Survival of *Listeria monocytogenes* introduced as a post-aging contaminant during storage of low-salt Cheddar cheese at 4, 10, and 21°C

S. Shrestha,* J. A. Grieder,* D. J. McMahon,† and B. A. Nummer*¹

*Department of Nutrition, Dietetics and Food Sciences, and

†Western Dairy Center, 8700 Old Main Hill, Utah State University, Logan 84322-8700

ABSTRACT

Traditional aged Cheddar cheese does not support *Listeria monocytogenes* growth and, in fact, gradual inactivation of the organism occurs during storage due to intrinsic characteristics of Cheddar cheese, such as presence of starter cultures, salt content, and acidity. However, consuming high-salt (sodium) levels is a health concern and the dairy industry is responding by creating reduced-salt cheeses. The microbiological stability of low-salt cheese has not been well documented. This study examined the survival of *L. monocytogenes* in low-salt compared with regular-salt Cheddar cheese at 2 pH levels stored at 4, 10, and 21°C. Cheddar cheeses were formulated at 0.7% and 1.8% NaCl (wt/wt) with both low and high pH and aged for 10 wk, resulting in 4 treatments: 0.7% NaCl and pH 5.1 (low salt and low pH); 0.7% NaCl and pH 5.5 (low salt and high pH); 1.8% NaCl and pH 5.8 (standard salt and high pH); and 1.8% NaCl and pH 5.3 (standard salt and low pH). Each treatment was comminuted and inoculated with a 5-strain cocktail of *L. monocytogenes* at a target level of 3.5 log cfu/g, then divided and incubated at 4, 10, and 21°C. Survival or growth of *L. monocytogenes* was monitored for up to 90, 90, and 30 d, respectively. *Listeria monocytogenes* decreased by 0.14 to 1.48 log cfu/g in all treatments. At the end of incubation at a given temperature, no significant difference existed in *L. monocytogenes* survival between the low and standard salt treatments at either low or high pH. *Listeria monocytogenes* counts decreased gradually regardless of a continuous increase in pH (end pH of 5.3 to 6.9) of low-salt treatments at all study temperatures. This study demonstrated that post-aging inoculation of *L. monocytogenes* into low-salt (0.7%, wt/wt) Cheddar cheeses at an initial pH of 5.1 to 5.5 does not support growth at 4, 10, and 21°C up to 90, 90, and 30 d, respectively. As none of the treatments demonstrated more than a 1.5 log reduction in *L. monocytogenes* counts,

the need for good sanitation practices to prevent post-manufacturing cross contamination remains.

Key words: *Listeria monocytogenes*, low-salt cheese, safety

INTRODUCTION

Traditional Cheddar cheese manufacturing, including pasteurization of milk and good manufacturing practices, minimizes the occurrence and growth of *Listeria monocytogenes* (Ryser and Marth, 1987; Genigeorgis et al., 1991; US FDA 2009). In addition, Bishop and Smukowski (2006) cite that intrinsic characteristics of hard and semi-hard cheese developed during fermentation and aging create a hostile environment for bacterial pathogens. Cheddar cheese is typically manufactured to a pH range of 4.9 to 5.4 (Lawrence et al., 1984), water activity (a_w) from 0.93 to 0.97, and water phase salt (WPS) from 4.6 to 5.4% (Lawrence and Gilles, 1980, 1982; Marcos et al., 1981). The US FDA (2009) has listed pH <4.4, a_w <0.92, and WPS >10% as growth limiting for *L. monocytogenes*. Therefore, it is believed that the multiple hurdles of low pH and WPS, together with the activity of starter and non-starter cultures contribute to the inhibition of *L. monocytogenes* growth in Cheddar cheese (Ryser, 1999) during both fermentation and aging. After aging, Cheddar cheese is typically further processed by both manufacturers and retailers into consumer-friendly blocks, shreds, or slices. *Listeria monocytogenes* is considered a common environmental contaminant in both manufacturer and retailer facilities and significant risk exists for contamination at this stage in processing (CFP, 2004–2006).

