A Research Note

Thermal Destruction of *Listeria monocytogenes* in Ground Pork Prepared with and without Soy Hulls

PATRICIA OLLINGER-SNYDER, FATHY EL-GAZZAR,¹
M. EILEEN MATTHEWS,* ELMER H. MARTH, and NAN UNKLESBAy²

¹Department of Dairy Science, Faculty of Agriculture, University of Assiut, Assiut, Egypt; and ²Department of Food Science and Human Nutrition, College of Agriculture, Food and Natural Resources, University of Missouri–Columbia, Columbia, Missouri

(MS# 94-8: Received 30 December 1993/Accepted 23 May 1994)

**ABSTRACT**

D-values and z-values were determined for *Listeria monocytogenes* Scott A cells heated in raw ground pork prepared with and without soy hulls and in a soy hull/water mixture. Products inoculated with ca. 10⁷ colony-forming units (CFU) per g were sealed in glass vials, immersed in a water bath, and held at 50, 55, 60, or 62°C for predetermined times. Survival was determined by testing heated samples with McBride listeria agar. The D-values for *L. monocytogenes* cells at 50, 55, and 60°C were 108.81, 9.80, and 1.14 min, respectively, when heating was in ground pork and 113.64, 10.19, and 1.70 min, respectively, when heating in ground pork with added soy hulls. At 62°C *L. monocytogenes* cells were inactivated too rapidly to permit determination of the D-value. The D-values for *L. monocytogenes* in the soy hull/water mixture at 50 and 55°C were 19.84 and 3.94 min, respectively. *L. monocytogenes* cells were inactivated too quickly to determine the D-value at 60°C. The z-values for *L. monocytogenes* in ground pork prepared with and without soy hulls were 5.45 and 5.05°C, respectively. If ground pork naturally contains 10⁷ *L. monocytogenes* cells per g and if we want to assure safety with a 4-D *Listeria* cook (reducing the *L. monocytogenes* population by four orders of magnitude), then according to results of this study, ground pork must be heated to an internal temperature of 60°C for at least 4.6 min and ground pork with added soy hulls for at least 6.8 min.

Key words: *Listeria monocytogenes*, ground pork, soy hulls

*Listeria monocytogenes* is widely distributed in the environment (15) and has been found in a wide variety of food products (25). According to Ryser and Marth (25), from 0 to 80% of raw meat and from 7 to 80% of European ground meat samples contained *L. monocytogenes*. In the United States, Buchanan et al. (8) found *L. monocytogenes* in 25 to 100% of ground meat samples and in 0 to 100% of fresh meat samples. Four large food related outbreaks of listeriosis (6, 13, 19, 26) are responsible for the considerable attention this pathogen has received; none of the outbreaks involved meat. However, precooked chicken (1), frankfurters (2, 4), Cajun pork sausage (3), and paté (22) have been implicated in cases of human listeriosis.

Investigators have reported that *L. monocytogenes* can survive in ground meat following some heat-processing methods (Table 1) (7, 11, 12, 18, 27). Because there are only a few reports on the thermal resistance of *L. monocytogenes* in ground meat, research was done to determine the thermal-death time for *L. monocytogenes* in raw ground pork. Since consumption of soy hulls may be beneficial to the health of humans (17, 20, 24), and since this study was part of a larger research project evaluating the benefits of incorporating soy hulls into ground pork, D-values were also determined for *L. monocytogenes* in raw ground pork prepared with soy hulls.

**MATERIALS AND METHODS**

Experimental product

Prepackaged ground pork (ca. 0.5 kg) was purchased at a local supermarket the day before each experiment and stored at ≤ 2°C in a refrigerator until analyzed. The recipe used to prepare patties with soy hulls was as follows: 314.0 g of ground pork, 124.5 g of autoclaved water, 28.5 g of soy hulls and 3.0 g of salt. The dry ingredients were weighed and placed into a 9.4 l mixing bowl. The water was weighed and added to the dry mixture, which was then mixed with a mixer (Hobart Corporation, Model C100, Troy, OH) on low speed for 10 s. The meat was then mixed with a mixer (Hobart Corporation, Model C100, Troy, OH) on low speed for 10 s. The meat was removed from the mixer, broken apart with a tablespoon, weighed, and added to the mixture, which was then mixed on low speed for 15 s. The mixer was turned off, and a spoon was used to scrape down the mixture from the sides of the mixing bowl. The product was then mixed on low speed for an additional 15 s.

