A Research Note

Inhibition of *Listeria monocytogenes* on Normal Ultimate pH Beef (pH 5.3-5.5) at Abusive Storage Temperatures by Saturated Carbon Dioxide Controlled Atmosphere Packaging

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ABSTRACT

Beef striploin steaks (each weighing between 60 and 100 g) of normal ultimate pH (average 5.45) were inoculated with a mixture of two food isolates of *Listeria monocytogenes*, packaged under saturated carbon dioxide controlled atmosphere or vacuum, and stored at either 5 or 10°C. Saturated carbon dioxide packaging but not vacuum packaging inhibited growth of *L. monocytogenes*. During storage under vacuum at 5°C, numbers increased from 6.2-log_{10} CFU per sample to 9.0-log_{10} CFU per sample in 23 days, and at 10°C numbers increased from 6.3-log_{10} CFU per sample to 9.5-log_{10} CFU per sample in 11 days. During storage under saturated carbon dioxide at 5°C, numbers decreased from 5.6-log_{10} CFU per sample to 5.0-log_{10} CFU per sample in 18 days, and at 10°C numbers decreased from 6.7-log_{10} CFU per sample to 5.2-log_{10} CFU per sample in 44 days.

The use of saturated carbon dioxide controlled atmosphere packaging to extend the storage life of fresh beef results in apparent destruction of *L. monocytogenes* even at abuse temperatures.

Key Words: *Listeria monocytogenes*, normal ultimate pH beef, saturated carbon dioxide controlled atmosphere packaging

*Listeria monocytogenes* is found on raw meat cuts (11), ground meat and sausages (2), and in the meat processing environment (11,14). More seriously, it has also been isolated from ready-to-eat foods, including cooked meat products (7,9,15). However, there has been, to our knowledge, no documented outbreak of listeriosis due to the consumption of meat or meat products.

For product stored at 1°C, the use of oxygen-free saturated carbon dioxide packaging (CAP) extends the storage-life of normal ultimate pH beef (pH 5.3 to 5.5) to over 20 weeks, compared with 12 weeks obtained using vacuum-packaging (VP) (4). A longer storage-life may be obtained when product is stored at -1.5°C, the optimum storage temperature (3). Given that *L. monocytogenes* is a contaminant of raw meat cuts, and that it can grow at refrigeration temperatures, it is possible that growth of this organism on preservatively packaged meat (e.g., meat pack-aged under vacuum or a controlled atmosphere) may pose a public health risk. This may be by cross-contamination of ready-to-eat foods, consumption of undercooked contami-nated products, or consumption of uncooked meat in dishes such as steak tartare.

During the process of rigor, glycogen is converted to lactic acid, which accumulates in the muscle, causing meat pH to drop to an ultimate level. Beef of high ultimate pH (>6.0) is produced when muscle glycogen is depleted before slaughter, and insufficient lactic acid is produced to lower the pH to a normal level (5.3 to 5.5). High ultimate pH beef is not normally preservatively packaged as a limited storage-life is obtained compared with that for normal ultimate pH beef (12).

Gill and Reichel (5) found that *L. monocytogenes* did not grow on high ultimate pH (>6.0) beef stored at 0 and 5°C under CAP, whereas growth occurred at 10°C. In contrast, *L. monocytogenes* grew on the same substrate at 0, 5 and 10°C under VP. Grau and Vanderlinde (6) found that *L. monocytogenes* grew on VP normal ultimate pH beef (pH 5.5 to 5.7) at 0 and 5°C.

Wimpfheimer et al. (16) reported that *L. monocytogenes* did not grow on minced raw chicken stored at 4, 10 or 27°C under an atmosphere of 75% CO_{2}:25% N_{2}. Similarly, Hart et al. (8) found that *L. monocytogenes* did not grow on skinless chicken breast meat of pH 5.8 stored at 1°C under either 30% CO_{2}:70% N_{2} or 100% CO_{2}, although minimal growth was observed at 6°C. However, in that study, growth was not obtained on skinless chicken breast meat of pH 5.8 under aerobic conditions at 1°C. In experiments with sliced frankfurters incubated under 20 to 80% CO_{2} with the balance being N_{2}, at 4, 7 and 10°C, only an atmosphere containing 80% CO_{2} was capable of preventing growth of *L. monocytogenes* at all temperatures after 6 weeks incubation (10). In contrast, Marshall et al. (13) reported that *L. monocytogenes* grew on precooked chicken nuggets stored at 3 and 11°C under an atmosphere of 80% CO_{2}:20% N_{2}, although the aerobe *Pseudomonas fluorescens*...
also grew under these conditions. They concluded that the packs may have leaked, or that the commercial gas mixture used contained oxygen.