It has been recognized for several decades that Americans consume unhealthy amounts of sodium in their food. Consuming too much sodium increases the risk for high blood pressure that can lead to a variety of diseases (IOM, 2001; Dickinson and Havas, 2007). It has been estimated that population-wide reductions in sodium could prevent more than 100,000 deaths annually (Danaei et al., 2009). The dairy industry has responded to these concerns by developing reduced-sodium cheese varieties. To be labeled as low-sodium, the product

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¹Corresponding author: brian.nummer@usu.edu

cannot contain more than 140 mg of sodium/50 g (US FDA, 2008), equivalent to 0.7% (wt/wt) NaCl. This is in comparison to the typical sodium content of 310 mg of sodium/50 g [1.6% (wt/wt) NaCl] in Cheddar cheese (Johnson et al., 2009).

Sodium chloride, along with pH, a_w , and lactic acid content are multiple hurdles contributing to the microbiological safety of traditional cheeses. Lowering the salt content (sodium chloride) may be a food safety and shelf-life concern (Johnson et al., 2009). The goal of this study was to evaluate whether low-salt Cheddar cheese at 2 different pH levels could support the growth of *L. monocytogenes* at refrigeration or abuse temperatures, simulating post-manufacturing contamination. Specifically, the objectives were to monitor the growth of inoculated *L. monocytogenes* at 4, 10, and 21°C in low-salt Cheddar cheese produced with low and high-pH compared with standard salt Cheddar cheese.

MATERIALS AND METHODS

Cheese Production

Cheddar cheese was made from 272 kg of pasteurized (73°C, 15 s) milk (Gary Haight Richardson Dairy Products Laboratory, Utah State University, Logan, UT), standardized to a protein-to-fat ratio of 0.85. Starter (34 g of *Lactococcus lactis*, DVS850; 12 g of *L. lactis* ssp. *cremoris*, DVS 213; and 6 g of *Lactobacillus helveticus*, LH 32; Chr. Hansen Inc., Milwaukee, WI) was added to the milk at 31°C, followed by CaCl₂ (0.12 mL/kg of milk) and annatto (0.07 mL/kg of milk; single strength; DSM Food Specialties USA Inc., Eagleville, PA). Milk was ripened for 30 min and then set using 20 mL of double-strength rennet (0.07 mL/kg of milk; Maxiren; DSM Food Specialties USA Inc.). After 30 min the curd was cut and allowed to heal for 5 min. Cheese curd was stirred for 25 min and then cooked by gradually raising the temperature to 39°C in 35 min. The curd was held at 39°C for 40 min until the pH reached 6.32. Whey was drained and curd was Cheddared (stacking block of cheese curd to expel additional whey and to knead the curd) until a pH 5.8 was reached. The curd was finally milled and divided into 4 approximately 7-kg portions. Treatment A curd (low salt and low pH) was held at 36°C until the curd reached pH 5.45 and 8.2 g of salt/kg was added. Treatment B curd (low salt and high pH) was washed with 3 L of water (at 12°C) to remove lactose and minimize acid production, and then salt was added at 8.2 g/kg. Treatment C curd (standard salt and high pH) had 24.2 g of salt/kg added. Treatment D curd (standard salt and low pH) was held at 36°C until the curd reached pH 5.25 and then 24.9 g of salt/kg was added. All treatments were hooped and

pressed overnight at ambient temperature. Blocks were vacuum packaged in PE-EVOH-PE 3.5-mil thickness bags (Vilutis and Co. Inc., Frankfort, IL) and aged for 10 wk at 6°C.

