EFFECT OF STORAGE AT 4 AND 10C ON THE GROWTH OF 
LISTERIA MONOCYTOGENES IN AND ON QUESO FRESCO*

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ABSTRACT

A five-strain rifampicin-resistant Listeria monocytogenes cocktail (ca. 3.0 log_{10} cfu/g) was introduced as a postpasteurization contaminant in queso fresco (QF) that was manufactured using a commercial procedure. L. monocytogenes was either inoculated into (IN) the curds before forming the cheese block or onto (ON) slices (52–66 g), individually vacuum-packed and stored at 4 and 10C. Growth was monitored for up to 35 days. Gompertz analyses showed small differences in lag time because of temperature, but growth rate and generation time were faster at 10C than at 4C. After 20 days for both the IN and the ON treatments, the maximum population density was 7.80 ± 0.17, regardless of the storage temperature. These results indicate that QF manufacture must be conducted using Good Manufacturing Practices and under hygienic conditions, and that the use of antimicrobials and/or postprocessing interventions is necessary to prevent the presence and growth of L. monocytogenes.

PRACTICAL APPLICATIONS

Queso fresco (QF) is a popular, rennet-set, Hispanic-style fresh cheese made from pasteurized milk known for its crumbly texture and nonmelting properties. However, its high moisture content, near neutral pH and moderate salt content provide the ideal conditions for growth of various spoilage and pathogenic bacteria, notably Listeria monocytogenes. Past studies have examined the growth of L. monocytogenes on QF but were typically conducted using retail cheese of unknown manufacturing, handling and storage procedures. In this study, the growth of L. monocytogenes was monitored in and on QF made according to a commercial procedure used in the U.S.A. These data and a primary model that was created from the data can be used as the basis for food safety assessments and process and formulation improvements, or to monitor the effectiveness of antimicrobials and process interventions in preventing the presence and growth of L. monocytogenes associated with QF.

INTRODUCTION

Queso fresco (QF) is a fresh, soft cheese that is bright white, crumbly and nonmelting when heated, with a mild, milky flavor and a distinct saltiness (Van Hekken and Farkye 2003). It originated in Mexico and other Latin American countries and is made traditionally using unpasteurized milk. In the U.S.A., fresh cheese is commercialized using only pasteurized milk and has a refrigerated shelf life of typically less than 60 days. Hispanic-style cheese now accounts for 2% of the total cheese production in the U.S.A. (National Agricultural Statistics Service, NASS 2011), with QF being one of the most popular varieties.

Despite the requirement for pasteurization, several outbreaks of listeriosis in the U.S.A. have been attributed to the consumption of soft cheese, including QF (Linnan et al. 1988;
Hise et al. (2004), either because pasteurization was not used or due to postpasteurization contamination of the cheese. QF provides excellent conditions for the growth and survival of *Listeria monocytogenes* due to its high moisture content (≥50%), near neutral pH and low levels of salt in the moisture phase (Bishop and Smukowski 2006). QF is susceptible to environmental contamination with *L. monocytogenes* due to the labor-intensive practices that are used to produce the cheese, with no postprocess lethality steps following pasteurization (Sandra et al. 2004; Soni et al. 2010).

Several steps in QF manufacture involve handling of the cheese, thus *L. monocytogenes* contamination may occur not only on the surface of the cheese but also in the interior of the cheese (Lin et al. 2006). The handling steps occur after milk pasteurization and include cutting and trenching of the curds, salting of the curds, milling and pressing of the curd. Even with strict hygienic measures in place to minimize microbial contamination at each step of the QF manufacturing process, recalls and sporadic cases of *L. monocytogenes* continue in the U.S.A. and worldwide.

The growth of *L. monocytogenes* on or in fresh, high-moisture Hispanic-style cheese such as QF was evaluated in most studies using retail commercial cheese (Genigeorgis et al. 1991; Lin et al. 2006; Uhlich et al. 2006; Soni et al. 2010). Because there are no standards of identity for QF in the U.S.A., manufacturers have the option of using a variety of processing techniques to enhance its properties, particularly its crumbliness and nonmelting characteristics. Prior to curd formation, manufacturers may homogenize the milk to enhance the nonmelting properties of QF (Guo et al. 2012a) or use starter cultures to control the pH during manufacturing process (Path 1991). Lactic acid cultures have also been used to inhibit the growth of *L. monocytogenes* in a Spanish QF (Mendoza-Yepes et al. 1999). After curd formation, manufacturers may milk or mix the curds with an agitator (Hwang and Gunasekaran 2001) to enhance the crumbliness of the QF. They may also choose to drain the molded curds, typically in the shape of a wheel, without pressing, to produce cheese with a loose structure that maintains its properties during shipping or press the curds to facilitate whey release to produce a firmer texture required for retail sales of the smaller blocks or pucks of cheese.

