Evaluation of Novel Micronized Encapsulated Essential Oil–Containing Phosphate and Lactate Blends for Growth Inhibition of *Listeria monocytogenes* and *Salmonella* on Poultry Bologna, Pork Ham, and Roast Beef Ready-to-Eat Deli Loaves

G. CASCO,¹ T. M. TAYLOR,² AND C. Z. ALVARADO¹*¹

¹Department of Poultry Science and ²Department of Animal Science, Texas A&M University, College Station, Texas 77843, USA

ABSTRACT

Essential oils and their constituents are reported to possess potent antimicrobial activity, but their use in food processing is limited because of low solubility in aqueous systems and volatilization during processing. Two proprietary noncommercial essential oil–containing phosphate blends were evaluated for antimicrobial activity against *Salmonella enterica* cocktail (SC)– and *Listeria monocytogenes* (Lm)–inoculated deli meat products made from pork, poultry, or beef. Four treatments were tested on restructured cured pork ham, emulsified chicken bologna, and restructured beef loaf: nonencapsulated essential oil with phosphate version 1 at 0.45% of final batch (EOV145; chicken and pork, or EEOV245 beef), micronized encapsulated essential oil with phosphate version 2 at 0.60% of final batch (EEOV260), a 2.0% potassium lactate (PL) control, and a negative control (CN) with no applied antimicrobial agent. Compared with the CN, none of the antimicrobial agents (EEOV260, EOV145, PL) successfully limited Lm or SC growth to <2.0 log cycles over 49 days or 35 days of refrigerated storage, respectively. The PL and EEOV260-treated ham loaves did show Lm growth limiting ability of up to 1 log cycle by days 35 and 42. On formed roast beef, the EEOV260 was able to extend the lag phase and inhibited the growth of Lm in the same manner as the PL. For SC–treated samples, the following effects were observed: in poultry bologna treated with EEOV260, a lag-phase extension was observed through 35 days of storage compared with the other samples. For pork deli loaves, the EEOV260 inhibited growth of SC at days 21 and 28 to the same level of efficacy as PL (0.5 log cycle). In roast beef samples, on day 35, the SC growth was inhibited ca. 0.5 log CFU/g by EEOV260 when compared with the CN. In conclusion the EEOV260 can function to replace PL to limit *Salmonella* and Lm growth in ready-to-eat deli products. Further testing is needed to ensure consumer acceptability.

Deli meats, such as bologna, roast beef, and ham, are ready-to-eat (RTE) products that are staple foods for many consumers and are formed by marinating poultry, pork, or beef with salt, phosphate, and other ingredients before being stuffed in casings and cooked (Title 9, U.S. Code of Federal Regulations §319.80, 81) (8, 9, 23, 38). The International Markets Bureau (14) reported that the United States processed meat (cooked, cured, fermented meats, and pâté) market demand for deli foods in 2010 was approximately $7.1 billion, and expected to grow to $7.8 billion for 2013. However, since deli meats are RTE foods and may be consumed without a cooking/heating step, there is a risk of foodborne disease from pathogenic bacteria such as *Listeria monocytogenes* (Lm), and though less common, *Salmonella enterica* serovar Typhimurium.

Lm, a gram-positive non–spore-forming psychrotroph, can be present in the postlethality environment of RTE foods, producing a risk of product cross-contamination. While the best method for controlling Lm on RTE deli products is the application of an appropriate lethality process, lowering the pH and water activity of deli meats is also known to cause an adverse growth environment for Lm (16, 22). The pathogen is tolerant to 10% NaCl, 300 ppm of nitrite (NO₂⁻), and is known to grow under refrigeration temperatures (1°C) (25). Since RTE deli meats have an increased risk of cross-contamination with Lm from the process environment and food contact surfaces during slicing or repackaging of the deli meats (1, 6, 21, 23, 25), the U.S. Department of Agriculture–Food Safety and Inspection Service (USDA-FSIS) has dictated that Lm is an adulterant in RTE products and enforces a “zero-tolerance” rule on this pathogen (43). *Salmonella enterica* serovar Typhimurium, a gram-negative infectious pathogen, is an endemic contaminant of poultry products. In the United States, of the 790 confirmed agent outbreaks reported in 2009 to 2010, *Salmonella enterica* accounted for 30% of the outbreaks and 7,039 (36%) illnesses. *Salmonella* also accounted for the most outbreak-related hospitalizations (49%) of the 1,184 confirmed hospitalizations (7). Kaitsa et al. (19) reported that 1.1% of retail RTE turkey samples from the Midwestern United States were

