Impact of Sodium Reduction on Survival of *Listeria monocytogenes* in Sliced Process Cheese

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Abstract

This project carried out the experimental research to investigate whether *Listeria monocytogenes*, a major food safety pathogen, is capable of surviving in sliced process cheese and if the survival is enhanced in low-sodium formulations. The experiments were conducted with three different brands of commercially available sliced cheeses (Brands A, B, C) packaged in bulk (slice on slice) and as individually wrapped slices. The effect of salt content and temperature were measured using surface inoculation of slices with pure cultures of 5 strains of *L. monocytogenes* and determining bacterial counts over time. The changes in *Listeria* counts were correlated with those factors as well as with pH, water activity and moisture concentration to identify the risk associated with low sodium cheese in comparison with regular cheese. At 4°C, the count of *L. monocytogenes* remained at approximately 4 log CFU/g for the entire 60 days of storage in any of the three brands of regular and reduced salt processed cheese in both SOS or individually wrapped slices. As the storage temperature increased, the *Listeria* counts declined reaching undetectable levels at any condition after 25 and 15 days at 23 and 30°C, respectively. At those temperatures, the survival rate in Brand C appeared to be shorter than the other two brands. In summary, the main finding of this research was that sodium reduction in processed cheese did not enhance survival nor promoted growth of *L. monocytogenes* at any temperature or brand tested.

Background Information

According to the standard of identity described in the Code of Federal Regulations, processed cheeses are defined as "the food prepared by comminuting and mixing, with the aid of heat, one or more cheeses of the same or two or more varieties" (21CFR 133.169). Cheddar, Swiss, Colby, Muenster and Gruyere are among the most common types of cheeses used for process cheese manufacturing. Process cheeses are required to be heated at 150^{o} F for 30 s and are required to have specific physicochemical characteristics. Pasteurized process cheese should meet the standard product criteria including maximum 43% moisture, 1% higher than its natural cheese, similar fat contents as natural cheese and pH values of 5.3 or higher. According to Glass et al (1998), samples of commercial sliced process cheese had a pH range of 5.56 to 5.81, water activity values from 0.918 to 0.929, water content from 39.1 to 40.3%, and salt concentrations of 2.35 to 2.62.

Because of the additional thermal treatment combined with intrinsic characteristics (pH, water activity, salt) that minimize microbial contamination and prevents the growth and survival of potentially pathogenic organisms, sliced process cheese has been rarely involved as a vehicle of listeriosis. In one of the few instances of recorded *Listeria* contamination of process cheese, in 2006 a recall of cheeseburgers due to detection of *L. monocytogenes* in the cheese slices was conducted by a ready-to-eat foods company (FDA, 2006). The recent recall of Swiss process cheese in Canada stresses the potential risk of environmental contamination with this highly virulent foodborne pathogen (CFIA, 2010). Fortunately, in none of these instances there were cases involved, but the previous occurrence of outbreaks linked with sliced meat products suggest a potential risk.

Salt has been traditionally added to dairy products for different purposes, but in different products such as cheeses, the concentration of sodium chloride is important for controlling microbial contamination and growth. The reduction of salt in cheese products prompted by the recent corroboration of its negative effects on health is leading cheese manufacturers to investigate the reformulation of low-sodium alternatives. However, these changes require a thorough evaluation of the potential food safety implications. The current project proposes a relatively simple and straight forward experimental approach to assess this risk in low-sodium sliced process cheese.

Objectives:
The overall goal of this project was to assess the ability of *Listeria monocytogenes* to survive in low-sodium commercial sliced process cheeses.

The specific objectives were:

Objective 1: Determine the effect of reduced-sodium on the survival of *Listeria monocytogenes* on commercial bulk sliced process cheese

Objective 2: Compare the survival rate of *L. monocytogenes* on individually wrapped slices with slice on slice (SOS, bulk) process cheese

Objective 3: Assess the role of sodium chloride content in combination with water

activity and pH to influence *L. monocytogenes* survival

Materials and Methods

Objective 1

a. Experimental design:

A series of experiments were conducted to determine the survival of *Listeria monocytogenes* in sliced (SOS) process cheese. American cheese with their corresponding low-sodium counterparts were obtained from three commercial manufacturers (A, B and C). Five different strains were inoculated into cheese slices and were evaluated for survival at different temperatures. The effect of different temperatures survival were assessed by periodical sampling every 3 days for as long as 21 days. Four different temperatures that were used for incubation temperatures are 4, 12, 23 and 30^{o}C. The experiments had a paired design and included the normal and the low-sodium counterparts. Each treatment were replicated at least twice in independent experiments.

