Control of *Listeria monocytogenes* by Monoglycerides in Foods

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**ABSTRACT**

Monoglycerides (MCs) including MC\(_{10}\), MC\(_{12}\), and coconut MCs were tested for inhibitory activity against *Listeria monocytogenes* strain Scott A in culture media and in several foods. MCs were inhibitory to *L. monocytogenes* in certain foods including beef frank slurry at pH 5.0 and 5.5 and seafood salad at pH 4.9 at 4°C, but were less active at 12°C than at 4°C. MCs were less inhibitory to *L. monocytogenes* in other foods including turkey frank slurry at pH 5.5, imitation crabmeat, cooked shrimp, summer sausage, yogurt, cottage cheese, and Camembert cheese. Combinations of MCs, particularly MC\(_{10}\) and MC\(_{12}\), showed increased activity in certain foods. The combination of MC\(_{10}\) (250 to 500 μg/ml) and MC\(_{12}\) (250 to 500 μg/ml) or a mixture of coconut-derived MCs (500 to 1,000 μg/ml) were inhibitory against *L. monocytogenes* in beef and turkey frank slurry. Certain chemical factors affected the degree of inhibition by the lipid compounds including pH, acidulants such as lactic acid, certain antioxidants, and lipid carriers. The results suggest that MCs could be used as preservatives in certain classes of minimally processed refrigerated foods when intrinsic antimicrobial activity is inadequate.

Because *L. monocytogenes* can withstand environmental stresses such as salt and acid and can grow at refrigeration temperatures, many minimally processed foods can foster the growth of relatively large populations of the pathogen. The potential for growth appears to be particularly important in foods that receive minimal processing and are expected to have extended shelf life under refrigerated storage. For these foods it often becomes necessary to add additional antimicrobial protection to control the growth and survival of *L. monocytogenes*. Our laboratory has investigated naturally occurring antimicrobials that have inhibitory activity against *L. monocytogenes* for applications in foods.

Lipids represent an important constituent in human and animal diets. Dietary lipids supply calories and essential fatty acids, act as vitamin carriers, and increase the palatability of foods. Besides their nutritional value, lipids have pharmacologic and antimicrobial properties (16). Trace quantities of certain fatty acids and monoglycerides (MCs) have been reported to inhibit microorganisms, especially gram-positive bacteria (15, 17) and fungi (17). Depending on the food, lipids can have antimicrobial activity at very low concentrations (e.g., in the order of 1 microgram per gram) and do not detract from desirable organoleptic properties.

Since 1980 several outbreaks of listeriosis have occurred in North America. Contaminated foods implicated in the outbreaks included coleslaw, milk, and soft cheeses (9, 13, 23). Microbiological surveys have documented that *Listeria monocytogenes* can be present in a wide range of retail foods including ready-to-eat meats, seafoods, and dairy products (6, 8, 10, 14, 22). The Centers for Disease Control and Prevention (CDC) reported an association between listeriosis and consumption of undercooked chicken or hot dogs (25). The frequency with which *L. monocytogenes* could be cultured from raw poultry and processed ready-to-eat meats suggested that these foods were plausible vehicles for transmission of this pathogen (20, 24). Fortunately, owing to increased vigilance to sanitation and food processing by the food industry, the incidence of listeriosis appears to be declining (27).

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against *L. monocytogenes* has also been observed by Oh and Marshall (19). The present study was undertaken to provide information on the inhibitory activity of individual as well as combinations of fatty acids and MCs in refrigerated foods. The present study differs from previous ones in evaluating a greater variety of compounds as carriers and potentiators of MC antimicrobial activity. As is the case with many other antimicrobials, their activity in media is often not representative of their efficacy in foods. We investigated the activity MCs in several foods, especially foods which have been shown or suspected to transmit listeriosis or to harbor the pathogen.

