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SURVIVAL AND GROWTH OF *LISTERIA MONOCTYOGENES* ON BEEF TISSUE SURFACES AS AFFECTED BY SIMULATED PROCESSING CONDITIONS

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ABSTRACT

The numbers of Listeria monocytogenes *Scott A were followed on experimentally inoculated lean and fat beef tissue handled under a variety of simulated storage conditions at 5°C*, including high and low moisture storage, simulated spray chilling, and vacuum packaging. Under high moisture storage conditions, L. monocytogenes *increased approximately 3 log cycles on both lean and fat tissue after 21 days. Similar growth was observed with vacuum packaged lean tissue. Under dry storage conditions, the population decreased approximately 2 logs during the first 14 to 21 days, but the counts remained constant for 42 days. Simulated spray chilling did not affect the growth or survival of* L. monocytogenes *on either lean or fat tissue.*

INTRODUCTION

In recent years, there has been an increasing awareness of *Listeria monocytogenes* as a food borne pathogen (Doyle 1985). It is estimated that there are approximately 1700 cases of listeriosis every year (Anonymous 1989), although the percentage of these cases transmitted by foods is unknown. Foodborne transmission of *L. monocytogenes* was initially associated with coleslaw and soft cheese (Brackett 1988), but has been epidemiologically linked to undercooked chicken and frankfurters (Schwartz *et al* . 1988). In a recent case of listeriosis in an immunocompromised patient, contaminated turkey franks were implicated as the probable vehicle of transmission (Anonymous 1989). This is apparently the first documented case of foodborne transmission of *L. monocytogenes* by meat products.

[1]Mention of a trade name, proprietary product or specific equipment does not constitute a guarantee or warranty of the product by the USDA and does not imply its approval to the exclusion of other products that may also be suitable.

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Although the bacterium is known to be present in meat and meat products, there is some controversy over the bacterium's ability to survive and grow. Khan *et al.* (1972) indicated that growth in and on sterile intact lamb tissue was temperature dependent, with growth at 8 °C and survival but no growth at 0 °C. Chung *et al.* (1989) indicated that growth of the bacterium was possible when inoculated on the surface of beef tissue at both 23 °C and 5 °C. However, Gouet *et al.* (1978) and Johnson *et al.* (1988) reported that *L. monocytogenes* survived but did not grow at 8 °C and 4 °C, respectively, in ground beef. Buchanan *et al.* (1987), reported that *L. monocytogenes* showed minimal growth in ground beef sterilized with ionizing radiation. Recently, Glass and Doyle (1989) related survival and growth of *L. monocytogenes* in processed meat products to the pH and product type. Growth was observed primarily in poultry products or those which had a pH of approximately 6 or greater. Clearly, these and other factors may affect the survival and growth of *L. monocytogenes* on intact beef tissues.

Intact tissues, in the form of carcass meats, are the raw materials for processed meats. Since these are potential sources of contamination, it is important to determine the fate of *L. monocytogenes* on these tissue surfaces. Traditionally, surface dehyration has been a factor in controlling microbial growth on beef carcasses. However, changes in processing techniques, such as spray chilling and vacuum packaging, have altered this control process. The objectives of this study were to determine the fate of *L. monocytogenes* on beef tissue as affected by tissue type (lean or fat), moisture level, and vacuum packaging.

MATERIALS AND METHODS

Bacterium

Listeria monocytogenes strain Scott A (FDA, Division of Bacteriology, Cincinnati, OH) was grown and maintained on tryptic soy agar (TSA; Difco) slants. Tryptic soy broth (TSB; Difco) was inoculated from the slants and incubated at 23 °C for 18 h. This temperature provided good growth and allowed for development of flagella. The cultures were harvested by centrifugation (3000 × G for 10 min, 5 °C), and the pellets resuspended in Butterfield's phosphate buffer (Pertel and McLure 1984). This preparation typically contained 10^8 colony forming units per mL. The culture was serially diluted in phosphate buffer to the desired concentrations.

Preparation of Tissue

Postrigor beef tissue was obtained as boneless trim from the abbatoir at the Roman L. Hruska U.S. Meat Animal Research Center. The tissue was separated into lean and fat tissue and frozen in sterile sealed bags at −20 °C. The tissue was then sliced into 0.5-cm thick slices and returned to the freezer until needed.

The tissue slices were partially thawed and sliced into 1.0 × 1.0 × 0.5-cm thick pieces (4 cm^2 surface). Tissue prepared in this manner typically contained fewer than 100 cfu/4cm^2. The tissue was surface sanitized by dipping in 80 °C water for 2, 15 s intervals (fat) or 2 min (lean). Alternately, the tissue samples were prepared from 0.5-cm thick slices which had been sterilized with gamma radiation to a dose level of 42 kGy. The tissue samples were inoculated to the desired level by immersing them in the diluted culture for 5 min at 23 °.

