate their relative liking on randomly coded frankfurter samples based on color and on odor. A 9-point Hedonic rating scale was used for evaluation where a scale of 9 = like extremely and 1 = dislike extremely was used.

Objective analysis of color included the measurement of L*, a* (redness), and b* from three different surface locations on each frankfurter using a Minolta Chromameter CR 200 (Osaka, Japan) calibrated to standard white plate. The illuminant used was C (6774K) and the measuring area was 8 mm.

**Statistical Analysis.** The design used was a completely randomized 4 x 16 factorials. Factors included four treatments and 16 sampling points (days 0, 1, 3, 5, 7, 12, 21, 28, 35, 42, 49, 56, 63, 70, 77 and 84) (n=3). Data were analyzed using analysis of variance and mean separation procedures of Statistical Analysis Software (SAS Institute, Inc., N.C). The model statement accounted for variation due to different factors and interactions. Differences among means were detected at the 5% level using the least significance difference (LSD) test.
RESULTS AND DISCUSSION

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

High-Inoculum Treatment ($10^5$ CFU)

The survival curves for *L. monocytogenes* in frankfurters dipped on antimicrobial treatments at 50°C, vacuum-packaged and kept at 4°C for 84 days are presented in Figure 1. In control samples, *L. monocytogenes* increased by 1.2 log CFU/g after 5 days of storage and further increased by 3.0 log CFU/g after 4 weeks. Subsequently, a gradual decrease of the *L. monocytogenes* population was observed from 5 through 12 weeks. A similar trend was observed for the growth of *L. monocytogenes* in control samples dipped at 45°C (Fig. 2), where it increased ($P < 0.001$) by 3.0 log CFU/g after 4 weeks and gradually decreased at the 5th week of storage (Fig. 2). Several studies have shown that *L. monocytogenes* grows readily on untreated frankfurters and other comminuted-type RTE products when vacuum-packaged and held at 2 to 7°C (Glass and Doyle, 1989; Schmidt and Kaya, 1990; Buncic et al., 1991; Palumbo and Williams, 1994; Mytle et al., 2006).

Dipping of inoculated frankfurters in antimicrobial (AA, MC+AA, or MC) solutions at 50°C achieved an instant reduction (day 0) of about 0.9 to1.6 log CFU/g relative to controls. After 4 weeks storage, treatments inhibited ($P < 0.001$) growth of *L. monocytogenes* between 3.4 and 5.1 log CFU/g when compared to controls. At the end of 12 week storage, *L. monocytogenes* populations were lower on treated samples than controls ($P < 0.001$).
Similar trends were observed for samples dipped in antimicrobial solutions at 45ºC as for those dipped at 50ºC; AA, MC, MC+AA solutions reduced the growth of *L. monocytogenes* (*P* < 0.001). Initial reductions (day 0) of about 1.19-2.0 log CFU/g were achieved by dipping in antimicrobial solutions. At the end of storage, *L. monocytogenes* populations on control, MC, MC+AA, and AA – treated frankfurters were 6.23, 3.51, 2.81, and 3.54 log CFU/g, respectively. MC+AA was the most effective treatment (*P*<0.001) for inhibiting growth of *L. monocytogenes*.

**Low Inoculum Treatment (10³ CFU)**

In frankfurters dipped in control solution at 50ºC, the initial population (day 0) of *L. monocytogenes* was 3.9 log CFU/g, and after 14 days of storage the population increased by 0.8 log CFU/g (Fig 3). The final count (day 77) was 3.0 log CFU/g which is significantly lower than the initial population at day 0 (*P* < 0.001).

Dipping of inoculated frankfurters in MC and MC+AA solutions at 50ºC achieved instant reductions (day 0) between 0.9 and 1.0 log CFU/g. During the entire storage period, antimicrobial treatments significantly inhibited the growth of *L. monocytogenes* on frankfurters when compared to controls (*P* < 0.001). The greatest growth inhibition occurred on frankfurters dipped in MC+AA solutions, while the least inhibition was observed for frankfurters dipped in AA only. From the 10th to 11th weeks of storage, *L. monocytogenes* was completely killed in frankfurters dipped in MC+AA, while samples treated with MC alone had a final count of 1.1 log CFU/g, which was significantly lower than the AA-treated samples (2.5 log CFU/g) (*P* < 0.001) (Fig 3).

