Research Note

Fate of Surface-Inoculated Escherichia coli O157:H7, Listeria monocytogenes, and Salmonella Typhimurium on Kippered Beef during Extended Storage at Refrigeration and Abusive Temperatures†

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ABSTRACT

The behavior of Escherichia coli O157:H7, Listeria monocytogenes, and Salmonella Typhimurium on kippered beef was evaluated. Individual pieces of the product were separately inoculated on the top and bottom surfaces with each three- to six-strain pathogen cocktail at ca. 6.0 log CFU per piece and stored at 4, 10, 21, or 30°C for up to 28 days in each of two trials. When kippered beef was inoculated with E. coli O157:H7, Salmonella Typhimurium, or L. monocytogenes and stored at 4, 10, 21, or 30°C for up to 28 days, pathogen numbers decreased ca. 0.4 to 0.9, 1.0 to 1.8, 3.0 to 5.25, and ≥5.0 to 5.25 log CFU per piece, respectively. Average D-values for E. coli O157:H7, Salmonella Typhimurium, and L. monocytogenes stored at 4 to 30°C for 28 days were ca. 41 to 4.6, 40.8 to 5.3, and 29.5 to 4.3 days, respectively. As expected, the higher the storage temperature, the greater the level and rate of inactivation for all three pathogens. These data establish that kippered beef does not provide an environment conducive to proliferation of these pathogens.

The meat snack market, which includes dried, salted, and/or cooked meats such as kippered beef and jerky products, continues to experience rapid growth in the United States as evidenced by an increase in sales from US$631.6 million in 1994 to nearly US$2.7 billion in 2004 (23). According to the U.S. Department of Agriculture (USDA) (26), kippered beef is a cured, dried product that is similar to beef jerky, but not as hard and dry. In brief, kippered beef is produced by marinating whole muscle strips of beef followed by cooking/drying and/or smoking for a specific time/temperature to a moisture-to-protein ratio (M:Pr) value followed by cooking/drying and/or smoking for a specific time/temperature to a moisture-to-protein ratio (M:Pr) value of ≥2.03:1 and ≥0.76:1 (26). Although there are no regulatory requirements stipulating a target value for water activity (a_w), due to its similarity to beef jerky it is assumed that to be shelf stable kippered beef should have a relatively low a_w (e.g., ≤0.80) (27). Although there have been no reported illnesses in the United States attributed to kippered beef, this product type was implicated in a 2003 recall of approximately 200 pounds due to possible contamination with Listeria monocytogenes (25). However, similar products such as beef jerky have been associated with foodborne illnesses and recalls due to Salmonella, Escherichia coli O157:H7, or L. monocytogenes (6, 7, 13, 28). Thus, it is possible that kippered beef could also serve as a vehicle for foodborne disease.

E. coli O157:H7, L. monocytogenes, and Salmonella Typhimurium collectively cause an estimated 65,610 illnesses, 4,635 hospitalizations, and up to 500 deaths each year in the United States (17). These pathogens have been associated with several outbreaks and a large number of voluntary and obligatory recalls of food products in many states over the last 10 years. Although manufacturing processes are designed to destroy these pathogens, many of the public health concerns linked to ready-to-eat meats have been due to postprocessing contamination (1) and the fact that such products are typically consumed without further heating and/or preparation.

Despite the recovery of L. monocytogenes from kippered beef and/or Salmonella and E. coli O157:H7 from similar products, such as beef jerky, to our knowledge there have been no studies published that have evaluated the ability of kippered beef to support growth/survival of these target pathogens. As part of our ongoing programmatic efforts to assist small to very small producers of specialty/ethnic meats, the objective of the present study was to evaluate the fate of E. coli O157:H7, L. monocytogenes, and Salmonella Typhimurium on the surface of kippered beef.

MATERIALS AND METHODS

Bacterial strains. The three strains of E. coli O157:H7 (EC505B, C7927, and SLH21788), five strains of L. monocytogenes.
genes (MFS2, MFS102, MFS104, MFS105, and MFS110), and six strains of *Salmonella Typhimurium* (H3278, G7601, H3402, H2662, H3380, and G8430) used in this study to inoculate the surface of kippered beef were confirmed, cultured, and maintained as described previously (21).