Experimental Design

(1) Lean and fat tissue samples which had been treated with 80 °C water were inoculated by immersion in the diluted culture for 5 min, attached to sterile, square jawed alligator clips (Radio Shack, Dallas, TX), and the clips suspended in 100 mL sterile beakers such that the tissue did not contact the sides of the beaker. Three moisture conditions were established: a "wet" beaker containing approximately 20 mL of sterile distilled water and sealed with Parafilm to prevent dehydration, a "dry" beaker, containing no water and loosely covered with foil to prevent contamination but allowing dehydration, and a variable condition in which the tissue was placed in a "wet" beaker for 24 h, after which it was transferred to a dry beaker. The wet condition is a worst case simulation, where a carcass surface or cut of meat would be kept moist. The dry condition simulates the surface of a beef carcass, which normally dehydrates on storage. The variable condition simulated the conditions found during spray chilling of carcasses. All of the tissue samples were incubated at 5 °C. The reported results are the average of two independent replications.

(2). Inoculated samples were placed in sterile stomacher bags (Tekmar, Inc., Cincinnati, OH) and then vacuum packaged in a Smith Supervac (Smith Equipment, Co., Clifton, NJ) to 98% vacuum in 3-mil thick bags. The packages were stored at 5 °C for up to 21 days. The reported results are the average of two independent replications.

(3). To determine if the method of tissue preparation (i.e., heat treatment vs. radiation) affected the response of the bacterium, irradiated lean meat was prepared as described in the initial experiments using wet and dry beaker conditions. The samples were analyzed over a 28 day period and compared to those of the blanched tissue. The reported results are the average of two independent replications.

Enumeration

The samples were aseptically removed from either the beakers or the vacuum packages and transferred to 99-mL phosphate buffer in sterile stomacher bags. The samples were stomached for 2 min in a Stomacher 400 (Tekmar, Inc., Cincinnati, OH) and serially diluted in phosphate buffer. As the storage time increased, it became necessary to process the samples from the dry beakers in

filter bags (Tekmar, Inc., Cincinnati, OH) to prevent the samples from rupturing the bags. The samples were surface plated on TSA or on modified McBridge agar with cyclohexamide omitted (MCB; Lovett *et al.* 1987) as indicated in the results. The plates were incubated at 32 °C for 24 to 48 h.

Water Activity

Water activity (a_W) was determined using a Newport Scientific hygrometer (Jessup, MD) and color code gray sensors (range 0.85 - 0.99 a_W). The tissue samples were transferred to dry beakers, and the sensors placed over the sample with a plastic adaptor. The adaptor was sealed over the beaker using Parafilm. The tissue sample and sensor were allowed to equilibrate at 5 °C for 24 h, and then water activity was determined by converting the reading on the instrument to percent relative humidity using the correction factors and calibration curves supplied by the manufacturer. Sensors were stored in dessicators for a minimum of 24 h before each use.

RESULTS

In high moisture environments, *L. monocytogenes* was able to grow to high levels irrespective of tissue type (Fig. 1). There was a 3 log increase on either tissue surface after 21 days, after which the population stabilized at this level for the remainder of the experiment. On fat tissue, the counts on both TSA and MCB correlated well, but there was a marked disparity with lean tissue. The TSA counts were substantially higher than the MCB counts, and the colonies on TSA exhibited two distinct morphologies: small, regular edged colonies which were typical of *L. monocytogenes* on TSA, and larger, irregular edged colonies. The morphological types were distributed in an approximate 2:1 (smooth to irregular) ratio on the plates. The larger colonies were assumed to be contaminants, and were isolated for further identification. However, these isolates demonstrated cellular morphology and motility typical of *L. monocytogenes*. Biochemical and seriological tests confirmed that these irregular colonies were in fact *L. monocytogenes*. The isolates reverted to typical *L. monocytogenes* colony morphology upon isolation. Biochemical characterization of the smaller, regular edged colonies confirmed that they were *L. monocytogenes*.