Proximate Analysis

Cheese pH, moisture content, a_w , and fat and salt content were determined after 10 wk of aging for each treatment (A to D). The cheese pH was determined by combining 20 g of finely grated cheese with 10 g of distilled water in a stomacher bag (model 400; Seward, Riverview, FL). Samples were homogenized in a stomacher (model 400, Seward) for 1 min at 260 strokes/min. The pH was measured using a Xerolyt combination electrode (model HA405; Mettler Toledo Inc., Columbus, OH) and an Accumet pH meter (Model AR 25; Fisher Scientific Inc., Pittsburgh, PA). The moisture content was measured using a microwave oven (model 907875; CEM Corp., Matthews, NC) at 70% power with an endpoint setting of <0.4 mg of weight change over 2 s. A water activity meter (AquaLab LITE; Decagon Devices Inc., Pullman, WA) was used to measure the a_w . The fat content was determined using the Babcock method 15.8.A (American Public Health Association, 1992). The salt content was measured by combining 5 g of finely grated cheese with 98.2 g of water and stomached for 4 min at 260 strokes/min. The slurry was filtered through a Whatman no. 1 filter paper and the filtrate was analyzed for sodium chloride using a chloride analyzer (model 926; Corning, Medfield, MA).

Inoculum Preparation

Five strains of *L. monocytogenes*, J1-177 (serotype 1/2b, human isolate), C1-056 (serotype 1/2a, human isolate), N3-013 (serotype 4b, food isolate), R2-499 (serotype 1/2a, sliced turkey isolate), and N1-227 (serotype 4b, food isolate) were obtained from the Utah State University culture collection of Jeff Broadbent. Stock cultures were maintained frozen (−80°C). Working cultures were prepared by transferring 0.1 mL of thawed frozen stock into 10 mL of fresh tryptic soy broth (TSB; Neogen Corp., Lansing, MI) and incubating at 37°C for 24 h. Individual strains were then grown in TSB for 24 h at 37°C before inoculation. The 5-strain mixture was prepared by combining 2-mL aliquots of each strain in a sterile, conical, 15-mL centrifuge tube. Cells were pelleted by centrifugation (1,509 × *g* for 15 min) and resuspended in 10 mL of fresh 0.1% peptone solution 3 times. Appropriate dilutions of washed cell suspensions were prepared in 0.1% peptone solution to achieve approximately 10³ to 10⁴ cells/g of cheese.

Table 1. Physicochemical characteristics of Cheddar cheese from 4 treatments

Characteristic	Treatment ¹			
	A	B	C	D
Moisture ² (% wt/wt)	39.0	39.3	35.9	34.2
Fat (% wt/wt)	33	33	33	33
Salt ² (% NaCl, wt/wt)	0.68	0.70	1.88	1.74
WPS ³	1.7	1.8	5.0	4.8
Water activity	0.98	0.97	0.95	0.95
pH at 1 wk	5.06	5.30	5.66	5.28
pH at 10 wk	5.11	5.50	5.77	5.28

¹A = low salt, low pH; B = low salt, high pH; C = standard salt, high pH; D = standard salt, low pH.

²Mean of 3 replicates.

³Water phase salt (WPS) = % salt × 100/(% salt + % moisture).

Sample Inoculation and Storage

Ten-week-old cheese treatments A to D were comminuted (Comitrol 1700; Urschel Laboratories Inc., Valparaiso, IN) to 3-mm particle size. A portion of each treatment was retained as an uninoculated control. The 5 strain mixture of *L. monocytogenes* was pipetted (10 mL/kg) dropwise into comminuted cheese treatments while mixing (model Classic; KitchenAid, St. Joseph, MI) at speed setting 1 for 5 min. Each inoculated cheese treatment (A to D) was subdivided into 3 equal portions. All inoculated portions (A to D) and uninoculated controls (A to D) were vacuum packaged in 2-ply nylon, 3.5-mil thickness bags (North Central Food Processing Supply, Sioux Falls, SD). Portions of each inoculated and uninoculated control were incubated at 4, 10, or 21°C for up to 90, 90, and 30 d, respectively.