*L. monocytogenes* is an environmental pathogen, yet no studies have been conducted to evaluate its viability when introduced as an environmental contaminant during manufacture of QF. The application of antimicrobials to the surface of QF has been proposed to control the growth of *L. monocytogenes* (Soni et al. 2010), but this approach may have little effect on controlling its growth on the interior of QF. Research is needed that focuses on environmental contamination of *L. monocytogenes* in a cheese manufacturing process to assist in food safety assessments for manufacture of this cheese, to make process improvements that prevent pathogen contamination, or if this is not sufficient, to develop postpasteurization processes that would eliminate this pathogen in the cheese and on its surface.

The objective of this study was to follow the growth and survival of *L. monocytogenes* in vacuum-packed slices of QF throughout refrigerated storage at 4°C and under moderate temperature abuse conditions at 10°C for up to 35 days: (1) with *L. monocytogenes* inoculated into the curds immediately after milling to simulate environmental contamination at the milling step and (2) with *L. monocytogenes* inoculated on both surfaces of QF slices. QF was manufactured in our laboratory according to a commercial cheese-making procedure used in the U.S.A.

**MATERIALS AND METHODS**

**Cheese Manufacture**

QF was manufactured in three separate trials according to the commercial procedure described in Guo et al. (2012a), with some modifications. Starter cultures were not used. Briefly, raw cows’ milk was collected from a local farm, stored overnight in a refrigerator at 4°C and then standardized to 3.5% (w/w) fat. The milk was homogenized using a two-stage homogenizer to pressures of 6.9 and 3.4 MPa, respectively, and then pasteurized at 72°C for 15 s (Universal Pilot Plant, Waukesha Cherry-Burrell, Philadelphia, PA). Pasteurization effectiveness was tested using the Charm Luminator T (Charm Sciences, Inc., Lawrence, MA), which detects the presence of alkaline phosphatase, with sensitivity to 0.005% raw milk.

Approximately 180 kg of milk was poured into a Kusel vat (Kusel Equipment Co. Watertown, WI) and heated while stirring with the mixing paddles until it reached 32°C, at which point 0.1% (w/w) of CaCl₂ (Spectrum Chemical Mfg. Corp., New Brunswick, NJ) was added. After 15 min, chymosin (CHY-MAX 73863, CHR. Hansen, Inc., Milwaukee, WI) was added in the ratio of 130 mL chymosin/1,000 L milk to coagulate the milk. After 30 min, the curds were cut with wire knives and then the temperature was increased by 1°C every 5 min until curd temperature reached 39°C. After the curds were cooked for 30 min at this temperature, 93 kg of the whey was drained from the vat and 0.012 kg salt (NaCl) was mixed into the remaining curd slurry in three applications with 10 min of stirring between each application. Additional whey was drained from the vat and the curds were then trenched, allowing the remainder of the whey to drain from the curds. Curd yield was 29 kg. Throughout the cheese-making process, the pH was monitored using an Oakton p510 series pH meter (Vernon Hills, IL). The cheese was then cut into approximately 5- to 10-cm-thick pieces and cooled in a 4°C refrigerator until an internal temperature of 21°C was reached. After cooling, the cheese was finely milled using a Bosch...
universal 6610UC/01 FD mill (Robert Bosch Hausgerate GmbH, Dillingen, Germany) equipped with a blade with five 14-mm-diameter holes.