* Author for correspondence: Tel: 979-845-4818; Fax: 979-845-1921; E-mail: calvarado@poultry.tamu.edu
positive for Salmonella. From 2005 until 2008, a total of 0.07% of the FSIS random testing (ALLRTE; \( n = 11,822 \)) samples and 0.04% of the risk-based testing (RTE001; \( n = 33,276 \)) programs tested positive for Salmonella enterica (44). Most Salmonella enterica contamination results from improperly cooked deli meat products or from postcook deficiencies in sanitary procedures (25, 44). Identification of Salmonella serovars in RTE products renders such products adulterated (44).

Current methods of Lm control include sodium or potassium lactate (PL) added to deli meat formula as a bacteriostatic agent (1, 24, 25). Sodium lactate (NaL) used at 3.0% of the batch weight was shown to extend the lag phase of Lm by 14 days compared with no-lactate controls for turkey deli loaves inoculated with \( 10^4 \) CFU/cm\(^2 \) Lm (6). Both sodium and PLs are regulated by FSIS to a maximum usage level of 4.8% in the final product (42). However, off-flavors may result in concentrations greater than 2% of PL in the final products (1, 6, 39). In addition, the incorporation of diacetate salts (up to 0.25%) as a flavor and antimicrobial agent has shown improved bacteriostatic effect when added with lactate salts (2%) to deli meats (22).

The demand for clean labels by consumers has driven extensive research on essential oils (EO) from seeds, barks, and flowers for their bacteriostatic and bactericidal activity against Lm and Salmonella Typhimurium (34, 40). The modes of action of these EO can be multiple, ranging from attacking the cell membrane, disrupting enzyme systems, to forming fatty acid hydroperoxides (40). Citrus oils and their constituents under in vitro conditions demonstrate bacteriostatic capacity ranging from a concentration of 0.125 to 10% (vol/vol) (11). The major component of citrus EO is limonene, a monoterpane constituting up to 98% of EO content in citrus oil (10, 28, 30). Espina et al. (10) reported mandarin EO showed a strong inhibition against Salmonella Enteriditis, and moderate inhibition of Lm determined via disc diffusion. The utility of EO in food systems is limited due to rapid volatilization and difficulty of dispersion in water-based marinades due to lipophilic properties of EO constituents.

Encapsulation and micronization are physical procedures that can be applied to EO to stabilize EO for addition to differing food systems (45). The use of supercritical fluids at specific temperatures and pressures to make a solution of EO and encapsulation substrate (e.g., polysorbate) allows not only for better extractions of the EO but also creates nano- or microscaled particles of EO that may be encapsulated via spray-drying processes (33, 47). Through encapsulation with carbohydrates or food-grade surfactants the volatilization of EO from food products can be slowed. The antibacterial capacity can be extended over time, achieving an extension of the bacterial lag phase in water-oil or oil-water emulsion systems (45). Rasenack and Müller (36) reported that micronized encapsulated poor water-soluble drugs have better rates of dissolution and increased particle surface area compared with their native crystal form. Micronized encapsulated EO have reduced particle size that may allow increases in particle surface area and solubility in aqueous phases, thus improving the availability of the EO compared with nonencapsulated micronized EO (36, 45). The objective of this study was to determine the efficacy of a sprayed dried micronized polysorbate encapsulated or an unencapsulated EO blend (citrus, onion, and garlic EO mixed in a sodium tripolyphosphate and potassium lactate base), and compared with PL on three types of RTE meat products (formed, cured chunked and formed, and emulsified deli loaf) against cocktails of Listeria monocytogenes (LC) and Salmonella serovars (SC).