Listeria monocytogenes Survival and pH Measurement

Treatments were first enumerated approximately 30 min after inoculation. Thereafter, treatments placed at 4 and 10°C were enumerated at 15, 30, 45, 60, 75, and 90 d. Treatments placed at 21°C were enumerated at 5, 10, 15, 20, 25, and 30 d. After sampling, cheese treatments were again vacuum packaged in 2-ply nylon, 3.5-mil thickness bags. For *L. monocytogenes* enumeration, 11 g of cheese was added to 99 mL of sterile 2% sodium citrate at 42°C and stomached at normal speed for 2 min (Duncan et al., 2004). Serial dilutions were prepared using 0.1% peptone water and plated in duplicate on PALCAM agar (Neogen Corp.) containing PALCAM supplement (Dalynn Biologicals Inc., Calgary, Alberta, Canada). Colonies were enumerated after 48 h of incubation at 35°C. For pH measurement, 10 g of cheese was stomached with 5 mL of distilled water for 30 s and measured using Double Junction pH Testr 30 (Oakton Instruments, Vernon Hills, IL).

Experimental Design and Statistical Analysis

Cheddar cheese with 2 salt levels [low = 0.7% (wt/wt) and standard = 1.8% (wt/wt)] at 2 target pH levels (low = 5.2 and high = 5.7 at 10 wk of aging) were prepared. The 4 treatments were low salt and low-pH (A); low salt and high pH (B); standard salt and high pH (C); and standard salt and low pH (D). Three replications of the experiment were conducted using the prepared cheeses. In each replication, comminuted cheese samples were inoculated and analyzed in duplicate for *L. monocytogenes* counts at 7 different day points. Data points are expressed as mean ± standard deviation. A repeated-measure design was used, where cheese type was the treatment between subjects and repeated measure was carried out at 7 different day points. Analysis of variance for repeated measures was performed using the MIXED procedure of SAS (version 9.1; SAS Institute Inc., Cary, NC). The effect of replication was blocked to avoid the variations for each replicate. The compound symmetry covariance structure was used based on goodness of fit as indicated by Akaike's information criterion. The Tukey method was used to determine the significance differences of mean values at an $\alpha = 0.05$ over all comparisons.

RESULTS AND DISCUSSION

Cheese Analysis

The average composition of uninoculated treatments A to D is shown in Table 1. The moisture content in low-salt treatments (A and B) was higher as compared with that of standard salt treatments (C and D). Less syneresis of curd during manufacturing of low-salt cheese yielded cheese with higher moisture content, which may have significance in microbiological activity. During syneresis, some added salt was also lost with whey and, therefore, the salt concentration measured

Table 2. pH of treatment and control Cheddar cheeses during storage at 4°C¹

Day	Treatment				Control			
	A	B	C	D	A	B	C	D
0	5.08	5.45	5.73	5.27	5.11	5.50	5.77	5.28
15	5.09	5.51	5.50	5.28	NA	NA	NA	NA
30	5.13	5.67	5.35	5.22	NA	NA	NA	NA
45	5.21	5.96	5.26	5.22	5.21	6.00	5.28	5.24
60	5.33	6.30	5.32	5.28	NA	NA	NA	NA
75	5.25	6.37	5.30	5.20	NA	NA	NA	NA
90	5.32	6.46	5.34	5.25	5.33	6.40	5.47	5.32

¹Treatments and control: A = low salt, low pH; B = low salt, high pH; C = standard salt, high pH; D = standard salt, low pH. Treatment and control cheeses were aged for 10 wk before the inoculation study. NA = not analyzed.