All experiments were conducted at the biosafety level 2 Food Safety Microbiology Laboratory located in the second floor of the ABLMS building.

b. Inoculum preparation

A total of five strains with distinctive ribotype of *L. monocytogenes* originally isolated from cheese outbreaks (strains 1038, 1042, 1042B) and from frankfurters implicated in human cases (strains 1053, 1044A) were used in this project. Stock cultures of all the strains were stored in glycerol at -55^{o}C and working cultures were prepared after streaking onto tryptic soy agar (TSA) incubated at 37^{o}C for 48 h. A single colony was picked and transferred to tubes containing tryptic soy broth (TSB) media which was incubated at 37^{o}C for 18 h to reach stationary phase and an approximate bacterial cell concentration to 10^{9} CFU/ml. Cultures of the five strains were evenly mixed and after serial dilutions of TSB cultures individual slices were inoculated on a single side with approximately 0.05 ml to obtain initial cell concentrations of approximately 10^{4} CFU/g. The inoculated slide was allowed to adsorb for 30 min and then covered with another slide. Two additional cheese slides

were placed on each of the exposed sides of the inoculated slices. The 6-slide stacks were placed into a sterile plastic bag, sealed and placed in the corresponding incubator.

c. Analytical methods

The bacterial count were determined at each of the sampling times as follows: duplicate 11 g samples of the central inoculated slice pairs were homogenized into 99 ml of buffered peptone water (BPW) in a stomacher for 2 minutes, serially diluted 10-fold into test tubes containing BPW to 10^{-4}, and 0.1 ml of each of this dilutions was spread-plated onto PALCAM agar, a selective media for *Listeria*. Petri plates were incubated at 35°C for 24-48 h. Physicochemical testing will be conducted on non-inoculated samples. Measurement of pH was done using a pH electrode (Beckman, Inc). Moisture contents were determined using a gravimetric method in a forced-air draft oven. Water activity was determined using a commercial portable meter (AquaLab, Inc.). Salt concentrations were measured determined through a reference laboratory, Research Analytical Laboratory, University of Minnesota, 135 corps Research, 1902 Dudley Avenue.

d. Statistical methods

The count of bacterial strains for each individual sample will be the average of at least two plate counts and this value were transformed to $\log_{10}$ CFU/mL. The results for at least two independent experiments of each treatment were be used to calculate the mean and standard deviation at each sampling day for each treatment. The average data points at each sampling time will be plotted or tabulated as a time course where the differences among control and treatment groups could be visualized. The detection limit for bacterial counts was <1 log CFU/ mL for all experiments. When the bacterial level was not detected, 0.9 log CFU/ mL were used for calculation in the analysis. Mean and standard deviations were determined using Microsoft Excel (Version 14.0; Microsoft, Corp; Redmond, WA). Statistical analyseswere performed by analysis of variance (ANOVA) and Duncan's multiple range test for differences ($P< 0.05$) between control and treatment groups using SAS software (9.2; Statistical Analysis systems, Cary, N.C., U.S.A.).

Objective 2

Objective 2 were conducted in a similar design as objective 1. The only difference is that the single cheese slice was individually wrapped in typical individual cheese wraps and sealed before incubating at different temperatures. There was a lack of commercially available individually wrapped sliced low sodium cheese. So fulfil the need, the commercial individually wrapped cheese was bought, the plastic was removed from the product, the cheese was discarded and the plastic was used to wrap the inoculated single slice process American cheese.

Results and Discussion*(Please write your discussion in past tense and use a format similar to a publication.)*

In this project, a series of experiments intended to address Objective 1 and 2 were completed. Three different commercial brands, A, B and C of American process cheese were used to determine the survival of *L. monocytogenes* on low-sodium in comparison to regular cheese levels at four different temperatures. Objective 1 was mainly addressed to identify the levels of *Listeria* with slice on slice (SOS) process cheese while experiments of objective 2 involved the individually wrapped (IW) commercial process cheese slices.

