EFFECT OF MARINADES ON SURVIVAL OF ACID-ADAPTED AND NONADAPTED *LISTERIA MONOCYTOGENES* ON BEEF JERKY

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Background

Jerky is a marinated and dried meat product considered as microbiologically safe due to antimicrobial hurdles involved in jerky processing, such as low (<0.70) water activity (a_w), salt, and preservatives (e.g., acetic acid and nitrite). Foodborne disease outbreaks linked to jerky consumption (4, 5) have increased interest in evaluating the efficacy of jerky processing, especially when prepared in home-type dehydrators, in inactivating foodborne pathogens. A recent report (6) by the Food Safety and Inspection Service of the United States Department of Agriculture (FSIS/USDA) indicated that prevalence of *L. monocytogenes* in jerky produced in federally inspected plants in the U.S. was 0.52%. Currently, FSIS applies a "zero-tolerance" policy for *L. monocytogenes* in ready-to-eat meat products including jerky. Products that are not in compliance with this policy are considered as adulterated under the provisions of Federal Meat Inspection Act. We recently have shown that use of marinades modified with food grade chemicals (acetic acid, lactate, ethanol, Tween 20) as pre-drying treatments resulted in faster declines in the numbers of *E. coli* O157:H7 and *Salmonella* during drying and during storage when bacteria were inoculated before drying or after drying on dried product simulating post-processing contamination, compared to control and traditional marination techniques (1, 2, 3). Although pathogens did not grow on jerky, extend of their survival was affected by the type of marinade. There is evidence that acid-adaptation of *L. monocytogenes* may enhance its survival in acidic foods as well as increase cross-protection to other types of stresses (7). To date, it is not known whether acid-adapted pathogenic bacterial cells survive better than nonacid-adapted cells during the jerky-making process.

Objectives

The objectives of our research have included evaluation of the effectiveness of various chemical-based pre-drying treatments (modified marinades) on survival of acid-adapted or nonadapted *L. monocytogenes* inoculated before or after drying on whole-muscle beef jerky.

Materials and Methods

A five-strain composite (meat or clinical isolates) of *L. monocytogenes* was used for inoculating beef slices. Each strain was grown in glucose-free trypticase soy broth (TSB) for nonacid adapted cells or in glucose-free TSB with 1% added glucose for acid-adapted cells for 24 h at 30°C prior to combining, centrifuging and diluting the inoculum to a final level of 7.0 log colony forming units (CFU)/ml. Beef slices (0.6 x 8.7 x 4.0 cm) were inoculated before drying or after drying with approximately 6.0 log CFU/cm^2. Inoculated meat slices were subjected to the following pre-drying marinade treatments: 1) Control, no treatment (C), 2) traditional marinade (for 1.0 kg of meat: 60 ml soy sauce, 15 ml Worcestershire sauce, 0.6 g black pepper, 1.25 g garlic powder, 1.5 g onion powder, and 4.35 g smoke-flavored salt) (TM), 3) modified marinade (for 1.0 kg of meat: 120 ml of soy sauce containing approximately 5.0 % ethanol as preservative, 30 ml of Worcestershire sauce, 0.6 g black pepper, 1.25 g garlic powder, 1.5 g onion powder, 4.35 g smoke-flavored salt, 3.6 ml of 60% food grade sodium-L-lactate preparation, and 16 ml of acetic acid to adjust the pH to 3.0) (MM), 4) dipped in 5% acetic acid for 10 min, then in TM (AATM), and 5) dipped in 1% Tween 20 for 15 min, then in 5% acetic acid for 10 min, followed by TM (TWTM). Marinated meat slices (4°C, 24 h) were dried at 60°C for 10 h in home-type dehydrators. After drying, the jerky slices were held in the dehydrators overnight, and then placed into sterile plastic bags for storage at ambient temperature (25 ± 1°C) for 60 d. Microbial population were determined after inoculation, and 0 (after 24 h marination at 4°C), 4, 7 and 10 h during drying, and on days 15, 30 and 60 of subsequent storage at 25°C. For post-processing contamination, bacterial populations were determined on days 0 (after inoculation), 7, 14, 28, 42, and 60. Bacteria were enumerated using tryptic soy agar with 0.1% sodium pyruvate (TSAP), and PALCAM agar. All plates were incubated at 30°C for 48 h. The enumeration detection limit was ~0.4 log CFU/cm^2. Enrichment of samples was done when countable colonies were not detected. The studies were replicated twice and two samples were analyzed per replicate.