**MATERIALS AND METHODS**

**Chemicals**

The preparation of coconut MCs was previously described (30). Fatty acids (C\(_{12:0}\), C\(_{14:0}\), C\(_{16:0}\), C\(_{18:0}\), C\(_{18:1}\), C\(_{18:2}\), and C\(_{18:3}\)) monocaprin (MC\(_{10}\)), and monolaurin (MC\(_{12}\)) were obtained from Sigma Chemical Company (St. Louis, MO). Stock solutions were prepared in absolute (100%) alcohol (Aaper Alchohol & Chemical Company, Shelbyville, KY). These lipid compounds were selected on the basis of previous screening of a large variety of fatty acids and MCs (29, 30). Tested in combination with MCs were carriers and potentiators including lysozyme, propyl gallate, BHA (butylated hydroxyanisole), potassium sorbate, lactic acid, glycine, propylene glycol, sodium citrate, and Tween 20 (Sigma Chemical Company, St. Louis, MO). TBHQ ( tert-butylhydroquinone) was purchased from Eastman Kodak Chemical Company, Rochester, NY.

**Bacterial and growth conditions**

*L. monocytogenes* strain Scott A was originally obtained from Elmer Marth University of Wisconsin—Madison. Previous studies indicated that other strains had comparative sensitivity to the MCs (29, 30). Stock cultures were maintained by monthly transfers on brain heart infusion (BHI) agar slants (Difco Laboratories, Detroit, MI) and stored at 4°C. Stock cultures were also stored in BHI broth plus 20% glycerol at ~70°C. For studies in media, cells from BHI agar slants were subcultured in BHI broth in test tubes (1.0 by 12.5 cm) containing 10 ml of BHI broth. Each tube was inoculated with one loopful of cells, and the tubes were incubated statically at 37°C for 18 h. For growth studies in foods, *L. monocytogenes* Scott A was first cultured in BHI broth at 37°C overnight prior to inoculation to foods.

**Preparation of foods**

1. **Preparation of ready to eat meats and seafoods.** Beef franks (Oscar Mayer, Madison, WI), turkey franks (Wampler-Longacre Inc., Broadway, VA), summer sausages (Hillshire Farm, Cincinnati, OH), imitation crab meat (Louis Kemp Seafood Co., Duluth, MN), cooked shrimp (Contessa Products, San Pedro, CA), and seafood salad (Compass Foods, Montvale, NJ) were obtained from a local supermarket. The meats and salads were coarsely ground in a Waring blender in 100-g portions. In half of the samples of beef and turkey frank slurries, the pH was adjusted with 5 N HCl to 0.5 unit lower than the original (e.g., from pH 5.5 to pH 5.0).

2. **Preparation of dairy products.** Four percent milkfat small curd cottage cheese (Compass Foods, Montvale, NJ) and plain yogurt (Dannon, Jacksonville, FL) were prepared in slurries as described above for meat. Camembert (Ino Foods, Merrill, WI) was either prepared in wedges for surface inoculation or slurries were prepared as described above for the wiener.

**Results**

**Activities of fatty acids and MCs in combination with potentiators against *L. monocytogenes* in BHI broth**

Fatty acids and MCs previously demonstrated to have inhibitory activity against *L. monocytogenes* Scott A in BHI broth (29, 30) were used in this study. Specifically, MC\(_{10}\), MC\(_{12}\), and coconut MCs were the most inhibitory MCs tested and were more inhibitory than fatty acids including C\(_{12:0}\), C\(_{18:2}\), and C\(_{18:3}\) (29, 30). On the basis of the present study and previous findings, MC\(_{10}\), MC\(_{12}\), and coconut MCs but not fatty acids were used in the testing of lipid dispersants and for the food experiments.
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TABLE 1. Inhibition of L. monocytogenes in brain heart infusion broth by monolaurin (50 μg/g) combined with various carriers

<table>
<thead>
<tr>
<th>Carrier used&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Carrier</th>
<th>MC&lt;sub&gt;12&lt;/sub&gt; + carrier</th>
</tr>
</thead>
<tbody>
<tr>
<td>MC&lt;sub&gt;12&lt;/sub&gt;</td>
<td>I&lt;sup&gt;c&lt;/sup&gt;</td>
<td>—</td>
</tr>
<tr>
<td>Sodium citrate</td>
<td>NI&lt;sup&gt;d&lt;/sup&gt;</td>
<td>I</td>
</tr>
<tr>
<td>Propylene glycol</td>
<td>NI</td>
<td>I</td>
</tr>
<tr>
<td>Glycine</td>
<td>NI</td>
<td>I</td>
</tr>
<tr>
<td>Tween 20</td>
<td>NI</td>
<td>NI</td>
</tr>
</tbody>
</table>

<sup>a</sup> The concentrations of carriers tested were as follows: sodium citrate, 0.1 to 0.2%; propylene glycol, 0.1%; glycine, 0.1%; and Tween 20, 0.1% (all in % [w/w]).