later in cheese (Table 1) was lower than the amount of salt added during manufacturing. This loss of added salt is higher in standard salt cheese due to comparative higher syneresis. The amount of salt added was based on the previous experience of manufacturing cheese with different salt concentrations. Due to less controllable lactose fermentation in low-salt cheeses along with proteolysis of cheeses during aging, the observed pH values at 10 wk in treatments varied slightly from targets. At all study temperatures, the pH (Tables 2, 3, and 4) of low-salt treatments (A and B) gradually increased throughout the incubation period, possibly due to proteolysis in cheese (final pH of 5.32 to 5.63 in cheese A and 6.46 to 6.87 in cheese B at different incubation temperatures). Stadhouders (1962) and Peichevski and Petrova (1979) reported comparatively higher proteolysis in washed cheese, as seen in cheese B in the present study. Stadhouders (1962) observed that cheeses that were manufactured by washing the curds for 20 min had about 1.7 times more rennet than the cheeses that were not washed. Also, Upreti et al. (2006) reported that cheeses with low lactose (as in cheese B in the present study, which has been washed to lower lactose content) exhibited significantly more proteolysis than cheeses with high lactose. Cheddar cheese is not typically curd washed. However, this pH increase has implications for other cheese varieties when considering

production with higher pH. For standard salt treatments (C and D), the pH decreased initially, possibly due to production of lactic acid, and later increased gradually (final pH of 5.34 to 5.91 in cheese C and 5.25 to 5.54 in cheese D at different incubation temperatures), again possibly due to proteolysis in cheese.

Survival of *Listeria monocytogenes*

For treatments A to D, the mean inoculum level was 3.55 to 3.78 log cfu/g (Tables 5, 6, and 7). *Listeria monocytogenes* numbers decreased between 0.14 to 1.48 log cfu/g in all treatments incubated at 4, 10, and 21°C for up to 90, 90, and 30 d, respectively. Counts of *L. monocytogenes* decreased (0.54 to 1.48 log cfu/g) significantly ($P < 0.05$) in all treatments incubated at 4°C for 90 d (Table 5). This is in agreement with the *L. monocytogenes* growth in the cheese model proposed by Tienungoon et al. (2000). That model predicts no growth at pH levels below 5.5 at 4°C, regardless of salt content.

The counts of *L. monocytogenes* decreased (0.66 to 1.27 log cfu/g) significantly ($P < 0.05$) in all treatments incubated at 10°C for 90 d (Table 6). Applying the salt and pH levels used in this study to the ordinal logistic regression model of *L. monocytogenes* growing in cheese (Bolton and Frank, 1999) indicates probabili-

Table 3. pH of treatment and control Cheddar cheeses during storage at 10°C¹

Day	Treatment				Control			
	A	B	C	D	A	B	C	D
0	5.08	5.45	5.73	5.27	5.11	5.50	5.77	5.28
15	5.21	5.73	5.22	5.14	NA	NA	NA	NA
30	5.18	6.13	5.19	5.16	NA	NA	NA	NA
45	5.32	6.44	5.32	5.26	5.33	6.34	5.31	5.27
60	5.41	6.65	5.47	5.38	NA	NA	NA	NA
75	5.38	6.65	5.57	5.36	NA	NA	NA	NA
90	5.54	6.87	5.91	5.44	5.42	6.84	5.88	5.38

¹Treatments and control: A = low salt, low pH; B = low salt, high pH; C = standard salt, high pH; D = standard salt, low pH. Treatment and control cheeses were aged for 10 wk before the inoculation study. NA = not analyzed.

Table 4. pH of treatment and control Cheddar cheeses during storage at 21°C¹

Day	Treatment				Control			
	A	B	C	D	A	B	C	D
0	5.08	5.45	5.73	5.27	5.11	5.50	5.77	5.28
5	5.19	5.76	5.30	5.19	NA	NA	NA	NA
10	5.15	6.06	5.15	5.17	NA	NA	NA	NA
15	5.22	6.28	5.23	5.26	5.20	6.25	5.22	5.23
20	5.40	6.42	5.41	5.40	NA	NA	NA	NA
25	5.45	6.60	5.57	5.39	NA	NA	NA	NA
30	5.63	6.70	5.85	5.54	5.58	6.59	5.77	5.51

¹Treatments and control: A = low salt, low pH; B = low salt, high pH; C = standard salt, high pH; D = standard salt, low pH. Treatment and control cheeses were aged for 10 wk before the inoculation study. NA = not analyzed.

ties of growth at 10°C for treatments A to D at 70.5, 98.8, 65, and 36.4%, respectively. Note that the Bolton and Frank (1999) model does not account for any effect from lactic acid starter cultures. This may account for the high probability of growth in the model where no growth occurred experimentally.