**Preparation and inoculation of kippered beef.** The two batches (one batch per each of two trials) of freshly manufactured, vacuum-packaged kippered beef were obtained from a producer/collaborator (Wild Bill’s Foods, Inc., Leola, PA) and stored at 4°C for up to 1 week prior to being used in an experiment. The commercially prepared kippered beef was formulated with lean beef trim, brown sugar, salt, dextrose, water, monosodium glutamate, natural hickory smoked flavor, spices, hydrolyzed soy protein, freeze-dried pineapple juice, soy sauce powder (soybeans, salt, and wheat), citric acid, flavorings, and sodium nitrite. The beef was sliced two times, placed in a mixer/grinder, mixed with the other ingredients, and then extruded/form into strips that were subsequently placed on a screen and cooked and dried at 180°F for 1.5 h to a M:Pr of <2:03:1 to >0.76:1. Prior to inoculation, the kippered beef was transferred aseptically from the original package onto sterile polystyrene foam packing trays (Koch Supplies, Kansas City, MO) and cut with the aid of a sterile knife into pieces (3.5 cm long by 2.5 cm wide by 0.3 cm high) weighing on average ca. 6.5 ± 1.5 g. Individual pieces were placed onto sterile polystyrene foam packing trays and separately surface inoculated on one side for 50 μl of each multidrain pathogen cocktail to a target level on average of ca. 6.0 log CFU/g. The inoculum was distributed across the entire surface area of each piece of kippered beef with the aid of a sterile L-shaped plastic spreader (Midsci, St. Louis, MO). The uncovered trays were placed in a laminar-flow hood and left for 15 min at room temperature (23 ± 2°C or 73.4 ± 3.6°F) for bacterial attachment. The pieces were then inverted, and the opposite side was surface inoculated with the same volume and levels of the pathogens, the cells were distributed across the meat surface with a sterile plastic spreader, and the inoculated strips were placed in the laminar-flow hood for another 15 min. Pieces that were not inoculated were used as controls. Pieces were individually repackaged in sterile polyethylene sampling bags, vacuum sealed to 95 × 10^5 Pa with a Multivac A300/16 vacuum-packaging unit (Sepp Hagemüller KG, Wolfertschwenden, Germany), and then stored at 4, 10, 21, or 30°C. For each of the two trials, at each storage temperature, triplicate samples were analyzed at each sampling interval for up to 28 days (N = 2 trials; n = 3 replicates per piece per sampling interval per trial).

**Microbiological analyses.** Pathogens were recovered using the USDA/ARS package rinse method (15) with 5 ml of sterile 0.1% peptone water. Pathogens were enumerated by spread plating 500 μl of the resulting rinsate essentially as described earlier (21); typical colonies were counted, and bacterial numbers were expressed as log CFU per piece. When pathogen levels decreased below the detection limit (<0.7 log CFU per piece) by direct plating, samples were enriched as previously described (21). The total aerobic plate count and total lactic acid bacteria count (LAB) were enumerated on days 0, 45, and 90 by spread plating 100 μl of the control rinsate or dilutions thereof onto brain heart infusion (BHI; Difco, Becton Dickinson Co., Sparks, MD) and onto deMan Rogosa Sharpe (MRS; Difco, Becton Dickinson) agar plates, respectively. MRS agar plates were incubated anaerobically (10.1% carbon dioxide, 4.38% hydrogen, and balance nitrogen; Bactron IV Anaerobic/Environmental Chamber, Sheldon Manufacturing Inc., Cornelius, OR) at 37°C for 48 h, and the BHI agar plates were incubated at 30°C for 72 h. Typical colonies were counted, and the bacterial numbers were expressed as log CFU per piece.

**Proximate composition analyses.** Chemical analyses were performed for each batch by using a composite sample (ca. 60 g total) comprised of ca. 10 pieces of kippered beef per each batch.

**RESULTS AND DISCUSSION**

Direct plating and/or enrichment of the two batches of kippered beef before inoculation revealed the absence of any indigenous *E. coli* O157:H7, *Salmonella Typhimurium*, or *L. monocytogenes* (data not shown). The average initial levels of total aerobic plate count and LAB on kippered beef were 7.08 log CFU per piece (range, 6.53 to 7.55 log CFU per piece) and 7.66 log CFU per piece (range, 6.65 to 8.68 log CFU per piece), respectively. Regarding proximate composition (Table 1), with a M:Pr ratio value of 1.28:1 this product is in compliance with the USDA/FSIS guidelines (M:Pr ratio, ≥2:03:1 and ≥0.76:1) and can be designated kippered beef (25). The aw of the product tested herein was 0.648 ± 0.021. In 2004, the USDA/FSIS issued compliance guidelines specifically for jerky-type products, stating that to be designed as shelf stable, it must have an aw value of ≤0.80 (27). Although the product is similar in its manufacture and proximate composition to beef jerky, to our knowledge, an aw value requirement and/or compliance guidelines have not been specified for the stability and safety of kippered beef.