<sup>b</sup> The initial population of L. monocytogenes was 10<sup>3</sup> to 10<sup>4</sup> CFU per milliliter of BHI broth. Growth tests for each condition were carried out in three independent tubes of BHI broth at 37°C for 6 days. Growth was determined by plating on BHI agar.

<sup>c</sup> I: inhibitory; no colonies of L. monocytogenes grew on BHI agar after 6 days at 37°C.

<sup>d</sup> NI: noninhibitory; the numbers of L. monocytogenes recovered was the same as the control (ca. 10<sup>6</sup> CFU/ml).

A potential limitation of lipids as antimicrobials is their distribution in lipid phases in foods, which could limit their contact with target microorganisms. To potentially increase interaction with L. monocytogenes, various carriers and potentiators were evaluated (Tables 1 and 2). Sodium citrate (0.1 or 0.2%), propylene glycol (0.1%), or glycine (0.1%) were not inhibitory by themselves, nor did they cancel the activity of MC<sub>12</sub>. Tween 20 (0.1%) also was not inhibitory by itself, but it did cancel the activity of MC<sub>12</sub> (Table 1). These data suggest that carriers other than ethanol such as sodium citrate, propylene glycol, or glycine could be used in foods.

Antioxidants and acidulants were evaluated as potentiators of MC<sub>12</sub> activity. In contrast to the results found with the carriers, certain antioxidants and acidulants in combination with MC<sub>12</sub> affected the growth rate, lag period, and maximum yield of L. monocytogenes in BHI broth at 37°C (Table 2). Antioxidants including BHA, TBHQ, propyl gallate (which at the concentrations used did not alter pH), and acidulants including lactic acid and acetic acid (which lowered the pH from 6.0 to 4.8 and 5.0, respectively) enhanced the activity of MCs in culture media (Table 2).

Activity of fatty acids and MCs against L. monocytogenes

The antibacterial activity of MCs against L. monocytogenes Scott A was evaluated in several foods. For maximum activity in most foods, a small quantity of ethanol (0.25 to 0.5%) was required in addition to fatty acids or MCs to improve solubility. Other carriers including sodium citrate (0.1%) and glycine (0.1%) were also used in certain foods, but these did not improve the activity over that obtained with ethanol and were not used in the majority of food experiments.

Activity of MCs against L. monocytogenes in meats

MCs were evaluated in slurries of meat products including summer sausages, beef frank, and turkey frank. In the various meats tested, MCs showed the highest listericidal activity in beef frank slurry. MC<sub>12</sub> (500 to 1000 μg/g), MC<sub>10</sub> (≥1,000 μg/g), MC<sub>10</sub> (500 to 500 μg/g) in combination with MC<sub>12</sub> (250 to 500 μg/g), and coconut MCs (500 to 1000 μg/g) had listericidal activity in beef frank slurries at pH 5.0 and 4°C (Fig. 1A and 1B). The antilisterial activities of MCs in beef slurry was enhanced when the pH was lowered by 0.5 unit (Fig. 1A and 1B). The addition of potassium sorbate (0.1 or 0.2%) or BHA (200 μg/g) together with MC<sub>12</sub> or MCs did not enhance the activity (data not shown).