Ground pork without soy hulls had no ingredients added. For soy hulls alone, 13.25 g of autoclaved water were added to 36.75 g of soy hulls. Both mixtures were mixed by hand with a stainless-steel spoon for 3 min. All utensils used to prepare these were autoclaved before each experiment.

Inoculation procedure

*Listeria monocytogenes* Scott A (serotype 4b), obtained from the Food Research Institute of the University of Wisconsin–Madison, was used in this study. Stock cultures were maintained through bimonthly transfer on tryptose agar (TA) (Difco Labora-
Table I. D-value studies of *Listeria monocytogenes* in ground meats.

<table>
<thead>
<tr>
<th>Meat Type Description</th>
<th>Initial Count (CFU/g)</th>
<th>Heating Container</th>
<th>Plating Medium</th>
<th>D-Values (min)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irradiated ground meat</td>
<td>$10^7$</td>
<td>laminated pouches</td>
<td>NP*</td>
<td>$D_{50^oC} = 13.18$</td>
<td>Farber (12)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$D_{sv^oC} = 6.39$</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$D_{mv^oC} = 3.12$</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$D_{tv^oC} = 1.01$</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$D_{av^oC} = ND^a$</td>
<td></td>
</tr>
<tr>
<td>Ground beef slurry</td>
<td>$10^7 - 10^8$</td>
<td>sealed jars</td>
<td>MDSA*</td>
<td>$D_{50^oC} = 0.63$</td>
<td>Boyle et al. (7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$D_{sv^oC} = 0.29$</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$D_{mv^oC} = 0.15$</td>
<td></td>
</tr>
<tr>
<td>Ground beef roast</td>
<td>$10^4 - 10^5$</td>
<td>sealed pouches</td>
<td>TA/\textit{g}LPMe*</td>
<td>$D_{50^oC} = 22.24/19.3\textit{f}$</td>
<td>Schoeni et al. (27)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$D_{sv^oC} = 15.37/17.5\textit{f}$</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$D_{mv^oC} = 4.47/3.5\textit{f}$</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$D_{tv^oC} = 2.56/2.82\textit{f}$</td>
<td></td>
</tr>
<tr>
<td>Lean ground beef (2% fat)</td>
<td>$10^7$</td>
<td>sealed tubes</td>
<td>Columbia CNA/\textit{LPMe}</td>
<td>$D_{50^oC} = 56.1/81.3\textit{h}$</td>
<td>Fain et al. (11)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$D_{sv^oC} = 2.4/2.6\textit{h}$</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$D_{mv^oC} = 0.5/0.6\textit{h}$</td>
<td></td>
</tr>
<tr>
<td>Fatty ground beef (30.5% fat)</td>
<td>$10^7$</td>
<td>sealed tubes</td>
<td>Columbia CNA/\textit{LPMe}</td>
<td>$D_{50^oC} = 34.5/71.1\textit{h}$</td>
<td>Fain et al. (11)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$D_{sv^oC} = 4.6/5.8\textit{h}$</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$D_{mv^oC} = 1.1/1.2\textit{h}$</td>
<td></td>
</tr>
</tbody>
</table>

* NP = Not provided.
* ND = No viable cells detected.
* Modified Doyle-Schoeni selective agar.
* Tryptose agar.
* *Listeria* plating medium.

The first number represents survival of *Listeria monocytogenes* as determined by enumeration on tryptose agar; the second number represents survival of *L. monocytogenes* that were stored in a refrigerator at 4°C until analyzed. Each heat treatment was duplicated.

**Moisture and pH measurements**

**Moisture.** The Association of Official Analytical Chemists (AOAC) Method No. 934.01 (15), with modification, was used to determine moisture content; however, sample size was increased from ca. 2 g to ca. 5 g.