**MATERIALS AND METHODS**

**Bacterial cultures and preparation of cocktails for product inoculation.** Bacterial cultures used to inoculate the deli meat samples were provided by Dr. Allen Byrd, USDA, Agricultural Research Service, Southern Plains Agricultural Research Center, College Station, TX, and were obtained from a United States commercial poultry products manufacturer. Cocktails of three bacterial isolates for each pathogen, revived from frozen stocks stored at \(-80^\circ\)C in tryptic soy broth (TSB; BD, Sparks, MD) containing 10% glycerol, were used for this study. Strains of Salmonella enterica Typhimurium, Salmonella enterica Heidelberg, and Salmonella enterica Enteritidis, naturally resistant to nalidixic acid and novobiocin, were revived from frozen stocks separately in 10.0 ml TSB supplemented with nalidixic acid (20 \( \mu \)g/ml) and novobiocin (25 \( \mu \)g/ml; Sigma-Aldrich Co., St. Louis, MO). Revived cultures were incubated at 36°C for 18 to 24 hours before performing a second passage in identical fashion to the first. After the second passage, strains were washed by centrifugation (Spectrafuge 6C, Labnet International Inc., Edison, NJ) for 10 min at 852 \( \times \) g. The supernatants were discarded and the pellet was suspended with phosphate buffer saline (PBS; Sigma-Aldrich Co.); this process was repeated twice in identical fashion. The washed pellets were then suspended in 10.0 ml PBS and mixed to a 1:1:1 ratio to generate the working cocktail (SC) of 8.1 \( \pm \) 0.2 log CFU/ml. A non-antimicrobial-resistant Listeria monocytogenes cocktail (LC) with isolates of Scott A (clinical outbreak), Brie 310 (goat cheese outbreak), and NADC 2783 (hamburger isolate) was prepared in a similar fashion as mentioned above. The individual isolates were incubated in TSB plus yeast extract (0.6%) and incubated at 36°C for 18 to 24 h without shaking. After the second passage the strains were washed as above and the suspended pellets were mixed on equal parts to a final concentration of 8.4 \( \pm \) 0.2 log CFU/ml.

**Deli product preparation.** All meat used in the deli meat loaves was purchased fresh from a local distributor and stored at 4°C for 1 day prior to manufacturing at the Rosenthal Meat Science and Technology Center at Texas A&M University (College Station). Manufacturing of deli loaves simulated industry processing methods with the use of pilot scale equipment. The four treatments manufactured for the poultry and pork loaves consisted of a no antimicrobial control negative (CN), a 2% PL control positive EO with phosphate formula 1 added at 0.45% of the final batch (EOV145), and micronized encapsulated essential oil with phosphate formulation 2 added at 0.60% of the final batch (EEOV260). The roast beef loaves contained the same controls but EO-incorporating treatments consisted of the EEOV260 (as described above) and a micronized encapsulated essential oil with phosphate formulation 2 added at 0.45% of the final batch (EEOV245). The 1 formuals contained sodium tripolyphosphate at 65%, potassium lactate at 30%, and the EO at 5% of the antimicrobial blend. Formulation 1 was not encapsulated in a polysorbate shell like formula 2.
Poultry loaves were produced using current industry standards and formulations (Table 1). Boneless and skinless chicken breast and thigh meat were chopped in a bowl chopper (Type K 64 UVA, Seydelmann, Stuttgart, Germany) at 2,000 rpm with pork fat for 3 min. Sodium chloride (1.5%; Morton Salt Inc., Chicago, IL) and a di- and triphosphate blend (0.45%; Carnal 822, Budenheim USA Inc., Columbus, OH) was added and the bowl chopper speed increased to 4,000 rpm. On the treatment formulations, the corresponding EO-containing blends were added in place of the phosphate. The remainder of garlic (1%; Readfield Meat & Deli, Bryan, TX), potassium lactate (2%; UltraLac KL-60, PL at 60), and thyme meat were chopped in a bowl chopper (Type K 64 UVA, Handtmann, Biberach, Germany) and stuffed into full barrier 15.2-cm-diameter by 70-cm-long plastic casings (ADV 5120, DeWied International, Inc., San Antonio, TX). The poultry loaves were clipped (model DCD6022, Poly-Clip System LLC, Mundelein, IL) and hung on stainless steel racks before being placed in a smoke house (model 1003, ALKAR System LLC, Mundelein, IL) and hung on stainless steel racks. Brines were prepared individually according to the formulation in Table 3. The brines contained NaCl (1.5%; Morton Salt Inc., Chicago, IL), a di- and triphosphate blend (0.45%; Carnal 822, Budenheim USA Inc.), PL (2%; UltraLac KL-60; PL at 60%), sodium erythorbate (0.05%; A.C. Leggs Inc., Calera, AL), and 6.25% cure (0.25%; A.C. Leggs Inc.), and were prepared following the corresponding formulation for each treatment one at a time (Table 2). For the treatments that contained the encapsulated EO no phosphate blend was added to the brine. The meat and the brine were placed into a vacuum tumbler (model VT-500, Leland Southwest Inc., Fort Worth, TX) and tumbled at 50 kPa and 6.0 rpm for 1.15 h. The tumbled meat batter was stuffed in casings, cooked to an internal temperature of 71°C for 8 h, and stored as described above. Beef clods were trimmed from excess fat and connective tissue and cut into cubes measuring 4 by 4 by 4 cm. Brines were prepared individually according to the formulation in Table 3. The brines contained NaCl (1.5%; Morton Salt Inc., a di- and triphosphate blend (0.45%; Carnal 822, Budenheim USA Inc.), PL (2%; UltraLac KL-60; PL at 60%), sodium erythorbate (0.05%; A.C. Leggs Inc., sugar (1.1%, Great Value brand; Wal-Mart Stores Inc.), and garlic (1%; Readfield Meat & Deli). The treatments that