Inoculation Procedures

After the cheese was milled, approximately 8 kg of the curd was reserved to prepare cheese slices that were contaminated with *L. monocytogenes* throughout (termed the IN treatment) and another 8 kg was reserved to prepare cheese slices that served as controls or were inoculated with *L. monocytogenes* only on the large surfaces of the slices (termed the ON treatment). To prepare the IN treatment slices, 8 kg of the curd was inoculated with a rifampicin-resistant *L. monocytogenes* cocktail to a target level of about $3.0 \log_{10} \text{cfu/g}$. The curds were thoroughly mixed, hand-packed into a sterile cheese hoop lined with a cheese cloth sanitized with 200 mg/L chlorine solution and stored overnight at 4°C in a refrigerator. The remaining uninoculated 8 kg of curds was also aseptically packed by hand into a sterile cheese hoop that was lined with a sanitized cheese cloth and stored overnight at 4°C. The next day the IN and the uninoculated cheese were removed from the hoops and cut into approximately 12.7 cm $\times$ 7.6 cm $\times$ 1 cm slices with each slice weighing from about 52 to 66 g. The uninoculated slices were then individually surface inoculated (ON treatment) with 50 $\mu$L per face of the rifampicin-resistant *L. monocytogenes* cocktail to a target level of $3.0 \log_{10} \text{cfu/g}$, while some were reserved to serve as controls. The slices from the IN and ON treatments and the controls were then vacuum-packed (Ultravac, Koch Company, LLC, Kansas City, MO) into sterile nylon polyethylene bags (Prime Source Vacuum Products, San Jose, CA) and stored at 4 and 10°C for up to 35 days. Three samples from each treatment and the controls were removed from the storage at various intervals to determine the growth of *L. monocytogenes*.

**Listeria monocytogenes Strains**

The inoculum was composed of five rifampicin-resistant (Rif; 100 g/mL; Sigma Chemical Co., St. Louis, MO) *L. monocytogenes* strains (MFS 53, MFS 1365, MFS 104, MFS 1363 and MFS 1394 (Uhlrich et al. 2006) ) that were confirmed, cultured and maintained as described in Porto et al. (2002). The isolates were combined to achieve a final concentration of about $10^5 \text{cfu/mL}$ to inoculate the curds of QF prior to hooping and slicing (IN treatment) or the surface of the QF slices (ON treatment). The levels of *L. monocytogenes* for the initial inoculum were estimated by direct plating appropriately diluted aliquots of the freshly grown cocktail onto Modified Oxford (Becton, Dickinson and Company, Franklin Lakes, NJ) agar plates. Following incubation at 37°C for ca. 48 h, typical colonies, e.g., black halos, were counted manually as the pathogen.

**Sampling and Enumeration of *L. monocytogenes***

At each sampling interval, three packages of cheese slices that were inoculated on the surface (ON treatment) with *L. monocytogenes* and three packages of slices from the inoculated curds (IN treatment) stored at 4°C were sampled on days 0, 1, 3, 6, 9, 12, 15, 20, 27 and 35, whereas the cheese slices stored at 10°C were sampled on days 0, 1, 2, 3, 4, 5, 6, 7, 9, 12, 20, 27 and 35. Prior to sampling, each package was sterilized with 70% ethanol and then cut open with ethanol-sterilized scissors. Each slice was weighed and then combined with 75 mL of sterile 0.1% peptone water (Waters, Stoughton, MA) and 100 $\mu$L of rifampicin agar plates. The plates were incubated at 25°C for ca. 7 days. The detection limit was $<0.9 \log_{10} \text{cfu/g}$.