TABLE 2. Effect of antioxidants and acidulants in combination with monolaurin (5 μg/g) on growth of L. monocytogenes in Brain Heart Infusion broth

<table>
<thead>
<tr>
<th>Potentiator</th>
<th>Lag&lt;sup&gt;a&lt;/sup&gt; (h)</th>
<th>T&lt;sub&gt;max&lt;/sub&gt;&lt;sup&gt;b&lt;/sup&gt; (h)</th>
<th>Y&lt;sub&gt;max&lt;/sub&gt;&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Lag&lt;sup&gt;a&lt;/sup&gt; (h)</th>
<th>T&lt;sub&gt;max&lt;/sub&gt;&lt;sup&gt;b&lt;/sup&gt; (h)</th>
<th>Y&lt;sub&gt;max&lt;/sub&gt;&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (no addition)</td>
<td>4.2 ± 1.0</td>
<td>0.7 ± 0.2</td>
<td>9.5 ± 0.5</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Control (MC&lt;sub&gt;12&lt;/sub&gt; alone)</td>
<td>12.4 ± 1.3</td>
<td>3.4 ± 1.3</td>
<td>8.0 ± 0.6</td>
<td>21.8 ± 2.7</td>
<td>7.0 ± 0.7</td>
<td>7.4 ± 1.1</td>
</tr>
<tr>
<td>BHA</td>
<td>13.8 ± 2.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.2 ± 0.4</td>
<td>7.5 ± 0.6</td>
<td>14&lt;sup&gt;d&lt;/sup&gt;</td>
<td>I</td>
<td>I</td>
</tr>
<tr>
<td>TBHQ</td>
<td>32.2 ± 3.5</td>
<td>8.0 ± 0.2</td>
<td>7.0 ± 0.7</td>
<td>I</td>
<td>I</td>
<td>I</td>
</tr>
<tr>
<td>Propyl gallate</td>
<td>18.3 ± 2.0</td>
<td>4.8 ± 0.5</td>
<td>5.2 ± 0.4</td>
<td>I</td>
<td>I</td>
<td>I</td>
</tr>
<tr>
<td>Acetic acid (pH 5.0)</td>
<td>14.4 ± 1.3</td>
<td>5.6 ± 0.8</td>
<td>5.8 ± 0.5</td>
<td>I</td>
<td>I</td>
<td>I</td>
</tr>
<tr>
<td>Lactic acid (pH 4.8)</td>
<td>17.9 ± 2.3</td>
<td>6.2 ± 1.1</td>
<td>5.2 ± 0.2</td>
<td>I</td>
<td>I</td>
<td>I</td>
</tr>
<tr>
<td>Potassium sorbate</td>
<td>15.7 ± 1.8</td>
<td>5.2 ± 0.6</td>
<td>7.8 ± 0.7</td>
<td>32.2 ± 3.5</td>
<td>8.2 ± 0.6</td>
<td>6.1 ± 0.6</td>
</tr>
</tbody>
</table>

<sup>a</sup> The concentrations of potentiators used were as follows: BHA, 100 μg/ml; TBHQ, 30 μg/g; propyl gallate, 200 μg/g; acetic acid, 0.1%; lactic acid, 0.1%; and potassium sorbate, 0.2%.

<sup>b</sup> The growth data were analyzed by a logistic model (II). Model parameters were used to estimate the lag period (Lag [h]), the doubling time (T<sub>d</sub> [h]), and maximum growth (Y<sub>max</sub> [CFU/ml]) of bacteria.

<sup>c</sup> Means are presented with one standard error. Two independent experiments were performed and each incubation was carried out in duplicate (n = 4).

<sup>d</sup> I: inhibitory; no colonies of L. monocytogenes grew on BHI agar after 6 days at 37°C.
The incubation temperature affected the antimicrobial activity of MCs in several foods. *L. monocytogenes* multiplied rapidly at 12°C in the presence or absence of MCs and reached high cell populations (10⁷ to 10⁸ CFU/ml) after 7 to 10 days of incubation in beef frank slurries (Fig. 1C). MC treated samples delayed growth of *L. monocytogenes* for 3 to 5 days, but did not kill significant numbers of cells and did not prevent eventual growth (Fig. 1C).

In contrast to the results with beef franks, the various MCs (MC₁₂, MC₁₀, and coconut MCs) preparations did not show significant inhibitory activity in turkey frank slurries at the normal pH of 6.1 or in summer sausage (pH 4.9) (data not shown). However, lowering the pH by 0.5 unit to 5.6 slightly improved the activity in turkey frank slurries at 4°C (Fig. 2). *L. monocytogenes* appeared to survive poorly in beef frank slurries compared to turkey frank slurries with or without treatment with MCs. This may have been partly due to the lower pH of beef frank slurries (pH 5.0 and 5.5) compared to turkey frank slurries (pH 5.6 and 6.1) or to the inherent inhibitory properties or ingredients of beef franks.