### Table 1. Treatment formulation for poultry deli loaves with 20 added solution

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>NA</th>
<th>PL</th>
<th>EOV145</th>
<th>EEOV260</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast 97/3</td>
<td>16.00</td>
<td>16.00</td>
<td>16.00</td>
<td>16.00</td>
</tr>
<tr>
<td>Thigh 85/15</td>
<td>44.00</td>
<td>44.00</td>
<td>44.00</td>
<td>44.00</td>
</tr>
<tr>
<td>Fat 15/85</td>
<td>20.00</td>
<td>20.00</td>
<td>20.00</td>
<td>20.00</td>
</tr>
<tr>
<td>Water</td>
<td>17.05</td>
<td>15.05</td>
<td>17.05</td>
<td>16.90</td>
</tr>
<tr>
<td>Salt</td>
<td>1.50</td>
<td>1.50</td>
<td>1.50</td>
<td>1.50</td>
</tr>
<tr>
<td>STPP</td>
<td>0.45</td>
<td>0.45</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Potassium lactate (60)</td>
<td>0.00</td>
<td>2.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Version 1 essential oil</td>
<td>0.00</td>
<td>0.00</td>
<td>0.45</td>
<td>0.00</td>
</tr>
<tr>
<td>Version 2 essential oil</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.60</td>
</tr>
<tr>
<td>Garlic</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Total</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
</tr>
</tbody>
</table>

*NA, no antimicrobial agent; PL, potassium lactate; EOV145, nonencapsulated essential oil with phosphate version 1 at 0.45 of final batch; EEOV260, micrized encapsulated essential oil with phosphate version 2 at 0.60 of final batch.

---

Chunked and formed ham loaves were manufactured according to formulations shown in Table 2. Fat from pork butts used in the production of the ham loaves was trimmed off and the meat ground (model 1056, Biro Manufacturing Co., Marblehead, OH) through a #32 3-hole kidney plate (Southwest Saw Corp., Houston, TX). The brines consisted of NaCl (1.5%; Morton Salt Inc., Chicago, IL), a di- and triphosphate blend (0.45%; Carnal 822, Budenheim USA Inc.), PL (2%; UltraLac KL-60; PL at 60%; Hawkins Inc.), sugar (0.02%; Great Value brand, Wal-Mart Stores Inc., Bentonville, AR), Na-Erythrobate (0.05%; A.C. Leggs Inc., Calera, AL), and 6.25% cure (0.25%; A.C. Leggs Inc.), and were prepared following the corresponding formulation for each treatment one at a time (Table 2). For the treatments that contained the encapsulated EO no phosphate blend was added to the brine. The meat and the brine were placed into a vacuum tumbler (model VT-500, Leland Southwest Inc., Fort Worth, TX) and tumbled at 50 kPa and 6.0 rpm for 1.15 h. The tumbled meat batter was stuffed in casings, cooked to an internal temperature of 71°C for 8 h, and stored as described above. Beef clods were trimmed from excess fat and connective tissue and cut into cubes measuring 4 by 4 by 4 cm. Brines were prepared individually according to the formulation in Table 3. The brines contained NaCl (1.5%; Morton Salt Inc.), a di- and triphosphate blend (0.45%; Carnal 822, Budenheim USA Inc.), PL (2%; UltraLac KL-60; PL at 60%; Hawkins Inc.), sugar (1.1%, Great Value brand; Wal-Mart Stores Inc.), and garlic (1%; Readfield Meat & Deli). The treatments that