**Sampling and Enumeration of Indigenous Bacteria**

Microbial analysis was performed on pathogen-free QF slices stored at 4 and 10°C for up to 28 days. Some of the slices were also sampled at 56 days of storage. Bacterial enumeration was performed similar to a method described previously (Reny et al. 2008). Briefly, a 10-g sample of cheese was homogenized in 90 mL of sterile 2% sodium citrate for 2 min using a Stomacher 400C (Seward Ltd., London) at 230 rpm. Serial dilutions (1:10) of the homogenized samples were prepared in sterile 0.1% peptone water and 100 $\mu$L of each dilution was used to inoculate plate count agar (PCA; Oxoid, Basingstoke, U.K.) or oxytetracycline glucose yeast extract agar base (OGE; Difco Laboratories, Detroit, MI) containing 0.1% oxytetracycline (Calbiochem, San Diego, CA) in a filter bag (Model XX-C003, Microbiology International, Frederick, MD), and then macerated for 60 s in a stomacher (Seward, Model 400C, Cincinnati, OH). Approximately 10 mL of the homogenous mixture was pipetted from the filter bag and transferred to a 15-mL conical tube. The mixture was serially diluted (1:10) in sterile 0.1% (w/w) peptone water, and then 100 $\mu$L was spread plated onto Modified Oxford (Becton, Dickinson and Company) plus 100 $\mu$L of rifampicin agar plates. The plates were incubated for 48 h at 37°C and *L. monocytogenes* colonies were counted manually (Luchansky et al. 2002). The detection limit was $<0.9 \log_{10} \text{cfu/g}$.
Identification of the aerobic bacteria was based on 16S rRNA gene sequence analysis, as described in Renye et al. (2008) and Guo et al. (2012b). A minimum of two colonies, representing the different colony morphologies observed, were selected for sequence analysis. The colonies were grown overnight in brain–heart infusion broth (Difco), and 1 μL of culture was used as template for polymerase chain reaction amplification of the 16S rRNA gene with the eubacterial oligonucleotide primers EubA and EubB (Cottrell and Kirchman 2000). Nucleic acid sequencing analysis was performed using an ABI PRISM 3730 DNA analyzer (Perkin Elmer, Wellesley, MA) with ABI PRISM Big Dye terminator cycle sequencing reagent and the EubA and EubB primer set. Sequences were analyzed using Sequencher 4.9 (Gene Codes Corp., Ann Arbor, MI) and compared with the available sequences in GeneBank using the National Center for Biotechnology Information BLAST search program (http://www.ncbi.nlm.nih.gov/BLAST/).

**Determination of Composition and pH of Cheese**

Moisture, fat, lactose and protein content were determined according to the methods reported in Guo et al. (2012a) for each block of cheese. The pH was measured on each side of the cheese slices inoculated with *L. monocytogenes* using a Model 611 pH meter (Orion Research Corp., Cambridge, MA) on each of the sampling days and at 41 days of storage. The pH of the control cheese was measured on days 1, 8 and 41 for the slices stored at 4°C and days 1, 17 and 41 for the slices stored at 10°C.

**Statistical Analysis**

The results from the three cheese-making trials for the IN and ON *L. monocytogenes* inoculations of the cheese slices were analyzed by analysis of variance (ANOVA) to determine the significance (*P* < 0.05) of the IN versus ON treatments or storage temperature effects (SAS, Statistical Analysis Systems Institute, Version 9.22, Cary, NC). ANOVA and mean separations were performed at each time point using Proc Mixed with the Bonferroni’s least significant difference (LSD) technique to determine significant differences (*P* < 0.05).

The data were also used to determine the parameters A, C, B and M in the Gompertz growth curve equation,

$$L(t) = A + C \exp\{-\exp(-(B(t - M))\}$$

(1)

to estimate the growth rate (GR), lag time (LT) and generation time (GT) using the method described in Gibson et al. (1988). $L(t)$ is the log count of *L. monocytogenes* at time $t$ in days. The variable $A$ defines the asymptotic log count as $t$ decreases indefinitely; $C$ defines the asymptotic amount of growth that occurs as $t$ increases indefinitely; $M$ is the time at which the absolute growth rate is at its maximum value; and $B$ defines the relative growth rate at $M$ (Gibson et al. 1988). GR, LT and GT were defined by the following equations:

$$GR = BC/e$$

(2)

$$LT = M - (1/B)$$

(3)

$$GT = \log_{10}(2)e/BC$$

(4)

The data for both the IN and the ON treatments at 4 and 10°C were fit using Proc NLIN of SAS. The parameters $A$, $C$, $B$ and $M$ were then compared using the Bonferroni’s LSD technique.

**RESULTS AND DISCUSSION**

**Cheese Composition**

QF is described as a cheese with a pH ≥ 6.1, moisture content between 46 and 57%; fat content between 18 and 29%; protein content between 17 and 21%; and salt content between 1 and 3% (Hwang and Gunasekaran 2001; Van Hekken and Farkye 2003). The average values of the moisture, fat, protein and salt contents from the cheese manufactured in this study fell within the reported compositional boundary values after storage for 1, 4 and 8 weeks as determined in a companion study (Tunick et al. 2011) on the characterization of QF. The average composition of QF in percent was as follows: moisture, 55.8 ± 0.7; protein, 17.9 ± 0.1; fat, 21.7 ± 0.4; lactose, 3.15 ± 0.15; and NaCl, 1.67 ± 0.14, with % salt-in-moisture (S/M) of 3%.