Treatments A, B, and D exhibited a significant ($P < 0.05$) inoculum decrease (0.48 to 1.11 log cfu/g) after 30 d at 21°C (Table 7). *Listeria monocytogenes* counts were 2 to 5 times lower in treatments A, B, and D at 21°C storage (Table 7) as compared with 4 and 10°C after 30 d (Tables 5 and 6). Except for treatment C, in which greater reduction in *L. monocytogenes* counts was not observed, other studies have seen this same effect of higher storage temperature. Genigeorgis et al. (1991) reported that low-pH, low-salt Monterey jack cheese (pH 5.0 and WPS 1.3%) surface inoculated with approximately 4 log cfu of *L. monocytogenes*/g decreased 2 log cycles in 13 d of storage at 30°C compared with 30 and 19 d of 4 and 8°C storage, respectively. Ryser and Marth (1987) inoculated pasteurized whole milk with 5×10^2 cells of *L. monocytogenes* (strain Scott A, V7,

or California)/mL and observed greater log reduction for strain V7 when prepared Cheddar cheese was aged at 13°C compared with 6°C. The rate of metabolism of *L. monocytogenes* is increased at higher temperature, which probably results in faster inactivation due to autolysis. Also, the greater activity of starter and non-starter cultures in cheese at higher temperatures presumably will inhibit *L. monocytogenes* due to microbial competition.

Treatments A and B were low salt compared with C and D. The low-salt treatments exhibited slightly lower *L. monocytogenes* reductions between 0.54 to 0.98 log cfu/g at either 4 or 10°C compared with standard salt treatments (0.69 to 1.48; Table 5 and 6) after 90 d. At 21°C incubation the low-salt treatments exhibited *L. monocytogenes* reductions between 0.48 to 1.11 log cfu/g compared with standard salt treatments (0.14 to 0.96 log cfu/g; Table 7) after 30 d. The data suggest that the low or standard salt levels used in this study did not greatly affect the survival of *L. monocytogenes* at the experimental incubation temperatures. These data are supported by previous studies. Larson et al.

Table 5. Survival (log cfu/g) of *Listeria monocytogenes* in different experimental treatments of Cheddar cheese during storage at 4°C¹

Day	Treatment ²			
	A	B	C	D
0	3.66 ± 0.22 ^{A,a}	3.67 ± 0.21 ^{A,a}	3.55 ± 0.48 ^{A,a}	3.78 ± 0.29 ^{A,a}
15	3.53 ± 0.19 ^{A,a}	3.67 ± 0.26 ^{A,a}	3.47 ± 0.21 ^{A,ab}	3.44 ± 0.29 ^{A,b}
30	3.32 ± 0.22 ^{A,b}	3.51 ± 0.18 ^{A,a}	3.32 ± 0.18 ^{A,bc}	3.22 ± 0.32 ^{A,c}
45	3.10 ± 0.20 ^{A,c}	3.27 ± 0.25 ^{A,b}	3.20 ± 0.23 ^{A,c}	3.05 ± 0.30 ^{A,d}
60	2.94 ± 0.29 ^{A,cd}	3.18 ± 0.24 ^{A,bc}	2.99 ± 0.24 ^{A,d}	2.98 ± 0.26 ^{A,d}
75	2.80 ± 0.23 ^{AB,de}	3.10 ± 0.22 ^{A,c}	2.78 ± 0.19 ^{AB,e}	2.58 ± 0.28 ^{B,e}
90	2.76 ± 0.27 ^{ABC,e}	3.13 ± 0.33 ^{A,c}	2.86 ± 0.25 ^{AB,de}	2.30 ± 0.13 ^{C,f}

^{A-C}Means followed by the same uppercase letters in the same row within each day of storage are not significantly different ($P \geq 0.05$).