### Table 2. Treatment formulation for ham deli loaves with 20 added solution

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>NA</th>
<th>PL</th>
<th>EOV145</th>
<th>EEOV260</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pork butts trimmed</td>
<td>80.00</td>
<td>80.00</td>
<td>80.00</td>
<td>80.00</td>
</tr>
<tr>
<td>Water</td>
<td>17.73</td>
<td>15.73</td>
<td>17.73</td>
<td>17.58</td>
</tr>
<tr>
<td>Salt</td>
<td>1.50</td>
<td>1.50</td>
<td>1.50</td>
<td>1.50</td>
</tr>
<tr>
<td>STPP</td>
<td>0.45</td>
<td>0.45</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Potassium lactate (60)</td>
<td>0.00</td>
<td>2.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Version 1 essential oil</td>
<td>0.00</td>
<td>0.00</td>
<td>0.45</td>
<td>0.00</td>
</tr>
<tr>
<td>Version 2 essential oil</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.60</td>
</tr>
<tr>
<td>Sodium erythorbate</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Sugar</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>Cure salt (6.25)</td>
<td>0.25</td>
<td>0.250</td>
<td>0.250</td>
<td>0.250</td>
</tr>
<tr>
<td>Total</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
</tr>
</tbody>
</table>
TABLE 3. Treatment formulation for roast beef deli loaves with 23 added solution

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>NA</th>
<th>PL</th>
<th>EEOV245</th>
<th>EEOV260</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef cloids 85–90 lean</td>
<td>76.92</td>
<td>76.92</td>
<td>76.92</td>
<td>76.92</td>
</tr>
<tr>
<td>Water</td>
<td>19.03</td>
<td>17.03</td>
<td>19.03</td>
<td>18.88</td>
</tr>
<tr>
<td>Salt</td>
<td>1.50</td>
<td>1.50</td>
<td>1.50</td>
<td>1.50</td>
</tr>
<tr>
<td>STPP</td>
<td>0.45</td>
<td>0.45</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Potassium lactate</td>
<td>0.00</td>
<td>2.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Version 2 essential oil</td>
<td>0.00</td>
<td>0.00</td>
<td>0.45</td>
<td>0.60</td>
</tr>
<tr>
<td>Sugar</td>
<td>1.10</td>
<td>1.10</td>
<td>1.10</td>
<td>1.10</td>
</tr>
<tr>
<td>Garlic</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Total</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
</tr>
</tbody>
</table>

Antimicrobial agents are often added to RTE deli products to eliminate or extend the lag phase of targeted microorganisms, including pathogens. These effects are important in the case of RTE deli loaves that are exposed to postlethality contamination as described by federal rules regarding postprocess contamination by Lm (9 CFR 430.4). Salts of lactate have been tested and are commonly used by the industry as bacteriostatic agents in RTE deli loaves (1, 22, 23). With the increased desire for cleaner labels, EO must validate that these compounds inhibit Lm on RTE deli loaves to no more than 2.0 log cycles over the course of a product’s intended shelf life prior to use (9 CFR 430.4).

All treatments (PL, CN, EOV145, EEOV245, and EEOV260) for the poultry bologna, pork ham, and roast beef deli slices were observed to contain the same number of cells of LC inoculum (5.4 ± 0.1 log CFU/g) on day 0 (Figs. 1 through 3). Antimicrobials (EOV145, EEOV260, and PL) applied in the poultry bologna extended the lag phase of LC to day 7 compared with the CN (P < 0.05; Fig. 1). However, both EO (EOV145 and EEOV260) antimicrobials were unable to inhibit LC growth to <2.0 log cycles thereafter, allowing an approximate 2.3-log increase in LC numbers at 14 days (Fig. 1). No further growth-limiting effects were observed thereon by any of the EO blends and LC numbers for EO-containing treatments were not different from the CN (P ≥ 0.05) on days 28 to 42. On the other hand, 2% PL in the PL poultry bologna provided an inhibiting effect (P < 0.05) on Lm when compared with the CN on days 21, 35, and 42 (Fig. 1).
Overall, micronized encapsulated EO and unencapsulated EO were capable of extending the lag phase of the LC to a level equivalent to PL on emulsified poultry bologna deli loaves. However, no antimicrobial agent successfully limited LC growth to \(2.0\) log cycles over 49 days of refrigerated storage (EEOV260, EOV145, PL).