When inoculated on SOS bulk process cheese at lower temperatures (4° C), the counts of *L. monocytogenes* onto American process cheese samples remained practically unchanged after 60 days period of experiments in regular and low-sodium cheese samples and there were no statistical difference (Fig. 1A). At 12° C, bacterial counts were static for 30 days and then gradually declined during last 20 days over 2 months period, bacterial cells survived longer in brand B than other brands (Fig 1B). However, at higher temperatures, at 23° and 30°C the numbers of *Listeria* sharply declined as soon as within the first 12 and 9 days of the experiment with brand C cheese. Interestingly, *Listeria* was detected for longer periods on brand A and B cheese at higher temperatures (23° and 30C) than brand C cheese (Fig 1C and 1D). Similar results of *L. monocytogenes* survival were obtained at different incubation temperatures in low-sodium cheese samples within different bands. Overall, no clear distinguishable patterns of *Listeria* survival were observed on low sodium cheese compared to regular cheese. (Fig 1).

The experiments of objective 2 were conducted with IW sliced process cheese to evaluate the survival of inoculated *L. monocytogenes* population on IW low- sodium cheese compared to regular sliced

cheese of brand A, B and C at different temperatures and times. Regardless the brands, microbial populations of inoculated *L. monocytogenes* did not show any change over 60 days period at 4°C incubation temperature (Fig. 2A). However, 12° C has minor effects on *L. monocytogenes* survival with Brand B cheese; the bacterial counts were reached to undetected level by 60 days on regular cheese compared as low counts on low sodium cheese (Fig. 2B). At higher temperatures, the numbers of *L. monocytogenes* were gradually declined and remained undetectable by 21 days at 23° C (brand B) and by 12 days at 30° C on both regular and low sodium cheese. (Fig.2C and 2D). At 23° C, bacterial cells survived longer on brand B cheese compared to other two brands. Similar survival patterns of *Listeria* were observed on low sodium cheese within different brands.

There were no significant changes that observed in *Listeria* survival in individually wrapped slices with previous bulk slice on slice cheese experiments. (Fig. 1 and 2). Similar survival patterns were observed on commercial sliced process American cheese within the different brands A, B and C at different incubation temperatures.

The pH, water activity, moisture of the samples of the three American cheese varieties (brand A, B and C) were measured and presented in Table 1. The sodium and potassium contents were also analyzed of the low-sodium and regular cheeses as mg/kg (ppm) (Table 1). In low-sodium cheese, sodium contents seemed to be replaced by potassium to compensate the salt concentration, which might explain the similar survival scenarios in both cheeses. Also, the low water activity and pH may have contributed in the bacterial population declining at higher temperatures (23° and 30°C) along with the antimicrobial effects of acids and other stresses in the cheese matrices. No difference observed at the moisture levels in between low-sodium and regular cheese samples.