^{a-f}Means followed by the same lowercase letters in the same column within each treatment are not significantly different ($P \geq 0.05$).

¹Data are presented as the mean values of 3 replications ± standard deviation.

²A = low salt, low pH; B = low salt, high pH; C = standard salt, high pH; D = standard salt, low pH. Treatment cheeses were aged for 10 wk before the inoculation study.

Table 6. Survival (log cfu/g) of *Listeria monocytogenes* in different experimental treatments of Cheddar cheese during storage at 10°C¹

Day	Treatment ²			
	A	B	C	D
0	3.66 ± 0.22 ^{A,a}	3.67 ± 0.21 ^{A,a}	3.55 ± 0.48 ^{A,a}	3.78 ± 0.29 ^{A,a}
15	3.47 ± 0.28 ^{A,b}	3.71 ± 0.22 ^{A,a}	3.56 ± 0.25 ^{A,a}	3.49 ± 0.25 ^{A,b}
30	3.28 ± 0.16 ^{A,c}	3.57 ± 0.27 ^{A,a}	3.37 ± 0.22 ^{A,b}	3.21 ± 0.23 ^{A,c}
45	3.11 ± 0.26 ^{A,d}	3.37 ± 0.25 ^{A,b}	3.18 ± 0.23 ^{A,c}	3.02 ± 0.33 ^{A,d}
60	3.00 ± 0.30 ^{AB,de}	3.31 ± 0.25 ^{A,bc}	2.99 ± 0.20 ^{AB,d}	2.80 ± 0.27 ^{B,e}
75	2.92 ± 0.29 ^{AB,e}	3.20 ± 0.26 ^{A,c}	2.83 ± 0.27 ^{AB,e}	2.46 ± 0.24 ^{B,f}
90	2.68 ± 0.26 ^{AB,f}	3.01 ± 0.28 ^{A,d}	2.72 ± 0.21 ^{AB,e}	2.51 ± 0.20 ^{B,f}

^{A,B}Means followed by the same uppercase letters in the same row within each day of storage are not significantly different ($P \geq 0.05$).

^{a-f}Means followed by the same lowercase letters in the same column within each treatment are not significantly different ($P \geq 0.05$).

¹Data are presented as the mean values of 3 replications ± standard deviation.

²A = low salt, low pH; B = low salt, high pH; C = standard salt, high pH; D = standard salt, low pH. Treatment cheeses were aged for 10 wk before the inoculation study.

(1999) reported *L. monocytogenes* survived in cheese brines from 5.6 to 24% with less than 1 log reduction for over 200 d at 4 and 10°C. Cole et al. (1990) demonstrated that at pH 7.0 it required greater than 8% salt to inhibit growth of *L. monocytogenes* at 5°C. Six and eight percent salt were required to inhibit *L. monocytogenes* growth at pH 5.13 incubated at 10 and 30°C, respectively (Cole et al. 1990). Ryser (1999) cites that Mehta and Tatini (1992) observed destruction of *L. monocytogenes* (strains Scott A and V7) in both 1.3% NaCl and 2.5% NaCl Cheddar cheese after 10 wk of aging at 7°C.

Treatments A and D were low-pH treatments compared with B and C. The low-pH treatments exhibited *L. monocytogenes* log reductions between 0.90 to 1.48 cfu/g at either 4 or 10°C compared with high-pH treatments (0.54 to 0.83; Tables 5 and 6) after 90 d.