Antimicrobials applied on a chunked and formed pork ham did not inhibit the LC from replication early on in the product shelf life (day 7; Fig. 2). The LC numbers on PL-treated product were lower \((P < 0.05)\) versus CN and EOV145 from days 14 to 42 (Fig. 2). Furthermore, hams treated with EEOV260 had lower \((P < 0.05)\) LC counts compared with the CN on days 21 to 42. Moreover, on days 35 and 42, EEOV260 limited growth of the LC to the same extent as the PL \((P < 0.05; \text{Fig. 2})\). Overall, by day 14, all treatments were unable to inhibit Lm growth \((>2.0\) log cycles) and failed to achieve USDA-FSIS limits for Lm control by antimicrobial agents on postlethality environment products. However, the PL and EEOV260 treatments on ham loaves limited LC growth by up to \(1.0\) log cycle compared with the CN by days 35 and 42 (Fig. 2).

**FIGURE 1.** Listeria monocytogenes cocktail (LC) growth in emulsified poultry deli loaves formulated with antimicrobial agent at 4\(^\circ\)C. Symbols depict mean surviving LC from three distinct replicates \((n = 15)\). Poultry loaves were formulated to contain (i) no antimicrobial agent (control), (ii) potassium lactate \((2\%)\), (iii) EOV145 \((0.45\%)\), or (iv) EEOV260 \((0.60\%)\). Differing letters within each sample day show average mean significant difference \((P < 0.05)\) between (a through c) EOV145, no antimicrobial agent and potassium lactate, and (z through x) EEOV260, no antimicrobial agent and potassium lactate treatments. PSE, pooled standard error of presented experimental means.

**FIGURE 2.** Listeria monocytogenes cocktail growth in chunked and formed cured pork hams formulated with antimicrobial agent at 4\(^\circ\)C. Symbols depict mean surviving Lm from three distinct replicates \((n = 15)\). Hams were formulated to contain (i) no antimicrobial agent, (ii) potassium lactate \((2\%)\), (iii) EOV145 \((0.45\%)\), or (iv) EEOV260 \((0.60\%)\). Differing letters within each sample day show average mean significant difference \((P < 0.05)\) between (a through c) EOV145, no antimicrobial agent and potassium lactate, and (z through x) EEOV260, no antimicrobial agent and potassium lactate treatments.

**FIGURE 3.** Listeria monocytogenes cocktail growth in formed roast beef deli loaves formulated with antimicrobial agent at 4\(^\circ\)C. Symbols depict mean surviving Lm from three distinct replicates \((n = 15)\). Beef loaves were formulated to contain (i) no antimicrobial agent, (ii) potassium lactate \((2\%)\), (iii) EEOV245 \((0.45\%)\), or (iv) EEOV260 \((0.60\%)\). Differing letters within each sample day show average mean significant difference \((P < 0.05)\) between (a through c) EEOV245, no antimicrobial agent and potassium lactate, and (z through x) EEOV260, no antimicrobial agent and potassium lactate treatments.
On beef deli loaves formulated with EEOV260, LC was inhibited from ≥2.0 log CFU/g through 21 days of product storage; numbers of LC were significantly lower (P < 0.05) than LC on PL-containing or CN products (Fig. 3). From 28 days through experimental completion (49 days), however, LC numbers on EEOV260-treated product were not different from PL-treated product and failed to achieve USDA-FSIS regulation for Lm control by antimicrobial agents on postlethality environment products (Fig. 3). On roast beef deli loaves treated with EEOV245, no lag-phase extension (P ≥ 0.05) was observed (Fig. 3), though from days 14 to 42 the numbers of LC on EEOV245-treated product were lower (P < 0.05) by 0.7 log CFU/g compared with the CN. The PL exerted higher (>1.0 log CFU versus CN; P < 0.05) inhibition capacity versus the EEOV245. Overall on formed roast beef the EEOV260 was able to extend the lag phase and inhibited the growth of LC in the same manner of the PL. Therefore, the use of the EEOV245 is not recommended for use to meet process requirements imposed by USDA-FSIS regulatory mandates in uncured emulsified poultry bologna, cured chunked, and formed pork loaves, or formed roast beef; however, EEOV260 could act as a replacer of PL in pork and beef roast loaves.