Activity of MCs against *L. monocytogenes* in cooked shrimp, imitation crabmeat, and seafood salad.

*L. monocytogenes* grew well in both imitation crabmeat (pH 6.5) and cooked shrimp (pH 7.1) at 4°C. Of the MCs tested, MC₁₂ was most inhibitory against *L. monocytogenes* in imitation crabmeat and cooked shrimp (Figs. 3 and 4), but still was only bacteriostatic, and eventually the pathogen...
 grew in these products. We investigated if certain antioxidants and carriers would enhance the activity of the MCs. Propyl gallate (200 µg/g) improved the activity of MCs and delayed growth of \( L. \) monocytogenes in both crabmeat and cooked shrimp (Figs. 3 and 4). MC\(_{12} \) at a concentration of 500 µg/g in combination with propyl gallate reduced the population of \( L. \) monocytogenes in both imitation crabmeat and cooked shrimp by 10- to 100-fold compared to the untreated control samples after 2 to 4 weeks of storage at 4°C. Propyl gallate alone did not show inhibitory activity compared to the control samples (Figs. 3 and 4). We tested the effects of MCs on survival of \( L. \) monocytogenes in seafood salad (pH 4.9). MC\(_{12} \) (1,000 µg/g) caused a 10- to 100-fold decrease from the initial cell count, whereas the control cell populations declined more slowly (Fig. 5).

**Activity of MCs against \( L. \) monocytogenes in dairy foods**

We previously showed that certain fatty acids and monoglycerides had antilisterial activity in skim milk but not in 2% milkfat or whole milk (29, 30). In this study, we investigated the activity in other dairy foods including cottage cheese, Camembert cheese, and yogurt. Fatty acids including C\(_{12:0}\), C\(_{18:2}\), and C\(_{18:3}\) were not effective against \( L. \) monocytogenes in cottage cheese or yogurt at concentrations of 1,000 µg/g or less (data not shown). In these acidic foods the populations of \( L. \) monocytogenes declined over several weeks but the inclusion of MCs did not enhance this rate.

Camembert cheese was studied since it has been involved in outbreaks of listeriosis. MC\(_{10}\), MC\(_{12}\), or coconut MCs were only slightly inhibitory against \( L. \) monocytogenes in Camembert cheese. MC\(_{12}\) at a concentration of 500 µg/g (Fig. 6A) or when combined with lactic acid (0.2%) prevented \( L. \) monocytogenes from growing for 2 to 3 weeks but did not kill significant numbers of cells and did not prevent eventual growth (Fig. 6A and 6B). Camembert cheese treated with MC\(_{12}\) by itself or together with lactic acid showed better results than MC\(_{10}\) and coconut MCs in both surface- and interior-treated samples (Fig. 6B). In contrast, MC\(_{10}\) or MC\(_{12}\) alone showed higher activity than MC\(_{12}\) combined with lactic acid or coconut MCs in cheeses treated only on the surface. The concentration of lactic acid (0.2%) decreased the pH of Camembert cheese by only 0.1 to 0.2 unit. MC\(_{12}\) at the concentration of 500 µg/g reduced the population of \( L. \) monocytogenes by a factor of 100 to 1000 during the first 2 to 4 weeks of storage at 4°C compared to the control, but later allowed growth under the experimental conditions (see Fig. 6A and 6B). These results indicated that MC\(_{12}\) was initially bacteriostatic but \( L. \) monocytogenes eventually overcame the inhibition. The results also suggested that MCs have increased activity when combined with lactic acid in certain foods.