**pH.** Five milliliters of distilled water were added to each sample (20 g) of ground pork prepared with or without soy hulls. Samples were placed in sterile stomacher bags (Tekmar Co., Inc., Cincinnati, OH) then mixed for 10 min in a stomacher™ (Seward Medical, Model 400, London, UK). A pH meter (Orion Research Inc., Model 610A, Cambridge, MA) was used for the determination, the standard combination electrode was directly inserted into each sample. Two readings were made and average values were calculated.

**Thermal inactivation**

One hundred grams of ground pork (57.49% moisture, pH 5.92) or 100 g of ground pork prepared with soy hulls (64.6% moisture, pH 5.57) or 50 g of the soy hull/water (26.5% water, pH 5.16) mixture were weighed, inoculated, and stirred with a stainless steel spoon for 3 min. Four grams of the product were weighed into tooled-neck glass vials (16 mm outside diameter by 50 mm height). Polyethylene closures with copper/constantan thermocouples (Omega Engineering Inc., Stamford, CT) attached were used to seal the vials. The capped vials were placed into racks, which were covered with weighted plastic mesh to prevent the vials from floating in the water bath (Precision Scientific, Chicago, IL) during heating.

The racks were placed into the water bath when the five thermocouples, attached to plastic rods extending from the bottom of the water bath, reached the test temperature. Timing began when the temperature of the product reached the test temperature. Heating and cooling times and temperatures were monitored and recorded with a multipoint recorder (Leeds and Northrup, Model 250, Milwaukee, WI). The tubes of product were removed at each prescribed time interval, cooled in a water/ice mixture (< 4°C) until the product reached an internal temperature of 4°C, and then stored in a refrigerator at 4°C until analyzed. Each heat treatment was duplicated.
Enumeration of L. monocytogenes

All samples were serially diluted in 0.1% peptone water and plated onto duplicate plates of McBride listeria agar (MLA) (21) with 0.5% lithium chloride added to inhibit gram-negative organisms. Plates were incubated for 48 h at 35°C. Colonies counted as L. monocytogenes were small, low, convex, finely textured, bluish gray, and weakly β-hemolytic. Initial counts (defined as counts at 0 time) were determined on unheated samples.

Uninoculated samples were cold treated. Twenty-five grams of sample were added to 225 ml of tryptose broth (TB; Difco), blended in a Stomacher™ for 5 min, stored at 4°C, and sampled after 2-, 4-, and 6-week intervals by streaking material onto MLA plates and incubating for 48 h at 35°C.

Statistical analyses

Linear regressions (23) were performed on all data for a given temperature and used to calculate D-values for L. monocytogenes. An analysis of variance (ANOVA) was used to determine significant differences in pH and moisture among treatments for the raw product and for D-values for L. monocytogenes in the products prepared with and without soy hulls. A level of P < 0.05 was used to establish significant differences.

RESULTS AND DISCUSSION

Statistically significant differences were found for moisture and pH. The pH was lower for ground pork with rather than without added soy hulls, which can be attributed to the low pH (5.16) of the soy hulls. As expected, the moisture content was highest for ground pork prepared with soy hulls, because of the water (124.5 g) added to this product. The D-values for L. monocytogenes in ground pork with and without added soy hulls were not significantly different. The D-values for L. monocytogenes ranged from 108.81 to 1.14 min for ground pork prepared without soy hulls and from 113.64 to 1.70 min for ground pork with soy hulls added; the z-values were 5.05 and 5.45°C, respectively (Table 2). Heating at 62°C inactivated L. monocytogenes cells at a rate too rapid to permit accurate measurement of the thermal-death time. The D-values for L. monocytogenes in the soy hull/water mixture were 19.84 min at 50°C and 3.94 min at 55°C. Since L. monocytogenes cells in the soy hull/water mixture were inactivated so quickly at 60°C, it was impossible to determine the thermal death time at that temperature.

