INHIBITION OF *LISTERIA MONOCYTOGENES* BY PRESERVATIVES IN VACUUM-PACKAGED BOLOGNA

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SUMMARY

A cooked bologna product was inoculated with *Listeria monocytogenes* and treated with additives applied by immersion either before or after immersing the slices of bologna in a culture suspension of *L. monocytogenes*. Growth of total mesophilic aerobic bacteria and *L. monocytogenes* in vacuum-packaged products at 4°C was rapid in control samples, where it exceeded 10^7 colony forming units (CFU)/g in 7-14 days of storage. Of the preservatives tested (all at 0.74 M solution), irrespective of sequence of exposure to the inoculum and preservative solution, potassium sorbate almost completely eliminated microbial growth during the 56-day storage period; sodium acetate was next in effectiveness, slowing down growth substantially; while sodium lactate showed no major antimicrobial activity. It should be stated, however, that the bologna was immersed in equimolar (0.74 M) solutions of the preservatives, and therefore, additional work is needed to determine whether a more concentrated solution of lactate or longer exposure time would be effective, or whether the concentration of sorbate could be reduced.

Introduction

Listeria monocytogenes is a human pathogen of major concern for the meat industry because of its ability to grow under refrigeration. In addition, fatality rates due to this pathogen exceed 20% (Ryser and Marth, 1991; Schuchat et al., 1991). Therefore, the United States Department of Agriculture has established a monitoring program for the pathogen in commercially cooked meat products. Thus, its control and possible destruction is of paramount importance in processed, ready-to-eat food items. Thermal processing of products should eliminate cells present in the formulation (Yen et al., 1992), but means of inhibiting multiplication of post-processing contamination introduced during product slicing and packaging would be beneficial. Common food preservatives with the potential of inhibiting *L. monocytogenes* include potassium sorbate, sodium acetate and sodium lactate (Sofos, 1989; 1993; Harmayani et al., 1993; Wederquist et al., 1994). The purpose of this study was to examine the antimicrobial activity of three different preservatives (potassium sorbate, sodium acetate and sodium lactate) as dipping solutions applied after a meat bologna had been prepared, but prior to vacuum-packaging. The cooked meat bologna slices were exposed to *L. monocytogenes* cells either before or after immersion in the preservative solutions.

Materials and Methods

The bologna formulation consisted of 50% beef trimmings, 30% pork trimmings, 10% ice, 3.5% non-fat dry milk, 2.5% sodium chloride, 2% dextrose, 1% dry mustard and 0.3% phosphate (mixture of sodium tripolyphosphate and sodium hexametaphosphate), while the remainder of the formulation included white pepper, nutmeg, liquid smoke, sodium nitrite and sodium erythorbate. The beef and pork trimmings (12-kg batches) were chopped with salt and the other nonmeat ingredients, and the smooth paste reached a temperature of 15.5°C. The product was then extruded into 7.62-cm diameter fibrous cellulose casings (Koch, Kansas City, MO), tension clipped, and cooked in a single tree smokehouse (Vortron, Beloit, WI) until the internal temperature of 66.7°C was reached. The cooking schedule involved dry heat at 60°C for 30 min; then the humidity was increased (36% RH) by heating at 71.1°C dry bulb and 51.7°C wet bulb for 30 min; and heating was finished at 76.7°C dry and 62.8°C wet bulb (47% RH). The bologna was then cold showered in the smokehouse for 15 min, and placed in a cooler for three days at 4°C. Samples were prepared by cutting the bologna into slices, which were then cut into quarters.

The first part of this study determined growth of *L. monocytogenes* on cooked bologna slices by first immersing the slices into a composite cell suspension (10^4 cells/mL) of *L. monocytogenes* consisting of nine strains (558, 65, Na-16, 97, N714, Scott A, N-7155, 7144 and Na-19) prepared individually in sterile tryptic soy broth (TSB; Difco Laboratories, Detroit, MI) for 48 hr at 37°C. After immersing in the cell suspension for 20 sec, the quarter slices of bologna were allowed to dry for 30 min, and then they were immersed for 20 sec in one of four individual solutions of potassium sorbate (granular, food grade; Monsanto, St. Louis, MO), sodium acetate (anhydrous powder, Matheson Coleman, Norwood, OH), sodium lactate (natural 60% SP liquid; Purac, Inc., Arlington Heights, IL) or sterile water. The solutions were prepared so that they contained a concentration of 0.74 M (g of preservative/molecular weight of preservative = 0.74 M) of each preservative. Thus, the solution consisted of 111.2 g potassium sorbate per liter of distilled water; 60.7 g of sodium acetate per liter of distilled water; or 138.2 g of a 60% solution of sodium lactate per 944.72 mL of distilled water. All samples were allowed an additional 30 min to dry on trays. Then they were vacuum-packaged (Multivac, Germany) two quarter slices per package in 15.2 x 20.3-cm vacuum bags (Koch, Kansas City, MO) at 120 mm Hg. The second part of this study involved slices of bologna which were first immersed in each of the above preservative solutions and then in the *L. monocytogenes* cell suspension (i.e., the above sequence was completely reversed).