When the tissue surfaces were allowed to dry out, *L. monocytogenes* demonstrated remarkable resistance to dehydration. The counts had decreased only 1.5 logs after 42 days on either tissue surface. Measurement of water activity of these samples indicated that the tissues had an $a_W < 0.85$ after 7 days. As the experiments progressed, the tissue samples became so hard that they would physically rupture the stomacher bags during homogenization. The filter bags provided adequate protection and largely prevented these ruptures, and were used with all dried samples. The difference between the TSA and McBride agar

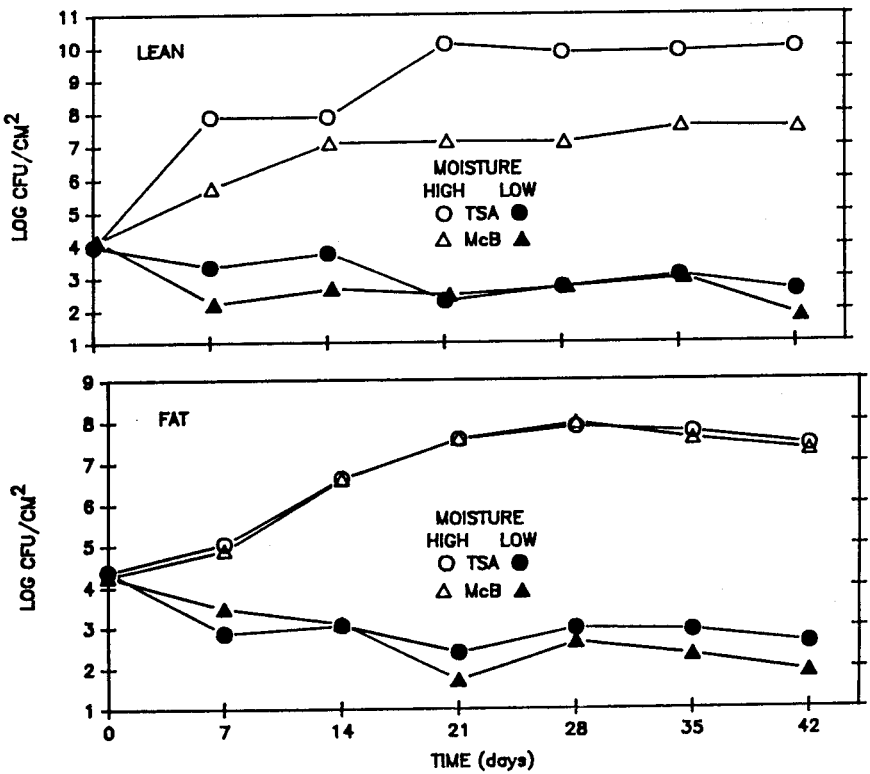

FIG. 1. SURVIVAL AND GROWTH OF *LISTERIA MONOCYTOGENES* ON LEAN AND FAT BEEF TISSUE AT 5 °C UNDER HIGH AND LOW MOISTURE CONDITIONS

counts may indicate the degree of stress on the bacteria. It would be expected that stressed cells would grow on TSA, but might not on the more inhibitory McBride agar.

Simulated spray chilling did not alter the growth or survival patterns of *L. monocytogenes* on lean or fat tissue (Fig. 2). There was no increase in numbers attributable to the one day high moisture environment. The counts on the high moisture environment samples increased 2 log cycles over 14 days, while the simulated spray chill samples decreased 1 to 1.5 log samples over the same time period. These trends correspond to the data presented in Fig. 1 for the high and low moisture environments.

The growth of *L. monocytogenes* under vacuum packaging conditions at 5 °C was studied using two different initial inoculum levels (Fig. 3). There was a lag in growth during the first 7 days, followed by rapid growth between 7 and 14 days. After 21 days, there was approximately a 4 log increase in bacterial numbers, irrespective of initial inoculum level.