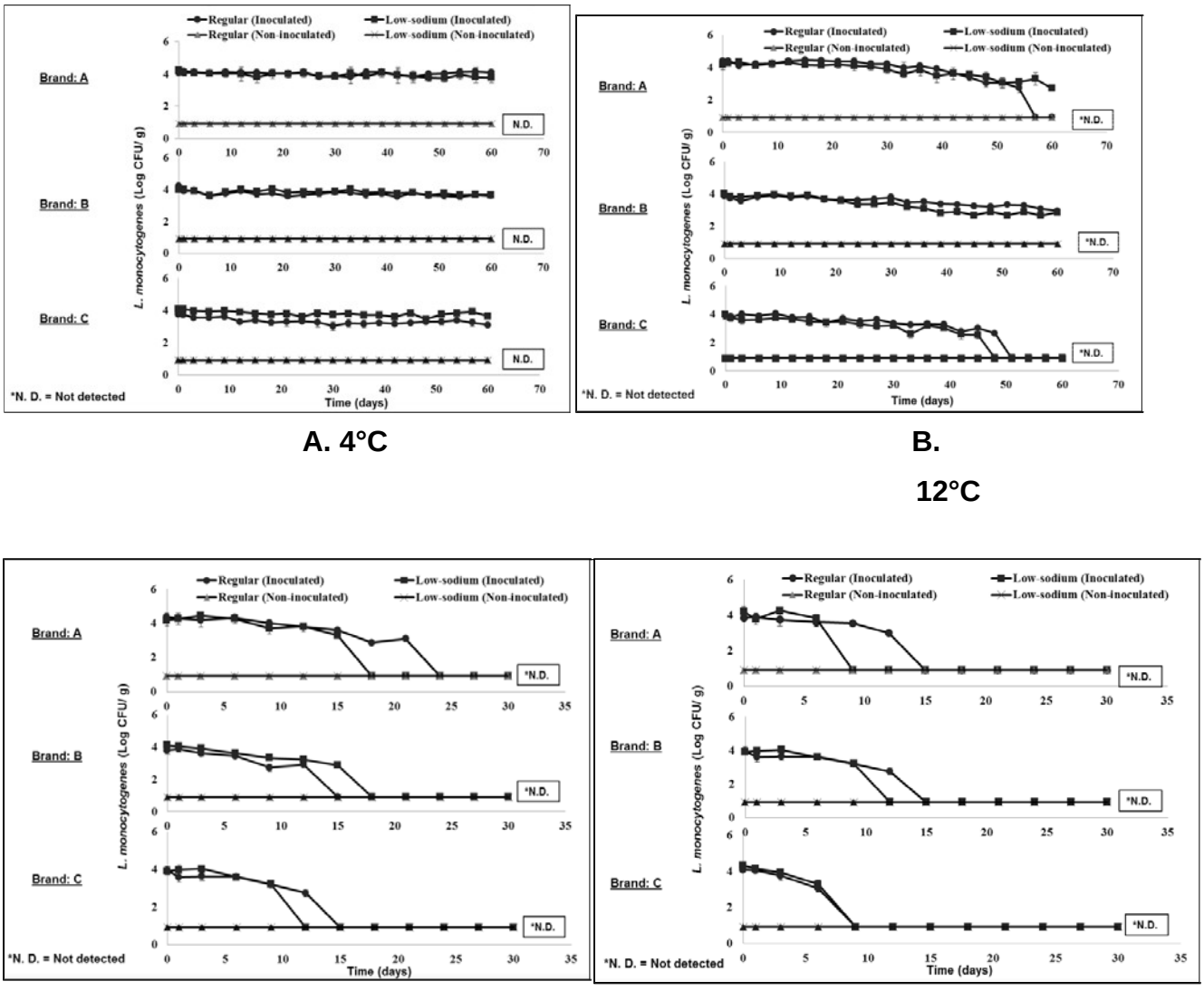

Figure 1. Survival of*Listeria monocytogenes* (log_{10} CFU/ g) on commercial bulk process sliced (slice on slice) low-sodium and regular American cheeseof brand A, B and C at 4°C (A), 12°C (B), 23°C (C) and 30°C (D).