At 21°C incubation, the low-pH treatments exhibited *L. monocytogenes* log reductions between 0.96 to 1.11 cfu/g compared with high-pH treatments (0.14 to 0.48 log cfu/g; Table 7) after 30 d. The data indicate that low pH provides a greater decrease in *L. monocytogenes* at all 3 incubation temperatures. Interestingly, despite reaching a pH between 6.46 and 6.87, the washed curd treatment B still did not permit *L. monocytogenes* growth at any of the incubation temperatures. The Bolton and Frank (1999) ordinal logistic regression model predicted a 98.8% probability that *L. monocytogenes* would grow in this cheese. It did have the least log reduction of *L. monocytogenes* of all treatments at either 4 or 10°C incubated for 90 d (Tables 5 and 6). Schaak and Marth (1988) reported significant inhibition of *L. monocytogenes* in lactic acid-cultured fermented milk when compared with that of the con-

Table 7. Survival (log cfu/g) of *Listeria monocytogenes* in different experimental treatments of Cheddar cheese during storage at 21°C¹

Day	Treatment ²			
	A	B	C	D
0	3.66 ± 0.22 ^{A,a}	3.67 ± 0.21 ^{A,ab}	3.55 ± 0.48 ^{A,a}	3.78 ± 0.29 ^{A,a}
5	2.88 ± 0.28 ^{B,b}	3.71 ± 0.27 ^{A,a}	3.58 ± 0.25 ^{A,a}	3.26 ± 0.33 ^{AB,bc}
10	2.60 ± 0.57 ^{B,cd}	3.54 ± 0.18 ^{A,abc}	3.55 ± 0.19 ^{A,a}	3.34 ± 0.27 ^{A,b}
15	2.77 ± 0.45 ^{B,bc}	3.46 ± 0.16 ^{A,bcd}	3.52 ± 0.28 ^{A,a}	3.13 ± 0.17 ^{AB,bcd}
20	2.51 ± 0.54 ^{B,d}	3.31 ± 0.15 ^{A,cde}	3.49 ± 0.23 ^{A,a}	3.03 ± 0.29 ^{AB,cde}
25	2.59 ± 0.46 ^{B,cd}	3.23 ± 0.22 ^{A,de}	3.39 ± 0.13 ^{A,a}	2.91 ± 0.39 ^{AB,de}
30	2.55 ± 0.34 ^{C,cd}	3.19 ± 0.28 ^{AB,e}	3.41 ± 0.20 ^{A,a}	2.82 ± 0.28 ^{BC,e}

^{A-C}Means followed by the same uppercase letters in the same row within each day of storage are not significantly different ($P \geq 0.05$).

^{a-e}Means followed by the same lowercase letters in the same column within each treatment are not significantly different ($P \geq 0.05$).

¹Data are presented as the mean values of 3 replications ± standard deviation.

²A = low salt, low-pH; B = low salt, high pH; C = standard salt, high pH; D = standard salt, low pH. Treatment cheeses were aged for 10 wk before the inoculation study.

trol even at the final pH of 5.99. Similarly, Gilliland and Speck (1972) noted that lactic cultures inhibited salmonellae and staphylococci at a pH of 6.6 and this may be the effect seen in *L. monocytogenes* inhibition in the present study.

This study demonstrated that post-aging inoculation of *L. monocytogenes* into low-salt (0.7%, wt/wt) Cheddar cheeses at an initial pH of 5.1 to 5.5 does not support growth at 4, 10, and 21°C up to 90, 90, and 30 d, respectively. In fact, a modest log reduction (~0.5 to 1.5 log cfu/g) of *L. monocytogenes* occurred when the cheese was stored at 4 or 10°C for 90 d or 21°C for 30 d. The results suggest that low- or reduced-salt cheeses are equally safe to their full salt counterparts and that salt may only be a minor food safety hurdle regarding the post-aging contamination and growth of *L. monocytogenes*. As none of the treatments demonstrated a substantial reduction in *L. monocytogenes* counts, the need for good sanitation practices to prevent post-manufacturing cross-contamination remains.

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