In general, when separately inoculated onto the surface

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**TABLE 1. Proximate composition of kippered beef**

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Value obtained (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salt (g/100 g)</td>
<td>3.70 ± 0.23</td>
</tr>
<tr>
<td>pH</td>
<td>5.83 ± 0.01</td>
</tr>
<tr>
<td>aw</td>
<td>0.648 ± 0.021</td>
</tr>
<tr>
<td>Moisture (g/100 g)</td>
<td>41.90 ± 0.14</td>
</tr>
<tr>
<td>Protein (g/100 g)</td>
<td>32.70 ± 0.42</td>
</tr>
<tr>
<td>M:Pr</td>
<td>1.28 ± 0.07</td>
</tr>
<tr>
<td>Fat (g/100 g)</td>
<td>5.06 ± 2.24</td>
</tr>
<tr>
<td>Carbohydrates (g/100 g)</td>
<td>14.25 ± 2.62</td>
</tr>
<tr>
<td>Ash (g/100 g)</td>
<td>6.08 ± 0.21</td>
</tr>
</tbody>
</table>

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*a Two trials were performed with each of two batches, using a composite sample (ca. 60 g total) comprised of ca. 10 pieces of kippered beef per each batch.

*b Mean of three batches ± standard deviation from analyses performed by the producer on two samples per each batch.

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**Statistical analyses.** The lethality/D-values, that being the time required in days to achieve a 1.0-log CFU/g reduction at a given storage temperature, were estimated by regression for pathogen lethality on pieces of kippered beef, using version 9.1.3 of the Statistical Analysis Systems software (SAS Institute, Inc., Cary, NC). The D-value is the absolute value of the inverse of the linear inactivation rate of the surviving cell fraction. Mean separations were performed using the Bonferroni least significant difference (LSD) test.
of kippered beef, all three pathogens were inactivated to a greater ($P \leq 0.05$) extent at the higher storage temperatures (21 and 30°C) than at the lower (4 and 10°C) storage temperatures (Table 2). In fact, there was relatively little inactivation of any of the pathogens even after 28 days at 4°C (0.43 to 0.89 log CFU per piece) or 10°C (0.97 to 1.81 log CFU per piece). However, after 10 days, storage at 30°C was significantly ($P \leq 0.05$) more deleterious toward all three pathogens than storage at 21°C, and storage at either 30 or 21°C was more deleterious than storage at 4 or 10°C. Our data are also in general agreement with previous studies showing that higher storage temperatures ($\geq 21^\circ$C) are more adverse toward cells of *L. monocytogenes*, *Salmonella Typhimurium*, and *E. coli* O157:H7 in or on ready-to-eat meats than colder storage temperatures ($\leq 15^\circ$C) (2, 8, 11, 22). The ability of these pathogens to grow and/or survive at lower temperatures compared to higher temperatures may be attributed to the intracellular accumulation of osmo- and cryoprotectant solutes, namely, glycine betaine, and/or to the adjustment of the fluidity of the cell membrane (14, 18, 20, 24). Moreover, the greater inactivation observed when the product was stored at 21 or 30°C can most likely be attributed to a combination of the high temperature of storage and the intrinsic factors of the product itself, such as a lower $a_w$ value and higher salt content. It could also be attributed to the ingredients used, such as smoke compounds, spices, soy sauce, citric acid, and/or sodium nitrite, which also have demonstrated antimicrobial activity.

Our results showed that when the surface of kippered beef was inoculated with *E. coli* O157:H7 and stored at 4, 10, or 21°C for up to 28 days, pathogen numbers decreased by 0.74, 1.35, and 3.99 log CFU per piece, respectively. When kippered beef was stored at 30°C, *E. coli* O157:H7 numbers decreased by $\geq 5.05$ log CFU per piece within 21 days of storage, and subsequent enrichment of these samples for up to 28 days revealed no viable cells of *E. coli* O157:H7. Likewise, when kippered beef inoculated with *Salmonella Typhimurium* was stored at 4, 10, or 21°C for up to 28 days, pathogen numbers decreased by 0.43, 0.97, and 2.99 log CFU per piece, respectively, whereas when stored at 30°C pathogen numbers decreased by $\geq 5.05$ log CFU per piece within 21 days of storage. However, cells of *Salmonella Typhimurium* were recovered from the surface of kippered beef for up to 28 days of storage at 30°C by enrichment. With regard to *L. monocytogenes*, when kippered beef was stored at 4, 10, or 21°C for up to 28 days,
pathogen numbers decreased by 0.89, 1.81, and ≥5.25 log CFU per piece, respectively. However, when kippered beef was stored at 30°C, numbers of *L. monocytogenes* decreased by ≥5.25 log CFU per piece within 10 days and viable cells were not recovered even by enrichment after 13 days of storage. Although there have been no studies published on the fate of pathogens associated with kippered beef, the inability of the three pathogens tested herein to grow on the surface of this product is consistent with published findings for comparable products. For example, Calicioglu et al. (3–5) monitored the fate of *E. coli* O157:H7, *Salmonella* Typhimurium, and *L. monocytogenes* inoculated postdrying on marinated and nonmarinated beef jerky. In general, their results showed that after 28 days of storage at 25°C, pathogen numbers decreased by ca. 3.5 to 5.0 log CFU/cm² on the surface of both marinated and nonmarinated beef jerky, with the greatest reductions observed for jerky prepared using modified marinades containing antimicrobials such as sodium lactate, acetic acid, and/or Tween 20. In another study, Ingham et al. (12) evaluated the survival of *L. monocytogenes* on the surface of beef jerky and related products stored at 21°C for up to 28 days. These authors reported that none of the 15 commercial beef jerky and related products tested supported growth of *L. monocytogenes*. In fact, pathogen numbers decreased by 2.3 to 5.6 log CFU on vacuum-packaged products stored at room temperature for 28 days. Collectively, these findings emphasize that if postprocessing contamination would occur, higher storage temperatures (≥21°C) in combination with low aw values (≤0.80) could be effective at controlling the subsequent outgrowth of *E. coli* O157:H7, *Salmonella* Typhimurium, and *L. monocytogenes* on the surface of dried meat snacks, such as kippered beef and jerky products.