These studies present conflicting evidence on the ability of *L. monocytogenes* to grow on meat and poultry under anaerobic conditions in the presence of carbon dioxide. Although *L. monocytogenes* grows under CAP and VP on high ultimate pH beef, and also on VP normal ultimate pH beef, it is not known if growth occurs on normal ultimate pH beef under CAP.

The objectives of this work were: (i) to determine whether *L. monocytogenes* can grow on normal ultimate pH beef packaged under CAP and stored at the abusive temperatures of 5 and 10°C and (ii) to compare growth obtained under VP and CAP conditions.

**MATERIALS AND METHODS**

**Organisms**

The strains used, *L. monocytogenes* L70 and L72, were isolates previously recovered in this laboratory from smoked mussels and smoked salmon, respectively (9).

**Preparation of inocula**

Shaken cultures of both strains were grown in brain heart infusion (Difco Laboratories, Detroit, MI) at 10°C to an OD<sub>600</sub> of approximately 0.8 over 3 days. The two cultures were combined in equal proportions to produce the inoculum. Serial 10-fold dilutions of the bacterial inoculum were prepared in 0.85% saline, to give inocula containing approximately 10<sup>6</sup> CFU/ml.

**Preparation of meat samples**

Beef striploins of pH 5.3 to 5.5 from cattle slaughtered 72 h previously were used. The striploins were stored at -1.5°C either aerobically or under VP until use.

The meat was trimmed of fat and sliced into thin steaks of approximately 100 × 150 × 5 mm, each weighing between 60 and 100 g. All equipment was cleaned and disinfected before use. The steaks were placed into sterile plastic stomacher bags (A. J. Seward and Co. Ltd., London, England) of high oxygen and CO<sub>2</sub> permeability (approximately 9,000 ml/m<sup>2</sup>/24 h at 20°C and 85% relative humidity, and 1,100 ml/m<sup>2</sup>/24 h at 38°C and 90% relative humidity, respectively).

Steaks were either uninoculated for use as controls or inoculated with 0.1 ml of diluted bacterial inoculum. The inoculum was pipetted onto the meat and was then rubbed into one surface of each inoculated steak by manually massaging the exterior of each stomacher bag over the meat surface. The stomacher bags were then evacuated and sealed.

Steaks to be stored under VP were further packaged by evacuating in packets of polyvinylidene chloride/ethylene/vinyl acetate co-extruded film with oxygen permeability of 30 ml/m<sup>2</sup>/24 h at 23°C and 1 atm (Cryovac<sup>™</sup>, W. R. Grace [N.Z.] Ltd., Porirua, New Zealand). Each packet contained up to five steaks.

Steaks to be stored under CAP were further packaged by evacuating and then flushing with CO<sub>2</sub> in packets of gas impermeable aluminum foil laminate (Borden Liquipac, Ellerslie, Auckland, New Zealand) using a prototype Captron II controlled atmosphere packaging machine (Securefresh Pacific Limited, Auckland, New Zealand). Each packet contained five steaks and about 2 L of CO<sub>2</sub> after sealing.

**Microbiological analyses**

Three inoculated steaks and one uninoculated steak were analyzed at each sampling time. Results are reported as the mean of the triplicate inoculated samples. The numbers of bacteria per sample were subjected to a logarithmic transformation before the standard deviations were calculated for each set of triplicate samples. The unoinoculated control samples were used to confirm that *L. monocytogenes* was not naturally present on the steaks.

Stomacher bags containing individual steaks were removed from the external VP or CAP packets and a 100-ml volume of dilution fluid (0.85% saline plus 0.1% peptone) was added to each stomacher bag. The steaks were pulverized using a Colworth stomacher 400 (Seward) for 2 min. Duplicate 0.1-ml volumes of suitable dilutions, prepared in dilution fluid, were spread on half-plates of selective agar. *Listeria monocytogenes* was enumerated on modified oxford agar (Oxoid, Columbia, MO), incubated at 35°C for 48 h. Lactic acid bacteria, the spoilage organisms normally dominant on stored preservatively packaged meat, were enumerated on MRS agar with added sorbic acid, incubated at 25°C in anaerobic jars (with CO<sub>2</sub>-enriched atmospheres) for 5 days (1). Half plates containing between 15 and 150 colonies were counted.

**Calculation of growth parameters**

Values for the specific growth rates were obtained by fitting the Gompertz equation, as modified by Zwietering et al. (17), to the data points.

**RESULTS**

**Changes in the population of *L. monocytogenes* at 5 and 10°C**

*Listeria monocytogenes* was not detected on any control sample.

At both 5 and 10°C, *L. monocytogenes* grew on VP normal ultimate pH beef steaks but did not grow on steaks stored under CAP conditions (Fig. 1).