Table 2. D-values and z-values for Listeria monocytogenes Scott A in ground pork prepared with and without soy hulls.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Ground Pork Without Soy Hulls</th>
<th>Ground Pork With Soy Hulls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>D-Value (min)</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>108.81 (5.09)</td>
<td>113.64 (6.67)</td>
</tr>
<tr>
<td>55</td>
<td>9.80 (0.47)</td>
<td>10.19 (0.88)</td>
</tr>
<tr>
<td>60</td>
<td>1.14 (0.07)</td>
<td>1.70 (0.07)</td>
</tr>
<tr>
<td>62</td>
<td>ND¹</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>z-Value (°C)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.05 (0.12)</td>
<td>5.45 (0.47)</td>
</tr>
</tbody>
</table>

¹ Values in ( ) are the standard deviations.
² ND, no viable cells detected.

Farber (12) reported a D-value of 3.12 min for L. monocytogenes in ground meat heated to an internal temperature of 60°C. Schoeni et al. (27) noted a D-value of 3.5 to 4.47 min for L. monocytogenes in ground beef roast heated to 60°C (Table 1). Boyle et al. (7) found a D-value of 0.63 min for L. monocytogenes inoculated into a ground beef slurry, sealed in glass jars, and heated to 60°C. According to Fain et al. (11), decimal reduction times ranged from 0.5 to 0.6 min at 63°C for L. monocytogenes heated in lean (2% fat) ground beef. In the same study, the authors found decimal reduction times ranging from 1.1 to 1.2 min at 63°C for L. monocytogenes in fatty (30.5% fat) ground beef.

Boyle et al. (7) inoculated L. monocytogenes cells into a meat slurry composed of 20% ground beef and 80% water. Hydrating soy hulls in the present study showed that the more water added, the greater the rate of thermal destruction. The D-values determined in the present study are lower than those reported by Farber (12) and Schoeni et al. (27), and higher than those reported by Boyle et al. (7). Farber (12) inoculated irradiated ground meat with five different strains of L. monocytogenes. The containers used by these investigators were different, as were the plating media. Different strains of L. monocytogenes and differences in the plating media, the composition of the ground meat, and the types of containers used could all account for differences between the D-values found by these investigators and those reported in the present communication. The D-values at 60°C in our study fall between the values of 2.4 and 2.6 min at 57°C and 0.5 and 0.6 min at 63°C observed by Fain et al. (11) for L. monocytogenes cells inoculated into lean ground beef.

El-Shenawy et al. (9) found that when L. monocytogenes cells were heated (50, 55, 60, 65, 70, and 75°C), more cells were injured at lower than at higher temperatures. Other investigators have shown that the greatest number of injured cells occurred when L. monocytogenes cells were frozen or heated to temperatures of generally < 54°C (14, 28). McBride listeria agar does not detect injured cells. However, three of the four temperatures used to determine D-values in the present study were > 54°C.

Results of this study indicate that ground pork needs to be heated to an internal temperature of 60°C for at least 4.6 min to achieve a 4-D Listeria cook (reducing the L. monocytogenes population by four orders of magnitude); thus, if the natural contamination of the pork is 10⁶ CFU of L. monocytogenes cells per g, the heat process would give a reasonable assurance of safety. Ground pork with soy hulls added needs to be heated for at least 6.8 min to achieve a 4-D Listeria cook. In practical terms, the treatment would result in a 100-g pork patty that would contain one Listeria cell. With the infective dose of L. monocytogenes being unknown (5, 10), but generally thought to be low for susceptible individuals — neonates, pregnant women, cancer patients, the elderly, and those with acquired immune deficiency syndrome or other illnesses affecting the immune system (10) — a Listeria cook > 4-D may be required to assure virtual absence of L. monocytogenes in ground pork.
consumed by highly susceptible persons. Therefore, it is important to minimize contamination and to prevent growth of *L. monocytogenes* cells in ground pork before cooking, so that initial numbers will be low.

**ACKNOWLEDGMENTS**

This research was supported by the College of Agricultural and Life Sciences, University of Wisconsin–Madison.

**REFERENCES**