Hard cheese such as Cheddar that are made with the use of starter cultures have low water activity, $a_w$, of approximately 0.95 because of their low moisture content of <39%, pH of 5.2 and typical salt content of 1.7% (Bishop and Smukowski 2006), which helps limit microbial growth. They do not support the growth of *L. monocytogenes* because of lactic acid development from the starter cultures (Ryser and Marth 1987; Genigeorgis et al. 1991; Bishop and Smukowski 2006) and the presence of secondary metabolites, such as bacteriocins. However, for soft cheese such as QF made without the use of starter cultures, the high moisture content, near neutral pH and moderate salt content provide limited protection against growth of microorganisms and $a_w$ is determined only by the presence of NaCl (Marcos and Esteban 1982). Using a nomograph that predicts the water activity of soft cheese (Marcos and Esteban 1982) for the general compositional ranges of moisture of 46–57% and salt content between 1 and 3% for QF (Van Hekken and Farkye 2003), $a_w$ ranges between 0.962, at 46% moisture and 3% salt content, and 0.999, at 57%
moisture and 1% salt content. For the cheese of this study with average salt content of 1.67% and moisture content of 55.8%, the value of $\alpha$ calculated using the nomograph was approximately 0.983.

The experimental values of pH for the control QF slices at 4°C as a function of days in storage were as follows: 6.75 ± 0.02 (1 day), 6.68 ± 0.03 (8 days) and 6.62 ± 0.02 (41 days). The experimental values of pH for the control QF slices stored at 10°C were 6.56 ± 0.02 (1 day), 6.09 ± 0.02 (17 days) and 5.77 ± 0.08 (41 days). These values of pH are consistent with the previously reported values for QF (Genigeorgis et al. 1991; Hwang and Gunasekaran 2001).

**Levels of Indigenous Bacteria**

The mesophilic counts following storage overnight at 4 or 10°C were determined as 3.54 ± 0.12 and 3.37 ± 0.31 log cfu/g, respectively, and were determined after incubating PCA plates at 30°C; to ensure that bacterial populations, which prefer an elevated growth temperature, were not missed in our analysis, we also incubated PCA dishes at 37°C. Results were similar in that there was an apparent lag in growth during the first 7 days of storage at 4°C, followed by a gradual increase in bacterial growth with an average of 4.0 ± 1.5 log10 cfu/g observed by 28 days of storage. Results obtained from QF samples stored at 10°C showed an increase in bacterial growth after only 7 days of storage and reached 8.07 ± 0.45 log10 cfu/g by 28 days of storage. Psychrotrophic bacteria were not detected in QF samples stored at 4°C for up to 28 days, but were observed (4.48 log10 cfu/g) after 56 days of storage in two of the three cheese samples analyzed. When QF was stored at 10°C, psychrotrophic bacteria were not detected. The presence of yeasts and molds were also detected by 28 days of storage at 4°C and 56 days of storage at 10°C.

Representatives of each colony type identified on PCA were selected for 16S rRNA gene sequence analysis to determine the predominant bacterial species in the QF samples. Bacillus species were identified in samples from 1 to 28 days; however, sequence analysis could not distinguish if the isolates were Bacillus cereus (99% identity, accession number EU333122.1) or Bacillus thuringiensis (99%, FR846529.1). Bacillus species are often identified as contaminants of pasteurized milk and fermented dairy products due to their ability to sporulate and survive the pasteurization process (Ahmed et al. 1983; Larsen and Jorgensen 1997; Zhou et al. 2008). Both species are considered potential opportunistic pathogens due to the presence of toxin-encoding genes; however, only one food-poisoning outbreaks has been linked to the presence of *B. thuringiensis* (Granum and Lund 1997; Rivera et al. 2000; Hansen and Hendrickson 2001). Previous studies have reported that refrigeration temperatures were effective in controlling the growth of *B. cereus* (Feijoo et al. 1997); thus, in this study, storage at 4 and 10°C may have prevented the continued growth of these bacteria, explaining why they were not identified on PCA inoculated with cheese samples stored for 56 days.

