The Effect of Liquid Smoke on *Listeria monocytogenes*

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ABSTRACT

Although no documented outbreaks of listeriosis have been associated with the consumption of meat in the United States, *Listeria monocytogenes* is common to the environment of processing plants. In an effort to control the potential hazard of the bacteria, which is common to the environment of processing plants, a number of studies have been conducted to evaluate the antimicrobial activity of smoke as a potential treatment for beef products. The purpose of this study was to investigate the antimicrobial effect of different liquid smoke products on *Listeria monocytogenes*.

**Materials and Methods**

**Experiment I**

Preparation of inoculum. *Listeria monocytogenes* LCDC 81-861, an isolate from raw cabbage and a serotype 4b strain, originally isolated by Dr. L. Beuchat, University of Georgia, was obtained from Campbell Institute for Research and Technology, Camden, NJ, courtesy of Dr. Donald L. Zink. This particular strain was chosen because it is a demonstrated pathogen. It was maintained on tryptic soy agar (Difco Laboratories, Detroit, MI) slants at 5°C. The organism was grown in tryptic soy broth (Difco) for 24 h at 37°C. The bacterial suspension was diluted using 0.85% saline:0.1 M sodium phosphate buffer (3:1) to give 7.3 x 10^8 cells/ml.

Preparation of liquid smoke. Four different smoke concentrates were prepared: CharSol-10, Aro-Smoke P-50, CharOil Hickory, and CharSol PN-9. One spray dried smoke (CharDex Hickory) was obtained from Red Arrow Products, Manitowoc, WI. Two different concentrations of smoke (0.5% and 0.25%) were prepared.
in a 0.85% saline:0.1 M sodium phosphate buffer (3:1). The pH of the mixture was 5.64. The diluted liquid smoke preparations (99 ml) were inoculated with 1 ml of the bacterial suspension containing 7.3 x 10^6 cells/ml in the 0.5% concentration of smoke and 3.2 x 10^6 cells/ml in the 0.25% preparation and held at room temperature. The viable cell counts were determined at 0, 4, and 24 h for the 0.5% liquid smoke preparation and extended to include 96 h for the 0.25% liquid smoke preparation. At each sampling time, a dilution series was made in 0.1% Phytone peptone (BBL, Cockeysville, Maryland) and 0.1 ml of each dilution was surface plated onto duplicate tryptic soy agar plates. Plates were incubated in a 5% CO₂ incubator at 37°C for 24 h and then counted for viable organisms.

**Experiment II**

**Preparation of inoculum.** Six different strains of *L. monocytogenes* were used in this experiment: *L. monocytogenes*, a serotype 4b derived from ATCC 19115; LCDC 81-861, a serotype 4b; M1; M2; M5 (all isolated from raw ground beef); and C6 (isolated from raw chicken). Strains M1, M2, M5, and C6 are all isolates that were obtained from Campbell Institute for Research and Technology and have not been serotyped. These strains were grown separately on TSA slants for 24 h at 37°C, then mixed together to allow for more variability by transferring 1 loopful of each strain from the TSA slant into a flask containing 100 ml tryptic soy broth which was then incubated for 24 h at 37°C. An appropriate volume of the bacterial suspension in TSB (about 1 ml) was transferred to a test tube containing 9 ml of 0.85% saline: 0.1 M sodium phosphate buffer (pH 7.2) to adjust the culture to a McFarland 1.0 nephelometer standard (6) which makes the cell density approximately 3 x 10^8 cells/ml. Further dilutions were made from this tube in 0.85% saline: 0.1 M sodium phosphate buffer to approximate 1 x 10^7 cells/ml in a total volume of 3 L. Direct plating was done on TSA plates that indicated the inoculum used had 1.13 x 10^7 cells/ml.

**Inoculation of beef franks.** Thirty-two beef franks (CFR 319.180 standard of identity for beef franks) were immersed (8 franks at a time) in the inoculum solution and removed, 2 franks at a time, which took 2-4 min. Upon removal from the inoculating solution, the franks were air dried on absorbing paper. One-half of the inoculated franks (sixteen) were dipped in full strength CharSol-10, then air dried. The remaining franks (sixteen) were immersed in non-inoculated salin-sodium phosphate buffer to serve as a control. Each of the above groups was divided into two samples (8 franks each) in order to test the viable colony forming units, after dipping, at zero time (actually 15 min. had elapsed between air drying and this first sampling) and after 72 h of storage in vacuum (22 in. Hg vac.) at 4°C. P.51B barrier bags (Viskase, Chicago, IL) were utilized in vacuum packaging the franks. The zero time samples were tested without packaging.

The isolation and identification of *L. monocytogenes* was a modification of the USDA-FSIS procedure (12). Each sample (227 g) was blended in 227 ml Listeria enrichment broth (Gibco) and 50 grams of the blended mixture was weighed and suspended in 200 ml Listeria enrichment broth to give a 1:10 dilution. Further dilutions of 1:100 and 1:1000 were made. These tubes were incubated in a 5% CO₂ incubator at 30°C for 24 h. After 24 h, 0.1 ml from each tube in each dilution was surface plated onto McBride’s Listeria agar (Difco) and incubated in a 5% CO₂ incubator at 30°C for 48 h.