Lloyd et al. (23) reported that formulating turkey loaves with 2.0% of either NaL or PL and dipping postlethality inoculated deli slices in a solution containing diacetate (0.25%) and NaL or potassium lactate salts (3.6%) resulted in an extended Lm lag phase through 56 days’ storage (4°C) compared with a nontreated control. Meat products dipped in formulations of ≥2.0% lactate salts can exhibit extended Lm lag phase in part due to interactions between lactate salts and pathogen cells. Vacuum-packaged turkey bologna formulated with 2.0% NaL and stored under 4°C was observed to allow a net increase of 1.3 log CFU/g of inoculated Lm by day 70 (46). Mbandi and Shelef (25) reported inhibited growth of Lm and a prolonging of the lag phase through 30 days on beef bologna formulated with 2.5% NaL and stored at 5°C for 45 days. Oussalah et al. (31) used alginate-based films treated with Spanish oregano EO as an inhibitor of Lm inoculated on ham slices stored at 4°C for 5 days; a 0.5-log-cycle reduction was attained compared with a control sample. In the current study, results obtained from formed roast beef for the PL are in agreement with previous research where net growth was limited to 1.8 to 2.0 log CFU/g at the end of the sampling periods (25, 29). However, lag-phase extensions from lactate salts reported on previous reports were not observed in this study. This observation is in agreement with the findings of Miller and Acuff (27), where beef top rounds were pumped with a 20.0% brine solution containing 2% NaL and were then sliced to 2-mm-thick slices. Sliced product was reported to be inoculated with 3.0 log CFU/cm² of Lm and stored under vacuum at 10°C for 28 days; NaL-containing product was compared with a control (0.0% NaL) sample. The NaL did not provide an extension of lag phase or inhibition of Lm; it was observed that by day 7, Lm had grown ≥2.0 log cycles and by day 28 reached 7.6 log CFU/cm², not differing from the control (27). However, it is possible that the incorporation of Na-diacetate (up to 0.25%) to the PL control could have altered the observed results for Lm in this experiment and possibly extended the lag phase, as suggested by published data (3, 23, 25, 37). On all the LC-inoculated deli loaves, a drop in Lm counts of ≥2 log CFU/g on day 49 was observed. This unexpected result is possibly explained by the out-competition caused by the natural microaerophilic biota present in the deli loaves (17).

Treatments (PL, CN, EO145, EEOV245, and EEOV260) for poultry bologna, pork ham, and roast beef deli products (Figs. 4 through 6) contained the same number of SC-inoculated cells (6.2 ± 0.09 log CFU/g) on day 0. Significant differences (P < 0.05) of 0.2 log cycle on day 0 were observed for poultry and pork (EO145) and beef (EEOV260) deli products (Figs. 4 through 6, respectively) compared with the CN and PL. However, these differences are not of practical significance and from the authors’ previous experience do not impact the results. In poultry bologna treated with EEOV260, a lag-phase extension is observed through 35 days of storage, and EEOV260 inhibited the growth (P < 0.05) of the SC compared with PL and CN samples (Fig. 4). Similar results were observed on poultry bologna treated with EO145 with respect to observed changes in SC numbers, maintenance of <2.0 log CFU/g growth during product storage, and achieving reduced growth versus PL or CN samples at 35 days of product storage (Fig. 4). When EO antimicrobials were applied to pork deli loaf formula, the EEOV260 inhibited (P
< 0.05) growth of SC at days 21 and 28 to the same level of efficacy as PL, although no differences were observed on the rest of the sample days (Fig. 5). Conversely, EOV145 did not exhibit inhibition of SC on pork loaves except on day 21, producing a 1.1-log CFU/g lower count in SC numbers versus CN (P < 0.05; Fig. 5). PL continued to exert inhibition (P < 0.05) of SC growth on days 28 and 35 compared with the CN while the EEOV260 was not different (P > 0.05) to the CN. On roast beef the EEOV245 was unable to inhibit SC growth until days 28 and 35 (ca. 0.5 log CFU/g compared with CN; P ≥ 0.05; Fig. 6). Similar results were observed for the EEOV260 when compared with the CN roast beef samples, were the SC growth is inhibited ca. 0.5 log CFU/g (Fig. 6).

