GROWTH OF *LISTERIA MONOCYTOGENES* ON SODIUM REDUCED HAM

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Abstract – Microbial challenge studies were performed on vacuum-packaged ham manufactured with specific levels of added NaCl to study the impact of sodium concentration on the survival and growth of *Listeria monocytogenes*. Ham was inoculated with *L. monocytogenes* in the presence or absence of the background microflora and stored 4°C. The length of the lag phase of *L. monocytogenes* was shortened by half in ham with 0.5% added NaCl compared with that observed in ham with 1% added NaCl. The presence of high numbers of background microflora significantly reduced the *Listeria* counts, regardless of the sodium content in the ham.

Key Words – *Listeria*, low salt, ready-to-eat meat.

I. INTRODUCTION

Sodium salts, especially sodium chloride, play an important role in the diet, as sodium is required for many physiological and intracellular functions in the human body. However, excess sodium intake can cause electrolyte imbalance that can lead to kidney overburden, cardiovascular disease and high blood pressure [1]. Health agencies including Health Canada, have asked for a sodium reduction in foods including ready-to-eat (RTE) prepackaged meat products, and strategies have been proposed to achieve this goal by 2016 (2). The specification for sodium reduced RTE meats includes the maximum sodium content for a "less salt" product is 800 mg per 100 g product.

Traditionally, sodium serves as a preservative in meat. A reduction in sodium may lead to an increase in the growth of food spoilage and pathogenic bacteria [3]. A major concern specifically associated with sodium-reduced RTE vacuum packaged meat such as sausages, hot dogs and ham, would be the potential for increased growth of *Listeria monocytogenes*, which is the causative agent for listeriosis. Listeriosis is a severe illness and may lead to a death. In Canada, there are between 100 and 140 cases of listeriosis reported each year (4).

The sodium content in meat may influence the growth of *L. monocytogenes*. A change in NaCl concentration influences the water activity (a_w) of the environment, which affects the growth of microorganisms. Sodium ions interact with bacterial proteins [2], which could have an impact on the growth of *Listeria*. The presence of a background microflora might also influence the growth of *Listeria* and its susceptibility to changes in NaCl concentrations. To date, there have not been any *in situ* studies specifically evaluating the effect of sodium and background microflora on the growth of *L. monocytogenes*.

To assess whether sodium reduction causes changes in the growth of *L. monocytogenes*, ham products formulated with specific concentrations of added NaCl levels (0.5%, 0.75% and 1% added NaCl) were manufactured. Ham was inoculated with *L. monocytogenes* and/or cocktails of background microflora (previously isolated from RTE meats) and stored at refrigeration temperature.

This research aims to improve the microbial safety of sodium reduced RTE meat products. The study was conducted *in situ*, and therefore provides insight on the growth of foodborne pathogens in meat products. The methods and models used in this study can be validated for future research in monitoring microbial survival, growth and metabolic activity.

II. MATERIALS AND METHODS

Bacterial strains and media

The strains of lactic acid bacteria used in this study were isolated from vacuum packaged sliced deli

meats purchased from local retail markets. These included: *Brochothrix thermosphacta* P107, *Carnobacterium maltaromaticum* E102, *Lactobacillus sakei* F201, *Leuconostoc gasicomitatum* C302 and *Leuconostoc gelidum* C101. These strains were cultured in All Purpose Tween broth (APT, Difco). Strains of *L. monocytogenes* used in the cocktail included strains that were previously determined to be heat-resistant and included: *L. monocytogenes* FS13, *L. monocytogenes* FS14, *L. monocytogenes* FS19 and *L. monocytogenes* ATCC 7644. These strains were cultured in Tryptic Soy Broth (TSB, Difco). APT agar was used for enumeration of the background microflora and lactic acid bacteria. PALCAM (Polymyxin, Acriflavine, Lithium chloride, Ceftazidime, Aesculin, Mannitol; Oxoid) agar (Oxoid) containing selective supplement (Oxoid) was used for enumeration of *Listeria*.