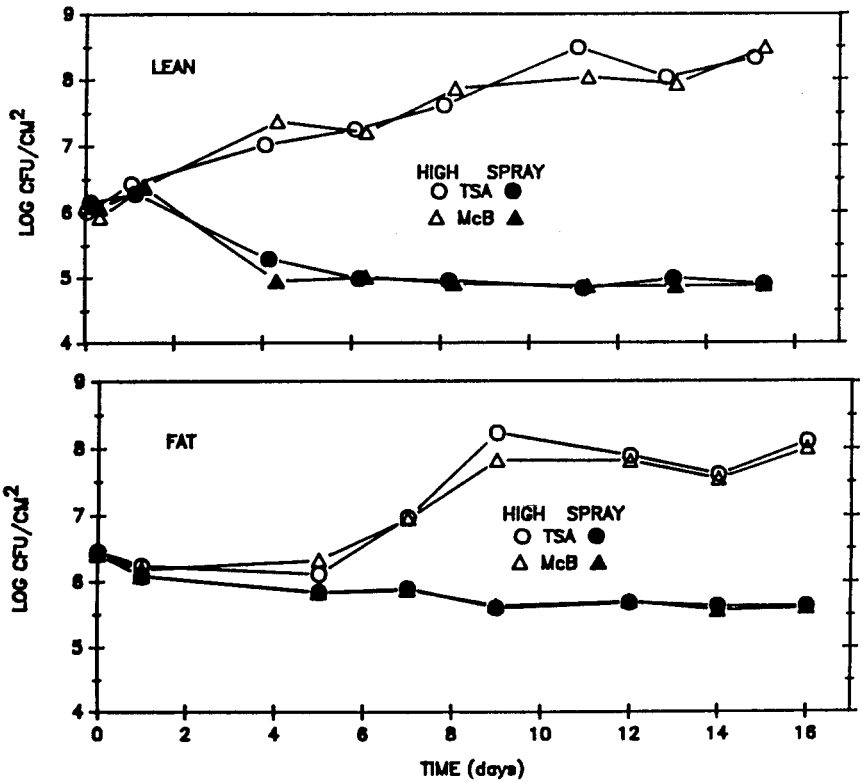

FIG. 2. SURVIVAL AND GROWTH OF *LISTERIA MONOCYTOGENES* ON LEAN AND FAT BEEF TISSUE AT 5 °C UNDER HIGH MOISTURE AND SIMULATED SPRAY CHILLING CONDITIONS

There was a concern that the method of tissue preparation, i.e. blanching, would affect the results. However, when blanched and irradiated lean tissue was compared under high and low moisture environments, the same growth and survival trends were observed (Fig. 4). The observed differences between the two sterilization techniques at day 0 were directly attributable to differences in the initial numbers of bacteria in the inoculum, and not due to any intrinsic difference in the samples.

DISCUSSION

Surface dehydration and refrigeration have traditionally been relied on to control bacterial growth on carcasses (Ayres 1955). *L. monocytogenes* can survive under both of these conditions, with only 1.5 to 2 log reductions on either lean or

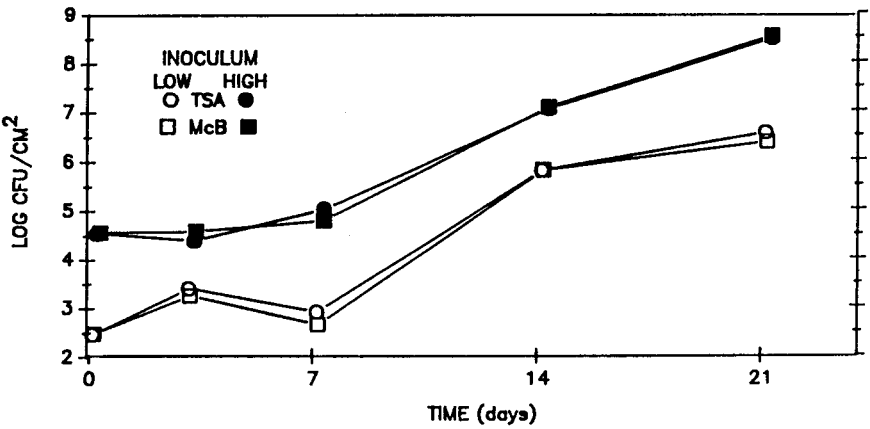

FIG. 3. GROWTH OF *LISTERIA MONOCYTOGENES* ON VACUUM PACKAGED LEAN BEEF TISSUE AT 5 °C WITH HIGH (ca. 3 × 10^4 cfu/cm²) AND LOW 2.5 × 10^2 cfu/cm²) INITIAL INOCULUM LEVELS

fat tissue after 42 days of storage (Fig. 1). This rate of survival was unexpected, particularly when the water activity ($a_w < 0.85$) of the tissue was considered. The counts had decreased only 1.5 logs after 42 days on either tissue surface. Doyle *et al.* (1985) reported approximately a 1 log reduction in *L. monocytogenes* after 28 days in nonfat dry milk stored at 25 °C, but after 42 days there were no viable cells. However, these bacteria had been subjected to heat stress during the spray drying process, and this in combination with a higher storage temperature (25 °C in NFDM vs. 5 °C in the meat studies) may be responsible for the somewhat shorter survival time. The tissue samples after prolonged storage were physically hard enough to rupture the standard stomacher bags, and required the use of filter bags to maintain the integrity of the bag.