Samples dipped in control solutions at 45ºC had initial *L. monocytogenes* counts of 3.6 log CFU/g which increased by 0.8 log CFU/g after 3 weeks storage. However, the population
gradually decreased after week 4. By the end of storage, the final count was 2.85 log CFU/g which is lower than the initial count at day 0 ($P < 0.001$).

Frankfurters dipped in MC, AA, MC+AA solutions at 45°C had lower $L. monocytogenes$ counts throughout storage when compared to controls ($P < 0.001$). The lowest survival rate ($P < 0.001$) was observed for samples treated with MC+AA. After 8th weeks of storage, samples treated with MC+AA had < 1.0 log CFU/g viable $L. monocytogenes$ (enrichment positive but no growth on oxford plates).

AA or MC alone significantly reduced ($P < 0.001$) the growth of $L. monocytogenes$ (Figs.1, 2, 3, and 4), and combinations of AA and MC consistently showed enhanced inhibitory effects compared to each antimicrobial used singly.

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 cellular environment are two commonly cited possibilities. Based on electron microscopic studies, monoglycerides have been proposed to act as non-ionic surfactants that may penetrate and become incorporated into the bacterial lipid membrane thereby altering its permeability (Greenway et al., 1979; Bergsson et al., 1998). In another electron microscopic study, Noseda et al. (1989) 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 (Freese et al., 1973; Viegas and Sa-Correia, 1991; Sun et al., 1998).
In this study, antilisterial activity of AA or MC+AA alone could be attributed to the pH lowering effect in the dipping solution. The enhanced inhibitory activity of MC+AA against *L. monocytogenes* was a result of complementary effect of MC and AA. These results were similar to those published by Oh and Marshall (1994). They found that fatty acid (monolaurin) alone was effective against *L. monocytogenes* and its activity was further enhanced with inclusion of organic acid (e.g. lactic acid, acetic acid, citric acid).

Low pH increased the susceptibility of *L. monocytogenes* to antimicrobial molecules. 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 cellular 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 (Brul and Coote, 1999). Accumulation of the released anions was toxic to bacterial cells (Eklund, 1985) and the released protons stress the intracellular pH homeostasis (Salmond et. al., 1984; Cole and Keenan, 1987; Bracey et al., 1998).

**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. The mean odor scores for control, MC, MC+AA and AA were 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 for MC samples (6.4 vs 5.9). Control and MC+AA samples had the same (*P > 0.05*) mean scores (6.8 and 7.0), which were significantly greater than AA or MC samples (Table 2). Objective color measurements revealed that treatments did not significantly affect (*P > 0.05*) the color of
samples (Table 3). L*, a* and b* values of different treatments were statistically similar ($P > 0.05$).

This study revealed that an additive inhibitory effect of combining MC and AA resulted in significant inhibition of *L. monocytogenes* growth without affecting frankfurter odor and color.

**CONCLUSION**

The ability of *L. monocytogenes* to multiply in vacuum-packaged RTE meats during refrigerated necessitates the post-processing use of antimicrobials. Our study revealed that 50 mM MC plus 1% AA exerted antilisterial activity in vacuum-packaged pork-beef frankfurters kept at 4°C, and that the combination of these two antimicrobials resulted in a highly significant growth inhibition of *L. monocytogenes*. MC+AA represents a potential post-processing antilisterial treatment of frankfurters that could be used by meat processors in compliance with the FSIS directive issued in June 2003.
REFERENCES


Table 1. Mean pH of dipping solutions and treated samples

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Dipping solutions</th>
<th>Frankfurters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.44±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.35±0.09&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>MC</td>
<td>5.28±0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.34±0.09&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>MC+AA</td>
<td>3.46±0.03&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.82±0.09&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>AA</td>
<td>3.51±0.03&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.83±0.09&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a-c</sup> Means within a column with different superscripts within column are significantly (<i>P</i> < 0.05) different