At 5°C under VP, the population of *L. monocytogenes* increased 2.8-log cycles, from 6.2-log<sub>10</sub> CFU per sample to 9.0-log<sub>10</sub> CFU per sample in 553.5 h (23 days). At 10°C under VP, the population of *L. monocytogenes* increased...
3.2-log cycles from 6.3-log_{10} CFU per sample to 9.5-log_{10} CFU per sample in 265.5 h (11 days). The growth rate of *L. monocytogenes* at 10°C was 2.6 times faster than that at 5°C.

At 5°C under CAP, the population of *L. monocytogenes* recovered decreased 0.6-log cycles, from 5.6-log_{10} CFU per sample to 5.0-log_{10} CFU per sample in 432 h (18 days). At 10°C under CAP, the population of *L. monocytogenes* recovered decreased 1.5-log cycles, from 6.7-log_{10} CFU per sample to 5.2-log_{10} CFU per sample in 1,056 h (44 days).

**Growth of lactic acid bacteria spoilage organisms at 5 and 10°C**

At 5°C, the growth rate of spoilage organisms was 1.8 times faster under VP than that under CAP (Fig. 2). At 5°C under VP, the population of spoilage organisms increased 5.5-log cycles from 4.0-log_{10} CFU per sample to 9.5-log_{10} CFU per sample in 553.5 h (23 days). Off odors, evident on opening the external VP packets prior to opening the individually packaged steaks, were detected at 337.5 h (14 days) when the spoilage organisms were 2.1-log cycles from maximum numbers but were not detected at 217.5 h (9 days). At 5°C under VP, spoilage organisms grew 2.7 times faster than *L. monocytogenes*.

At 5°C under CAP, the population of spoilage organisms increased 6.3-log cycles from 2.9-log_{10} CFU per sample to 9.2-log_{10} CFU per sample in 1,104 h (46 days). Off odors, evident on opening the external CAP packets prior to opening the individually packaged steaks, were not detected with CAP at any time, including the final sampling time of 1,104 h (46 days).

At 10°C, the growth rate of the spoilage organisms was similar on both VP and CAP steaks (Fig. 2). The population of spoilage organisms on steaks stored at 10°C under VP increased 5.9-log cycles from 3.9-log_{10} CFU per sample to 9.8-log_{10} CFU per sample after 337.5 h (14 days). Off odors were detected at 265.5 h (11 days) when the spoilage organisms were approaching maximum numbers but were not detected at 217.5 h (9 days). At 10°C under VP, spoilage organisms grew two times faster than *L. monocytogenes*.

At 10°C under CAP, the population of spoilage organisms increased 6.2-log cycles from 3.7-log_{10} CFU per sample to 9.9-log_{10} CFU per sample after 312 h (13 days). Off odors were detected at 588 h (24 days), by which time the spoilage organisms had been at maximum numbers for 276 h (11 days) but were not detected at 312 h (13 days).

**DISCUSSION**

*Listeria monocytogenes* grew to substantial numbers on VP normal ultimate pH beef stored at the abusive temperatures of 5 and 10°C. This supports the findings of Grau and Vanderlinde (6), who reported growth of *L. monocytogenes* on lean beef of pH 5.5 to 5.7 stored at 5.3°C under VP. In the present study, the onset of spoilage at both 5 and 10°C, evidenced by off odors, did not precede growth of *L. monocytogenes*. At these abusive storage temperatures, VP beef is likely to support growth of *L. monocytogenes* before spoilage is obvious. Therefore, VP beef should not be exposed to these abusive storage temperatures, as such exposure could result in a public health risk if the product contained *L. monocytogenes*.

*Listeria monocytogenes* did not grow on normal ultimate pH beef stored under CAP at either 5 or 10°C. In fact, the number of *L. monocytogenes* recovered in both cases declined over the course of the experiment. This may reflect either actual death of the inoculated organisms with time, or failure to recover sublethally injured cells on the selective agar.

Gill and Reichel (5) showed growth of *L. monocytogenes* (strain ATCC 19111) on high ultimate pH (>6.0) beef packaged under CAP and stored at 10°C. The data obtained in the present study show that two food-derived strains of *L. monocytogenes* did not grow on normal ultimate pH (5.3 to 5.5) beef packaged under CAP and stored at 5 and 10°C. While different strains were used in these two studies, it is likely that the low pH of normal pH beef adds a sufficiently high "hurdle" that growth of *L. monocytogenes* under CAP cannot occur at 10°C, while on high pH beef this impediment is lessened, permitting growth at 10°C. It seems, therefore, that exposure of normal ultimate pH beef stored under CAP to temperatures up to 10°C does not permit growth of *L. monocytogenes*, and cells may lose viability under these conditions.

These data show there is no risk from the growth of *L. monocytogenes* due to the extended storage-life of CAP normal ultimate pH beef, and thus, there is no additional public health risk associated with these products. Exposure of CAP beef to abusive temperatures, while not risking...