Ham ingredients

Pork leg meat was obtained from a federally inspected meat processing facility. Sodium chloride, sodium erythorbate, dextrose monohydrate, sodium tripolyphosphate (STPP) and Prague powder were supplied by Griffith Laboratories™. Water-proof nylon casings (75 mm in diameter) were purchased from UniPac.

Ham production

Pork leg meat was defrosted at 4°C overnight and chopped into ca. 4 cm x 4 cm x 4 cm cubes. The meat was ground through a 1/8" plate (Mini-matic; Hollymatic Corp.). Meat and other ingredients [ice (20% of meat weight), STPP (0.6% of meat weight), sodium erythorbate (0.1% of meat weight), dextrose (3% of total batch weight), Prague powder (0.46% of meat weight), NaCl (0.5% of total batch weight)] were combined and tumbled (VAS-40, Glass®) under vacuum (-800 mbar) at 4°C for 10 min and rested for 1 h under vacuum. Meat batter was stuffed into casings, clipped (Tipper Tie Technopack™) and stored overnight at 4°C. The hams were cooked at 100°C in a kettle (M-04, Groen) for 10 min and then at 80°C until the final internal temperature reached 71°C. Cooked ham was sliced 3 mm thick.

Ham inoculation and packaging

Spoilage bacteria and *Listeria* spp. were cultured from frozen stock in appropriate media at room temperature (LAB and *Brochothrix*) or 37°C (*Listeria* spp.). All strains were subcultured once and incubated at 22°C overnight before inoculation. Bacterial cultures were centrifuged at 4,000 *x g* for 10 min at room temperature. Cell pellets were suspended in 0.85% (w/w) saline and diluted. Individual slices of ham were inoculated with saline, cocktail of spoilage bacteria (4.0 log CFU/slice), a cocktail of *Listeria* spp. (3.0 log CFU/slice) or both the spoilage cocktail and the cocktail of *Listeria* spp. Ham slices were vacuum packaged individually (C200; Multivac) and stored at 4°C.

Water activity

The water activity (a_w) of ham was measured using a water activity meter (AQUA LAB Rapid Read) following the manufacturer's instructions.

Sampling

After 1, 4, 7, 15, 22, 28 and 36 days of storage, sterile 0.85% saline was added to each package and the packaged was stomached for 2 min. Serial dilutions were prepared in saline and samples were plated onto APT or PALCAM agars. Cell counts were determined on APT agar after 48 h incubation at 22°C and on PALCAM agar after 24 h incubation at 37°C.

III. RESULTS AND DISCUSSION

Cell counts of *L. monocytogenes* and/or background microflora from different sampling times were determined. The cell count of *L. monocytogenes* in ham with 1% of NaCl was significantly higher than that in products with 0.5% NaCl and after 36 d of storage, the difference was 2 log CFU/slice. The lag phase of growth (time for *L. monocytogenes* to initially increase growth by1 log CFU/slice) for samples formulated with 0.5%, 0.75% and 1% NaCl were 7, 9 and 15 days, respectively (Figure 1).

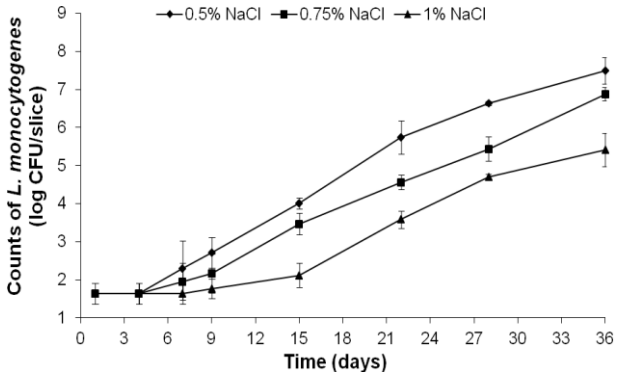

Figure 1. Mean log counts of *L. monocytogenes* on vacuum packaged ham formulated with 0.5, 0.75 or 1% NaCl and stored at 4°C. (Mean ± SD, n=3)

Sodium concentration also influenced the growth rate of the background microflora with faster growth in samples with a lower sodium concentration (Figure 2), but at the end of storage, there was no significant difference in maximum numbers of lactic acid bacteria.