**Effect of potentiators on activity of MCs in foods**

Certain adjuncts tested with MCs in foods did not enhance the activity compared to the MCs alone (data not shown). However, coconut MCs increased activity of MCs when combined with lactic acid in Camembert cheese and cooked shrimp (Figs. 6A and 6B). The results also suggested that MCs have increased activity when combined with lactic acid in certain foods.
shown). Carriers and potentiators tested in foods included glycine (0.1%), sodium citrate (0.1%), propylene glycol (0.1%), Tween 20 (0.1%), potassium sorbate (0.1 and 0.2%), lysozyme (200 μg/g), and BHA (200 μg/g). These were tested alone and in combination with MCs, but they did not improve the activity of MCs alone applied in ethanol (data not shown).

DISCUSSION

Owing to its wide distribution and resistance characteristics, L. monocytogenes is a particularly difficult organism to control at the farm level and in processing plants. Contamination of foods by L. monocytogenes is a particularly difficult problem in products that are not pasteurized or acidified. Although improved sanitation procedures, good manufacturing practices, raw material testing, and processing procedures have greatly minimized the Listeria problem (27), current technologies do not permit the complete eradication of L. monocytogenes from the processing environments and from all finished products. The safety and shelf life of minimally processed refrigerated foods could be enhanced by the use of barriers including naturally occurring antimicrobials. Unlike many antimicrobials, certain lipids and MCs are permitted for use by the Code of Federal Regulations. For many years, MCs have also been used in the food industry as emulsifying agents. MCs appear compatible with both commercial requirements and public and regulatory concerns.

The results of the present study indicate that MCs are effective inhibitors against L. monocytogenes Scott A in certain foods, particularly acidic foods, at refrigeration temperatures. The best results were obtained with combinations of MCs (MC\textsubscript{10}, MC\textsubscript{12}, or coconut MCs) or MCs alone especially in beef frank slurries at 4°C. Certain antioxidants potentiated the activity of MCs in shrimp and imitation crabmeat; in particular, propyl gallate increased the activity of MC\textsubscript{12} and delayed growth of L. monocytogenes. In Camembert cheese, lactic acid significantly enhanced the inhibitory activity of MCs in surface- and interior-treated samples (whole cheese) but not in cheese treated only on the surface. It is possible that the different properties in the surface and interior structure of Camembert or the natural microflora affected MC activity. Previous research in meat systems demonstrated that antimicrobial activity was increased when lactic acid and MC\textsubscript{12} were combined (4, 7, 28). Low temperatures and pHs often promote the antimicrobial activity of food preservatives (1), as was also observed in the present study.
MCs showed no significant inhibitory activities in dairy foods tested including yogurt and cottage cheese. In these foods, *L. monocytogenes* died off in the controls probably due to the low pH (4.0 to 4.9). The survival or growth of *L. monocytogenes* in foods generally correlated well with the initial pH of the products. The best growth occurred when the pH was near or above 6, and little or no growth occurred when the pH was below 5. In the acidic products (pH less than about 4.5), *L. monocytogenes* died off during several weeks of incubation whereas in the products with higher pH it tended to survive or grow. These results emphasize that pH is a major environmental factor controlling survival of *L. monocytogenes* in foods.

Carriers were evaluated in an attempt to replace or reduce the quantity of ethanol required as a carrier for MCs. Glycine, sodium citrate, or propylene glycol could reduce the amount of ethanol as a carrier in culture media but not in the foods tested. In general there may be a number of factors which could nullify MC activity in food systems such as distributed or localized regions of high pH, adsorption or complexation to starch, proteins or other macromolecules, or phase separation by fats or nonpolar compounds such as Tweens (3). Also, microorganisms often adsorb at the interface of oil and water phases, but can be found free in the aqueous phase during motile periods (17). These motile cells could escape contact with the lipophilic substances. More work needs to be done in finding satisfactory carriers for MCs or compatible carriers in combination with MCs for use in food systems.

As with all food antimicrobials, MCs have limitations. The necessity of using MCs at high concentrations (≥500 µg/g) in certain foods could have an adverse impact on the sensory characteristics of the foods. They are selectively active against specific groups of organisms including gram-positive bacteria and molds, but they completely lack activity against gram-negative bacteria. The absence of activity against gram-negative bacteria can be overcome by disrupting the outer membrane with chelating agents or other antimicrobials and emulsifiers. In conclusion, our results suggest that MCs could be useful as preservatives in refrigerated foods against *L. monocytogenes* in certain minimally processed refrigerated foods.

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