Results and discussion

Effect of agar media. In general, bacterial populations recovered were higher on TSAP compared to PALCAM (data not shown), depending on the pre-drying treatment and sampling time. These results suggest that nature of reduction (injury vs. death) in bacterial populations, as estimated by the differences in counts between nonselective (TSAP) and selective (PALCAM) agar, was affected by pre-drying treatment and time of storage. Although recovery is not expected on jerky, injured cells may cross-contaminate the environment or other foods and may repair their damage becoming a health concern.

Pre-processing contamination. Populations of *L. monocytogenes* were significantly ($P<0.05$) lower on TWTM, AATM and MM products than on C and TM during the 10 h of drying and until day-15 of storage (Fig 1A). There was no significant difference in survival of bacteria between acid adapted and nonadapted *L. monocytogenes* inocula. Complete elimination of the pathogen (enrichment negative) by 60 d occurred in MM, AATM and TWTM, regardless of acid adaptation. These treatments were also found to be significantly effective in reducing the numbers of *E. coli* O157:H7 and *Salmonella* during drying compared to C and TM (1, 3). However, unlike *L. monocytogenes*, acid-adaptation of these pathogens resulted in increased sensitivity to drying.

Post-processing contamination. When bacteria were inoculated on jerky after drying, simulating potential post-processing contamination, bacterial counts recovered from MM, AATM and TWTM treatments were significantly *($P<0.05$)* lower than those recovered from C and TM products up to day-42 or day-60, depending on recovery media and acid-adaptation resulting in a two-part grouping of the inactivation curves (Fig. 1B). This finding was similar to *E. coli* O157:H7 (2). Bacterial populations that were acid-adapted and nonadapted were not significantly *($P\geq0.05$)* different in TWTM, AATM and MM products during the 60-d storage period, regardless of recovery media. In contrast, populations of acid-adapted bacteria were significantly *($P<0.05$)* lower than those of nonadapted in treatment C on day-60 (TSAP only) and in treatment TM on day-28 (PALCAM only). These results indicated that acid-adapted *L. monocytogenes* were either equal to or slightly more susceptible to the conditions described in this study than nonadapted inocula depending on the pre-drying treatment or recovery media. Combination of hurdles of low pH and low a_w during storage at growth permitting temperature (25°C) in treatments MM, AATM and TWTM may explain the increased effectiveness of these treatments in inactivating bacteria. In addition, disturbance of pH homeostasis

(MM) or potentially weaker attachment of bacteria to the food the surface due to surfactants (TWTM) may have resulted in increased sensitivity of bacteria to other hurdles such as low pH.

Conclusions

The results of the present study indicated that acid-adapted cells of *L. monocytogenes* did not exhibit any increased resistance during drying and storage of beef jerky compared to nonadapted cells. The results also revealed that using food grade chemicals as pre-drying treatments improved the effectiveness of the meat-drying process for inactivating *L. monocytogenes,* and provided residual antimicrobial effects against possible post-processing contamination with *L. monocytogenes*, compared to the traditional jerky-making process. Sensory attributes of the final products are being evaluated.