Enumeration of *L. monocytogenes* was done using the 3 tube Most Probable Number procedure (8). The colonies were examined through a transillumination microscope angled at 45°. Plates containing bluish gray colonies which were also found to be Gram-positive and catalase positive were identified as *L. monocytogenes* colonies. No further tests were performed to confirm *L. monocytogenes* since our purpose in this investigation was not to isolate naturally occurring *L. monocytogenes* colonies but to be able to distinguish between *L. monocytogenes* and lactic acid bacteria. Gram staining, catalase reaction and the characteristic bluish gray color of the colonies on selective media (MLA) was sufficient to make this distinction.

**RESULTS**

Figure 1 depicts the survival curve for *L. monocytogenes* LCDC 81-861 in 0.85% saline: 0.1 M sodium phosphate buffer (pH 5.64) with 0.5% liquid smoke preparations.

Figure 2 represents survival of *L. monocytogenes* LCDC 81-861 in 0.5% liquid smoke preparations. The number of viable cells detected in the control after 4 h showed an initial decline in *Listeria* level that remained unchanged after 24 h. The time required for the reduction of microbial numbers below detection was variable among the different types of liquid smoke used. CharSol-10, Aro-Smoke P-50, and CharDex Hickory exhibited similar antimicrobial effects by eliminating the viable cell population within 4 h. CharSol PN-9 and CharOil Hickory also demonstrated similar but weaker antimicrobial properties by eliminating *Listeria* numbers within 24 h.

Figure 2 represents survival of *L. monocytogenes* LCDC 81-861 in 0.25% liquid smoke preparations. The control again exhibited an initial reduction in the *Listeria* level within 4 h while bacterial numbers then remained largely unchanged after 96 h. Within 4 h, CharSol-10 and
Aro-Smoke P-50 again demonstrate their effectiveness as strong antimicrobial agents by reducing the viable cell population to an undetectable level. CharDex Hickory liquid smoke was less effective when its concentration was lowered to 0.25%. At this concentration of CharDex Hickory, 24 h were required for the elimination of microbial numbers below detection whereas at a 0.5% concentration, only 4 h were necessary for the elimination of viable cells. CharSol PN-9 and CharOil Hickory also showed a reduction in their antimicrobial activity when their concentrations were lowered to 0.25% by requiring 48 h and 96 h respectively to lower the Listeria level below detection.

In untreated beef franks (no liquid smoke) that had been vacuum packaged and stored at 4 ± 1°C, the numbers of L. monocytogenes remaining after dipping did not change after 72 h (Table 1) indicating favorable storage conditions for the survival of L. monocytogenes. On the other hand, the Listeria population decreased more than 99.9% in the smoke treated franks vacuum packaged and stored at 4 ± 1°C for 72 h. Furthermore, the CharSol-10 liquid smoke used in treating the franks reduced the viable cell counts by more than 60% at zero time in Trials I and II and reduced Listeria numbers below detection in Trial III.

**DISCUSSION**

The continuous decline in the viability of L. monocytogenes, suspended in saline buffer to which 0.25 or 0.5% liquid smoke was added provides strong evidence of the antimicrobial effectiveness of liquid smoke against this bacterium. Donnelly, Ziegler and Acton (5) recommended a level of 0.5% liquid smoke as optimum to give color and flavor to fermented sausage but would not alter consumer acceptance. This indicates that liquid smoke can be used as an aid to control the hazard of L. monocytogenes in processed meat products without reducing their acceptability.

Some of the liquid smoke products like CharSol-10 and Aro-Smoke P-50 possess higher antimicrobial activity than the others tested in this study. This is probably due to a higher polar phenol content (10) of these products which would intensify their antimicrobial properties. Since these phenols are water soluble, it is also reasonable to assume that the rate of transfer of these components into the bacterium would be higher than for those which are oil soluble. As a result, rate of kill of the microbes would be increased.

All liquid smoke products used were effective over time in eliminating L. monocytogenes in pure culture. In addition, CharSol-10 demonstrated its effectiveness in controlling L. monocytogenes upon dipping or spraying the franks in full strength liquid smoke if contamination occurs post peeling.

**REFERENCES**

11. Ho, J. L., K. N. Shands, G. Friedland, P. Eckind, and D. W. Fraser. con’t. on p. 638

**TABLE 1.** The effect of CharSol-10 on frankfurters inoculated with 6 strains of L. monocytogenes.

<table>
<thead>
<tr>
<th>Trial No.</th>
<th>Treatment</th>
<th>0 h</th>
<th>72 h</th>
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<td>I</td>
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<td>2.36</td>
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<tr>
<td>I</td>
<td>Smoke</td>
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<tr>
<td>II</td>
<td>Control</td>
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<td>2.36</td>
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<tr>
<td>II</td>
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<td>0.00</td>
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<tr>
<td>III</td>
<td>Control</td>
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<td>2.36</td>
</tr>
<tr>
<td>III</td>
<td>Smoke</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

*The log of the number of cells per ml of the inoculum solution was 2.90.
*The log of the number of cells per ml of the inoculum solution was 3.05.

Turscott and McNab, con't. from p. 628


Messina et al., con't. from p. 631

12. McClain, D. Isolation and identification of Listeria monocytogenes from meat, USDA-FSIS, 322ARC, Beltsville, MD.