The packaged samples of all treatments of both parts of this study were stored at 4°C and two samples from each treatment were analyzed at weekly intervals for days of storage. Approximately 20 g of bologna from each sample was weighed, and an initial 3:1 dilution was made and blended (2 min) using sterile phosphate buffer solution (pH 7.2) in Stomacher-lab-blender (model 400, Tekmar, Cincinnati, OH) bags. The pH of each blended sample was determined with a Corning flask combination electrode attached to a Corning pH meter (Corning Glassworks, Medfield, MA) at this time. Tryptic soy agar (Difco) was used to determine total aerobic mesophilic bacteria counts by spread plating and incubating at 35°C for 48 hr. Testing for *L. monocytogenes* was carried out by spread plating on lithium chloride-phenylethanol-moxalactam-tellurite (Shelef, 1989) agar. Inoculated plates were incubated at 35°C for 48 hr. Colonies counted as *L. monocytogenes* appeared black, smooth, slightly raised, and 1-3 mm in diameter.

Results and Discussion

Both sequences of treating the product with preservatives and inoculating with *L. monocytogenes* yielded very similar results. Therefore, the results of both sequences of inoculation and preservative application are discussed together. The initial numbers of the *L. monocytogenes* inoculum were similar among treatments (Tables 1 and 2). Sodium lactate, at the concentration used (0.74 M solution for 20 sec), appeared to have very little effect in inhibiting the growth of *L. monocytogenes*. Rate of growth in this treatment was almost identical to that in the control samples, which reached 5.58-5.75 log CFU/g after 7 days of storage at 4°C and 7.42-8.89 log CFU/g after 14 days. Similarly, the sodium lactate treatment increased to 5.27-5.38 and 6.92-7.07 log CFU/g after 7 and 14 days of storage, respectively. Sodium acetate, at the concentration used (0.74 M solution for 20 sec), appeared to have a moderate effect in inhibiting *L. monocytogenes* growth (Tables 1 and 2). The initial inoculum levels of 2.93-3.33 log CFU/g reached 6.23-6.45 log CFU/g after 56 days of storage. Potassium sorbate, at the concentration used (0.74 M solution for 20 sec), appeared to be the most effective method of preservation, since it almost completely inhibited growth of *L. monocytogenes* (Tables 1 and 2); the initial inoculum level of 2.75-3.14 log CFU/g reached 1.78-4.82 log CFU/g after 56 days of storage.

The effects of the preservatives on total aerobic mesophilic bacteria were very similar to those observed for *L. monocytogenes* (Tables 3 and 4). The pH changes during storage of the various bologna treatments are illustrated in Tables 5 and 6, and correlate well with the microbial data obtained. The initial pH values were in the range of 6.76±0.02 to 6.89±0.01. After 14 days of storage, the pH values of the sorbate and acetate treated products remained relatively constant, while those of the control and the lactate treatments decreased dramatically. At 28 days of storage, the pH values of the lactate and control treatments were 5.62-5.64 and 5.20-5.25, respectively. These results suggest that potassium sorbate had the greatest inhibitory effect on *L. monocytogenes* of the preservatives used in this study, and at the concentrations and conditions of exposure examined.

Conclusions

The use of certain preservatives applied as a dipping solution had a positive effect in inhibiting/preventing the growth of *L. monocytogenes* in a bologna-type processed meat product inoculated and treated with the preservative after processing. At the concentration used in this study (exposure for 20 sec to a 0.74 M

solution), potassium sorbate was the most inhibitory, with sodium acetate second in effectiveness, while sodium lactate was ineffective. Additional studies are needed to determine whether higher concentrations of lactate are effective or whether theconcentration of sorbate could be reduced without loss of antimicrobial activity. The effectiveness of the preservatives was similar, irrespective of the sequence of exposure to the inoculum and the preservative solution.

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Tables

Table 1. Effects of food preservatives on growth of *Listeria monocytogenes* on bologna slices inoculated after processing and slicing during storage of bologna (the bologna slices were first immersed in the preservative solution (0.74 M) and then in the inoculum).

Table 2. Effects of food preservatives on growth of *Listeria monocytogenes* during storage of bologna slices at 4°C (the bologna slices were first immersed in the inoculum and then in a preservative solution).

Table 3. Effects of food preservatives on growth of total bacteria during storage of bologna slices at 4°C (the bologna slices were first immersed in the preservative solutions and then in the inoculum).

Table 4. Effects of food preservatives on growth of total bacteria during storage of bologna slices at 4°C (the bologna slices were first immersed in the inoculum and then in a preservative solution).

Table 5. Effects of food preservatives on product pH during storage of bologna slices at 4°C (the bologna slices were first immersed in a preservative solution and then in the inoculum).

Table 6. Effects of food preservatives on product pH during storage of bologna slices at 4°C (the bologna slices were first immersed in the inoculum and then in a preservative solution).