Under high moisture conditions, *L. monocytogenes* was capable of rapid growth. These results confirm the work of Khan *et al.* (1972) and Chung *et al.* (1989). The lack of growth reported in ground beef (Gouet *et al.* 1978; Johnson *et al.* 1988) has been attributed to a variety of factors, including inhibitory compounds in the tissue (Gray 1948). However, other possibilities may include the increased availability of oxygen to the cells for growth on intact tissue and the water activity of ground beef. While ground beef is generally considered to be a high moisture food, it has been reported that some strains of the *Moraxella-Acinetobacter* group are incapable of growth in ground beef exudate (Snyder and Maxcy 1979). When water was added to the exudate, rapid growth occurred. However, the potential for inhibitory compounds would still exist, since the addition of water would dilute any inhibitory compound present. The research presented in this manuscript does not support the theory of inhibitory compounds, at least not to the same degree as previously reported, since tissue

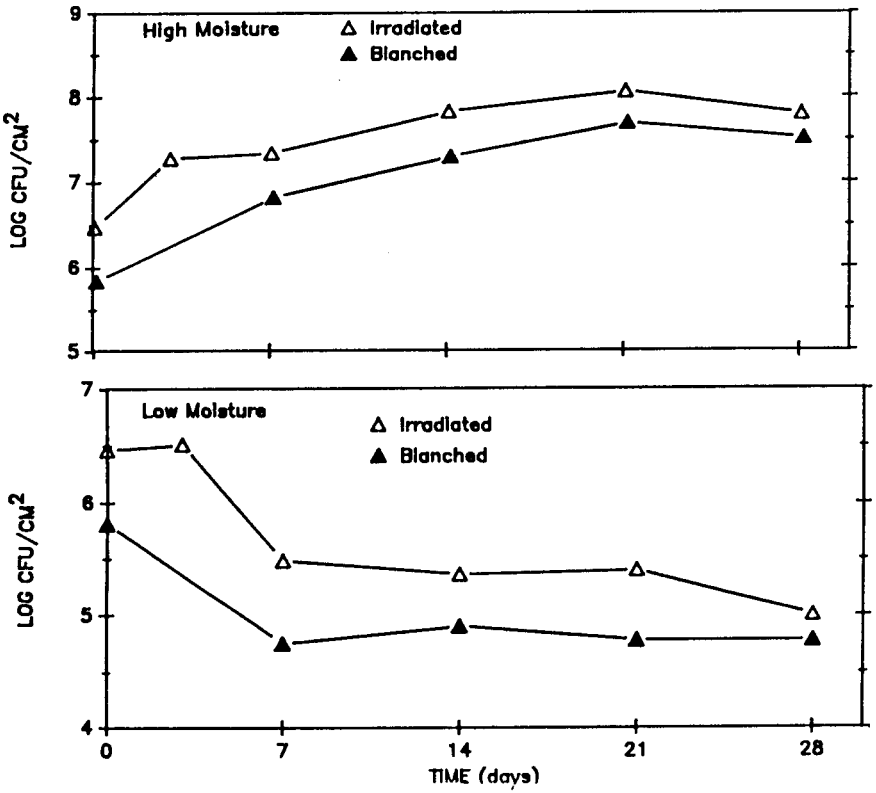

FIG. 4. GROWTH OF *LISTERIA MONOCYTOGENES* ON LEAN BEEF TISSUE UNDER HIGH AND LOW MOISTURE CONDITIONS AT 5 °C AS AFFECTED BY METHOD OF TISSUE PREPARATION

preparation (slicing) would disrupt the tissue cells and result in a release of these proposed compounds. There was no apparent inhibition of *L. monocytogenes*, with growth patterns typical of other bacteria under similar conditions.

Spray chilling has become the dominant method of chilling beef carcasses in the United States. Briefly, the process involves spraying the hot carcasses with chilled water in a forced air refrigeration unit, with the objective of obtaining more rapid chilling of the carcass. After 18 to 24 h, the carcasses are transferred to a conventional refrigeration unit. Spray chilling conditions were simulated in these experiments by holding the tissue in a high moisture environment. The simulated conditions did not result in any appreciable increase in the populations of *L. monocytogenes* after 24 h (Fig. 2), and the survival pattern after transfer to dry storage conditions was similar to tissue samples which had not been subjected to high moisture chilling (Fig. 1).

After chilling, beef carcasses are broken down into primal and subprimal portions, and these are frequently vacuum packaged for shipment. Johnson *et al.* (1988) reported survival but not growth of *L. monocytogenes* in vacuum packaged ground beef stored at 4 °C for 14 days. They reported that gas permeability of the packaging material did not affect the results. This lack of growth appears to be attributable to the ground beef and not to vacuum packaging. *L. monocytogenes* grew well on vacuum packaged lean tissue held at 5 °C (Fig. 3), with approximately a 3 log increase over 14 days, irrespective of inoculum level. The potential for growth on vacuum packaged beef cuts in apparently a real concern.

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