Salmonella serovars are reported to not have capacity to grow and replicate at temperatures below 5.3 to 6.2°C, in a strain-dependent fashion (15, 31). However, the results reported show an increase of ca. 1.0 log cycle by day 35 on all treatments. Though the cause is unknown, this may be due in part to, or a combination of, nutrient availability, natural microflora synergism, and/or specific Salmonella serotypes that completed a slow growth under refrigeration (2, 35, 41). Mbandi and Shelef (25) reported that Salmonella inoculated on beef bologna under storage at 5 and 10°C were reduced to undetectable levels by days 30 and 20, respectively. Under temperature abuse (10°C), NaL (2.5%), and sodium diacetate (0.2%) addition to the product formulation produced a reduction of Salmonella to non-detectable levels at 20 days’ storage, compared with other treatments used on beef bologna (25). Additionally, refrigerated storage systems used in this study may not have maintained constant and even cooling, although digital temperature indicators and bulb thermometer readings were daily checked to verify cooler temperature. Contrary to previous reports, PL treatments were not able to inhibit growth of SC to undetectable levels on meat types tested. Houtsma et al. (13) demonstrated that the inhibitory effects of NaL is lower for gram-negative than for gram-positive bacteria, and observed that Salmonella had higher variation between strains minimum inhibitory concentration (e.g., Salmonella Heidelberg had higher minimum inhibitory concentration than Salmonella Typhimurium, 893 and 714 mM NaL, respectively) compared with Lm strains. Miller and Aucuff (27) inoculated 20% injected beef top rounds containing 2.0% NaL with Salmonella Typhimurium at 3.0 log CFU/cm² and stored them at 10°C for 28 days; no differences in Salmonella numbers were observed on NaL-formulated rounds when compared with rounds formulated without NaL, while 4% NaL inhibited Salmonella growth for 28 days. Oussalah et al. (31) reported 1.4- to 1.8-log CFU/cm² reductions of Salmonella Typhimurium on beef bologna and ham slices containing an alginate-based film with either savory, oregano, and cinnamon EO as antimicrobials at 5 days of storage (4°C). Minced sheep

![Figure 5](http://meridian.allenpress.com/jfp/article-pdf/78/4/698/1688168/0362-028x_jfp-14-273.pdf)  
**FIGURE 5.** Salmonella cocktail (composed of Salmonella Typhimurium, Salmonella Heidelberg, and Salmonella Enteritidis) survival in chunked and formed cured pork hams formulated with antimicrobial agent at 4°C. Symbols depict mean surviving SC from three distinct replicates (n = 15). Hams were formulated to contain (i) no antimicrobial agent, (ii) potassium lactate (2%), (iii) EOV145 (0.45%), or (iv) EEOV260 (0.60%). Differing letters within each sample day show average mean significant difference (P < 0.05) between (a through c) EOV145, no antimicrobial agent and potassium lactate, and (z through x) EEOV260, no antimicrobial agent and potassium lactate treatments.

![Figure 6](http://meridian.allenpress.com/jfp/article-pdf/78/4/698/1688168/0362-028x_jfp-14-273.pdf)  
**FIGURE 6.** Salmonella cocktail (composed of Salmonella Typhimurium, Salmonella Heidelberg, and Salmonella Enteritidis) survival in formed roast beef deli loaves formulated with antimicrobial agent at 4°C. Symbols depict mean surviving SC from three distinct replicates (n = 15). Beef loaves were formulated to contain (i) no antimicrobial agent, (ii) potassium lactate (2%), (iii) EEOV245 (0.45%), or (iv) EEOV260 (0.60%). Differing letters within each sample day show average mean significant difference (P < 0.05) between (a through c) EEOV245, no antimicrobial agent and potassium lactate, and (z through x) EEOV260, no antimicrobial agent and potassium lactate treatments.
meat treated with 0.6 and 0.9% oregano EO showed enhanced bacteriostatic effect at 10°C over samples stored at 4°C (12).

EO formulae 1 and 2 were unable to provide reduction of SC numbers when applied to chunked and formed cured ham and formed roast beef. However, on emulsified poultry bologna the EEOV260 is able to extend the lag phase and inhibit the SC at levels comparable with PL-treated samples. Thus, the EEOV260 could be used as a PL replacer for emulsified poultry bologna, but the EO cannot be considered as replacers of PL for either, chunked and formed pork or formed roast beef deli loaves. The encapsulation and micronization of EOs tested can provide the possibility of replacing PL for control of Ln in ham and roast beef products only. In addition, EO can also help inhibit the growth of Salmonella (Typhimurium, Heidelberg, and Enteritidis) on emulsified poultry bologna. Moving forward, more research is needed to validate EO effectiveness on LC and SC present in meat RTE products either through new manufacturing procedures or formula, in order to identify products that meet USDA-FSIS regulations for postlethality environment products.

ACKNOWLEDGMENTS
Author Casco’s salary was supported by the Department of Poultry Science, Texas A&M University (College Station, TX). Research costs were supported by Texas A&M AgriLife Research (College Station, TX).

REFERENCES