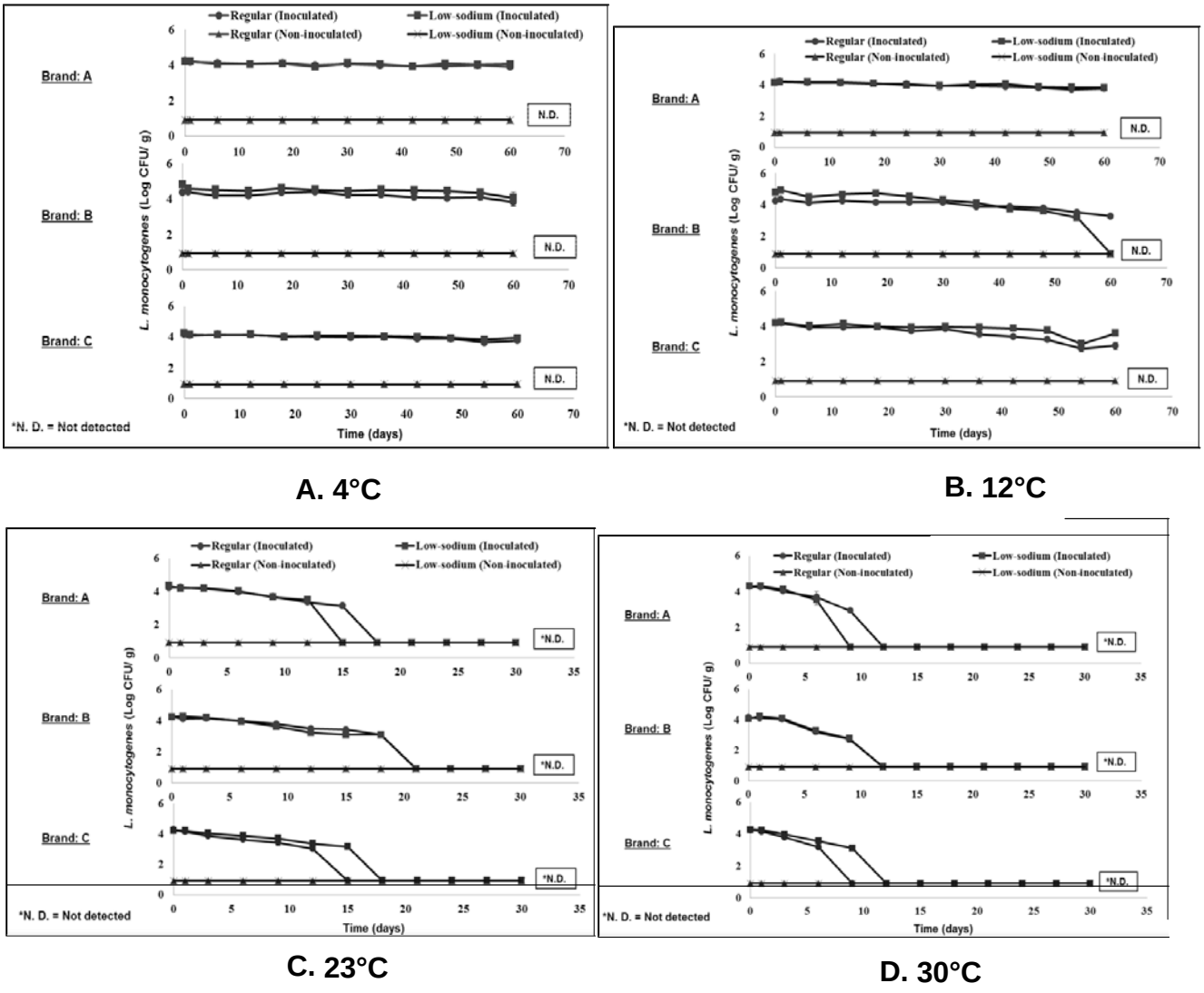

Figure 2. Survival of*Listeria monocytogenes* (log_{10} CFU/ g) on commercial process sliced (individually wrapped) low-sodium and regular American cheese of brand A, B and C at 4°C (A), 12°C (B), 23°C (C) and 30°C (D).

Table 1. Physicochemical measurements of regular and low-sodium sliced process American cheese of different brands.

Frequency	Brands	Sliced process American cheese	
		Regular	Low-sodium
pH	A	4.54 ± 0.13	4.67 ± 0.12
	B	5.00 ± 0.04	4.16 ± 0.11
	C	4.63 ± 0.15	4.85 ± 0.11
Water activity (a_w)	A	~ 0.89	~ 0.90
	B	~ 0.91	~ 0.95
	C	~ 0.89	~ 0.91
Moisture (%)	A	38.74	38.71
	B	39.64	39.65
	C	40.71	39.08
Sodium (mg/kg)	A	16099.0	9020.6
	B	16621.0	10995.0
	C	17287.0	12304.0
Potassium (mg/kg)	A	2111.4	12606.0
	B	1145.2	898.1
	C	2441.8	5975.1

Conclusions

This study was conducted to identify the effects of sodium reduction on *L. monocytogenes* survival on commercially process sliced American and Swiss cheese of three different brands. Our results indicated that there were no differences of *L. monocytogenes* survival patterns on slice on slice or individually wrapped process American Cheese at different temperatures. In all low sodium cheese, salt levels were replaced by potassium salts which contribute to the similar survival patterns as on regular cheese. Overall, *Listeria* cells showed the capacity to survive longer at lower storage temperatures compared to higher temperatures. Due to the unavailability of low sodium Swiss Cheese, all the experiments were done with American cheese.

This study provided the first actual data of *Listeria* cell survival on commercially available low sodium American cheese. This study will benefit the industries by addressing the important issue of the fate of *L. monocytogenes* on low sodium cheese and involved risks in case of cross contamination at different incubation temperatures.

References

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