Pertinent literature

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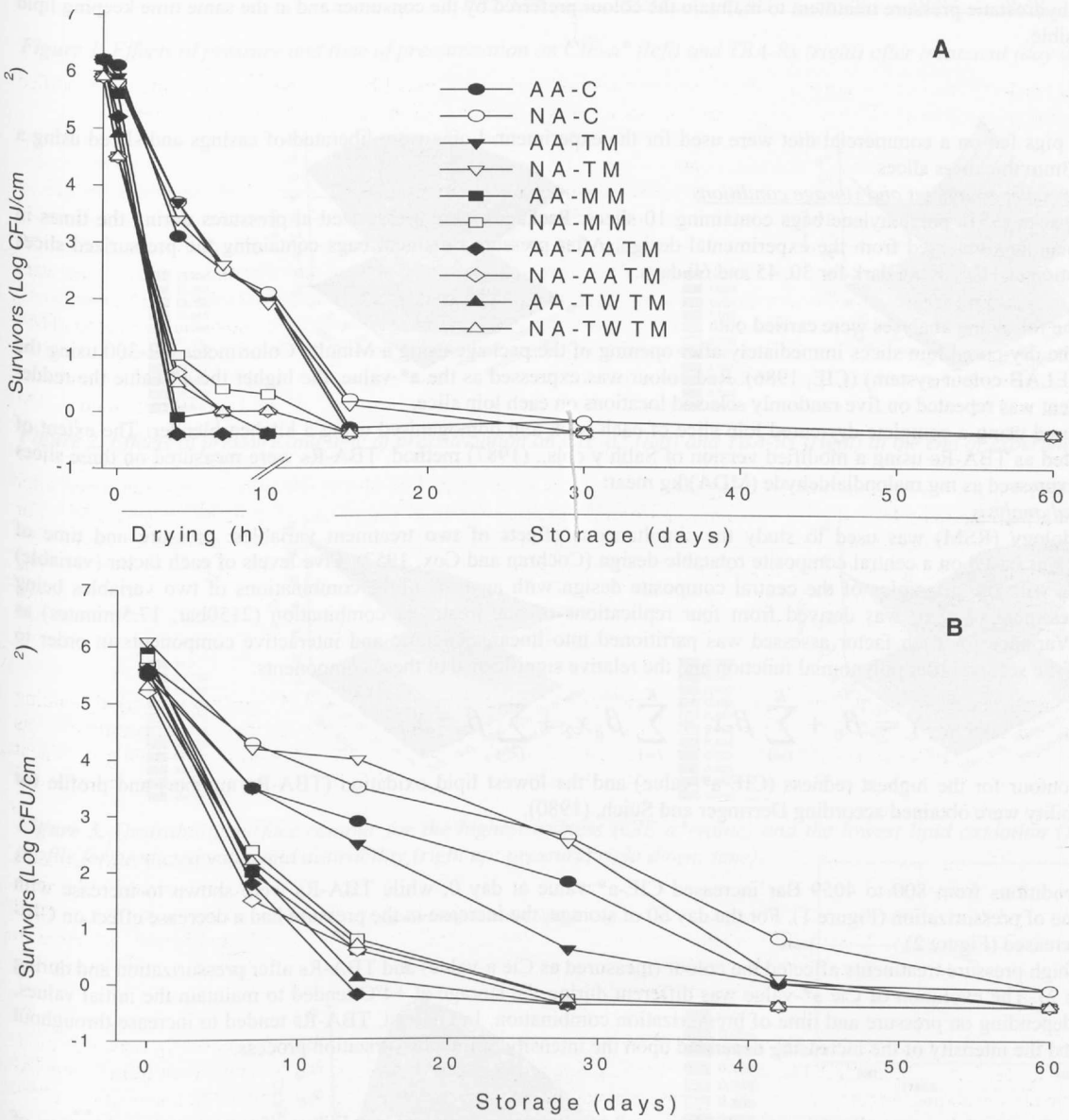

Figure 1. Survival of acid-adapted (AA) and nonadapted (NA) *Listeria monocytogenes* on beef jerky: A, during processing [marination, drying (60°C, 10 h) and subsequent storage (25°C, 60 d)] when inoculated before drying; B, during storage (25°C, 60 d) when inoculated after drying. Pre-drying marination treatments were control (C), traditional marinade (TM), modified TM (double amount, 1.2% sodium lactate, 5% EtOH, and 9% acetic acid (MM), acetic acid dip (5%) then TM (AATM), and Tween 20 dip (1%), then acetic acid dip (5%) followed by TM (TWTM). Results are mean bacterial counts as determined on PALCAM agar (n=4).