+ Standard error of the means

Table 2. Mean panel scores for color and odor of frankfurters

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Color</th>
<th>Odor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.76±0.31&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.40±0.34&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>MC</td>
<td>6.36±0.31&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>5.96±0.34&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>MC+AA</td>
<td>7.04±0.30&lt;sup&gt;dc&lt;/sup&gt;</td>
<td>6.44±0.30&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>AA</td>
<td>5.88±0.26&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.00±0.34&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a-c</sup> Means within a column with different superscripts within column are significantly (<i>P</i> < 0.05) different

+ Standard error of the means

Hedonic score descriptor: 9 = like extremely; 1 = dislike extremely

Table 3. Objective color measurements (L*, a*, and b*) of frankfurters following different treatments

<table>
<thead>
<tr>
<th>Treatments</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>
Figure 1. Antimicrobial activity of monocaprylin as dipping solution (50°C) for frankfurters inoculated with 10^5 CFU/g L. monocytogenes

Control
50 mM MC
50 mM MC + 1% AA
1% Acetic acid (AA)
Figure 2. Antimicrobial activity of monocaprylin as dipping solution (45°C) for frankfurters inoculated with $10^5$ CFU/g *L. monocytogenes*.
Figure 3. Antimicrobial activity of monocaprylin as dipping solution (50° C) for frankfurters inoculated with 10³ CFU/g L. monocytogenes

- Control
- 50 mM Monocaprylin
- 50 mM MC + 1% AA
- 1% Acetic Acid (AA)
Figure 4. Antimicrobial activity of monocaprylin as dipping solution (45°C) for frankfurters inoculated with $10^3$ *L. monocytogenes*
Experiment 2: To determine the antibacterial effect monocaprylin as an ingredient in turkey breast slices

Deli turkey breast manufacturing:

Deli turkey breasts were manufactured following the general procedure of Schwarz et al. (1999). Fresh, unpumped turkey breasts (200g each) were injected (10%) with brine solution containing salt (1.5%, w/v), sodium tripolyphosphate (STPP, 0.5%, w/v), with or without 50 mM monocaprylin (dissolved in ethanol). Disposable syringes and needle were used to inject the brine into the turkey breast muscle. To ensure that brine was uniformly injected throughout the turkey breast, a grid (2cm x 2cm) was drawn onto a piece of cheese cloth and injections were made at each intersection of grid lines. Following injection, turkey breasts were tumbled for 1 hr and cooked to an internal temperature of 76°C (USDA required minimum) in a commercial smoke house. The cooked turkey breasts were cooled and sliced (0.3 mm thick).

Each turkey breast slice was inoculated with 100 μl of the 5-strain mixture of L. monocytogenes to obtain an inoculation level of ~ $10^5$ CFU/slice. The inoculum was spread uniformly over the sample by a sterile bent glass rod. The inoculated slices were kept at room temperature for 15 min to facilitate attachment of L. monocytogenes onto slice surface, and vacuum packaged and stored at 4°C for 7 days. L. monocytogenes counts on each slice was determined on days 0, 3 and 7. Duplicate samples were used for treatment and control, and the study was replicated thrice.

Results:

The counts of L. monocytogenes on monocaprylin-treated breast slices remained approximately the same throughout the storage period. However, the pathogen counts increased by approximately 1.0 log CFU on the control slices.
Table 1: Effect of monocaprylin on *L. monocytogenes* on turkey breast slices

<table>
<thead>
<tr>
<th>Treatments</th>
<th>0</th>
<th>3</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.79</td>
<td>5.14</td>
<td>5.77</td>
</tr>
<tr>
<td>50 mM MC</td>
<td>4.68</td>
<td>4.65</td>
<td>4.71</td>
</tr>
</tbody>
</table>

**CONCLUSION**

Monocaprylin as an ingredient in turkey breast slices primarily inhibited the growth of *L. monocytogenes* on the slices.

**REFERENCE**