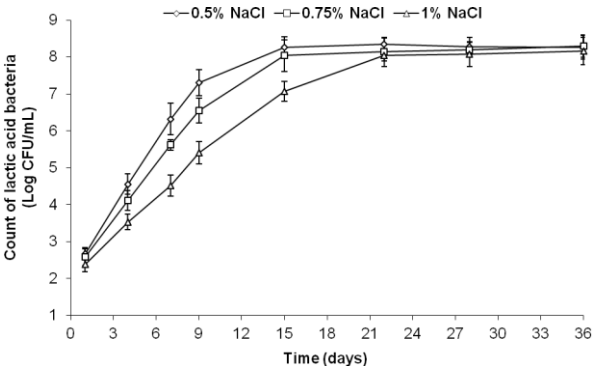

Figure 2. Mean log counts of the background microflora on vacuum packaged ham formulated with 0.5, 0.75 or 1% NaCl and stored at 4°C. (Mean ± SD, n=3)

In products inoculated with cocktails of spoilage bacteria and *Listeria* spp., the presence of spoilage microflora reduced the maximum growth of *L. monocytogenes* at the end of the 36 days of storage. The growth rate and length of the lag phase of *L. monocytogenes* were not influenced by the sodium level in the ham (Figure 3). This suggests that the competition from the background microflora overcame the effect of sodium reduction on the growth of *Listeria*.

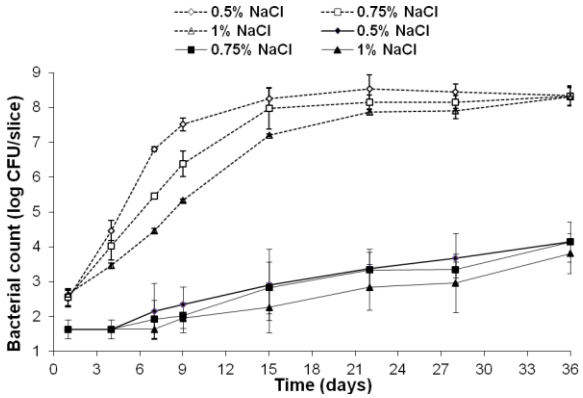

Figure 3. Mean log counts of the background microflora (solid lines) and *L. monocytogenes* (dotted lines) on vacuum packaged ham formulated with 0.5, 0.75 or 1% NaCl and stored at 4°C. (Mean ± SD, n=3)

No difference in a_w was detected among the hams formulated with various concentrations of NaCl. The a_w of all samples was between 0.982 and 0.985. This was contradictory to what is suggested in literature that NaCl influences the growth of microorganisms by altering a_w in the environment. It could be the fact that reducing the level of added NaCl from 1% to 0.5% was not enough to cause a detectable change in a_w. It also suggests that different sodium levels, regardless whether it alters a_w in meat or not, can influence the growth of *L. monocytogenes*. It would be useful to design studies on ham products produced with a wide range of added NaCl and assess the relationship between NaCl and a_w.

IV. CONCLUSION

The added NaCl concentrations of ham products used in this study (0.5%, 0.75% and 1%) were close to the specifications sodium reduction in RTE meats (Health Canada). It was found that *L. monocytogenes* had a shorter lag phase and grew faster in ham with 0.5% NaCl than that with 1% NaCl. The presence of a background microflora minimized the impact of lower NaCl concentrations.

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