The mean *D*-values for *E. coli* O157:H7, *Salmonella* Typhimurium, and *L. monocytogenes* in kippered beef stored at 4, 10, 21, and 30°C are shown in Table 3. Since in this study a ≥1.0 log reduction was not achieved at 4°C for any of the three pathogens tested, the *D*-value at 4°C was generated by extrapolation using the linear regression of the data. That being said, as expected, the generated *D*-values decreased with increased storage temperature. Although differences in the extent and rate of inactivation among the three pathogens tested were evident by empirical inspection of these data, such differences among these pathogens were not significant (*P ≥ 0.05*) when analyzed statistically. These results are in agreement with our previous work wherein inactivation rates for all three pathogens on the surface of a soudjouk-style sausage decreased with an increase in storage temperature (22). Thus, storage temperature has a marked influence on the inactivation of *E. coli* O157:H7, *L. monocytogenes*, and *Salmonella* Typhimurium on the surface of dried/fermented meat products.

We and others have validated the lethality of processes and/or have developed interventions to better manage public health concerns related to the fate of pathogens associated with salami, pepperoni, summer sausages, soudjouk, and jerky (2, 8–10, 19, 21, 22). However, to the best of our knowledge, the present study is the only or first scientific report describing the fate of foodborne pathogens on the surface of kippered beef. Thus, our results validated that refrigerated storage of kippered beef prevents pathogen outgrowth, whereas storage at ambient temperature, as is typical at retail, is effective in reducing levels of *E. coli* O157: H7, *L. monocytogenes*, or *Salmonella* Typhimurium that may on occasion be present on the surface of such products as a result of postprocess contamination. These data can also be used as guidelines by both policy makers and producers to enhance the safety of kippered beef and related products.

**ACKNOWLEDGMENTS**

We extend our appreciation to Greg B. Rhiner (Wild Bill’s Foods, Inc., Leola, PA), as well as to the following individuals in the Microbial Food Safety Research Unit (USDA/ARS/ERRC, Wyndmoor, PA), who contributed in large measure to the successful completion of this study by sharing their time, talents, resources, and/or opinions: John Phillips, Brad Shoyer, Jean Smith, Latika LeSeane, Ellen Sanders, Tina Sharp, Marcela Soto, John Cherry, Rosemary Martinjuk, and Dimitra Dourou. On behalf of Renata Jacob, we also express our gratitude to Marisa A. R. d’Arce, Carmen J. C. Castilho, and Marília Oetterer (University of São Paulo, Piracicaba, Brazil) for their support and helpful suggestions.

**REFERENCES**

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**TABLE 3. D-values for *E. coli* O157:H7, *Salmonella* Typhimurium, and *L. monocytogenes* inoculated onto the surface of kippered beef**

<table>
<thead>
<tr>
<th>Storage temp (°C)</th>
<th><em>E. coli</em> O157:H7</th>
<th><em>Salmonella</em> Typhimurium</th>
<th><em>L. monocytogenes</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>41.06 ± 16.12</td>
<td>40.78 ± 21.57</td>
<td>29.49 ± 17.25</td>
</tr>
<tr>
<td>10</td>
<td>19.05 ± 3.44</td>
<td>24.14 ± 7.01</td>
<td>14.13 ± 3.83</td>
</tr>
<tr>
<td>21</td>
<td>7.30 ± 0.51</td>
<td>8.43 ± 0.88</td>
<td>4.98 ± 0.47</td>
</tr>
<tr>
<td>30</td>
<td>4.60 ± 0.20</td>
<td>5.34 ± 0.34</td>
<td>4.30 ± 0.35</td>
</tr>
</tbody>
</table>

* D-values represent the absolute value of the inverse of the linear inactivation rate of the surviving cell fraction. The D-value at 4°C was generated by extrapolation using the linear regression of the data. Means with different letters within rows are significantly (*P ≤ 0.05*) different.