The other gram-positive bacteria identified in this study were *Enterococcus gallinarum* (98%, HQ721269), which was found in two cheese samples stored at 4°C for 1 day, and *Enterococcus faecalis* (98%, HQ721277.1), which was present in all cheese samples analyzed on day 56. Their predominance after prolonged storage conditions is most likely due to their ability to tolerate and grow at low temperatures, and in the presence of salt concentrations typically found in QF cheeses (1.7%), which was previously reported to affect the microbial population in QF cheese stored at 4°C (Guo et al. 2012b). Enterococci are common contaminants of raw milk on dairy farms (Kagkli et al. 2007) and are considered to play a critical role in the flavor development of several raw milk cheese (Foulquie-Moreno et al. 2006), specifically Mexican QF (Reny et al. 2008). Enterococci are also considered desirable due to their production of bacteriocins, which have broad spectrum activity against foodborne pathogens including *L. monocytogenes* (Reny et al. 2009); however, their potential as opportunistic pathogens has prevented this group of lactic acid bacteria from being designated as “generally regarded as safe” cultures. Safety assessments are ongoing to determine if specific enterococcal isolates could be designated as safe for use in the production of fermented foods (Ogier and Serro 2008). Gram-negative bacteria identified in this study included the Enterobacteriaceae species: *Escherichia hermannii* (98%, EF059869.1), *Pseudomonas aeruginosa* (98%, HQ995502.1) and *Enterobacter asburiae/cloacae* (98%, HQ242719.1/HM030748.1), all of which are considered potential human pathogens. In this study, storage at 4°C appeared to control the growth of these organisms as *E. hermannii* and *E. asburiae/cloacae* were not identified in samples stored for 28–56 days. Storage at 10°C was less effective at controlling the growth of these bacteria as all three were identified in cheese samples throughout the storage. In addition to the contaminants identified under mesophilic growth conditions, psychrotrophic spoilage organisms were identified on PCA plates incubated at 5°C, and included *Stenotrophomonas* sp. (97%, HQ670713.1), *Pseudomonas fluorescens* (99%, GU198122.1) and *Enterobacter aerogenes* (98%, FJ796202.1).

**Viability of *L. monocytogenes* on QF**

Pasteurization effectively eliminates the risk of *L. monocytogenes* contamination of cheese from milk, but QF manufacture includes pasteurization steps that may introduce *L. monocytogenes* into the curds or on the surface of the cheese. In this study, *L. monocytogenes* (ca. 3.0 log10 cfu/g cheese) was introduced into the curds of QF after salting and milling to simulate environmental contamination of
L. MONOCYTOGENES INOCULATED ON QUESO FRESCO SLICES AND STORED AT 4 OR 10C. (B) GROWTH OF L. MONOCYTOGENES INOCULATED ON QUESO FRESCO SLICES AND STORED AT 4 OR 10C. The error bars represent standard deviations.

The decrease in pH to 5.77, for the QF stored at 10C, most likely resulted from lactic acid formation from the breakdown of lactose by microorganisms remaining from the pasteurized milk used to make the cheese (Tunick et al. 2011), which was not sufficient to suppress the growth of L. monocytogenes. In this study, the total aerobic counts showed the indigenous microbial load to be approximately $4.0 \log_{10} \text{cfu/g}$ (4C) and $8.0 \log_{10} \text{cfu/g}$ (10C) by 28 days of storage. The initial load of indigenous bacteria was about $3.0 \log_{10} \text{cfu/g}$. Results from this study showed L. monocytogenes counts reached the expected levels when compared with the previous reports (Genigeorgis et al. 1991; Soni et al. 2010), suggesting that the indigenous microbial load did not inhibit listerial growth.

Although no differences in the growth of L. monocytogenes were observed for the IN or ON treatments in this study, others have noted differences in the growth of L. monocytogenes between the surface and interior of soft cheese, which were attributed to differences in pH values between the surface and interior of the cheese, as reported in Lin et al. (2006). In accordance with this study, no difference in the pH or growth of L. monocytogenes for cheese samples inoculated on the surface or the interior of cheese samples was noted for retail QF (Lin et al. 2006).

It is unlikely that contamination by L. monocytogenes during the salting step or on the surface of QF would occur at the level of approximately $3.0 \log_{10} \text{cfu/g}$ used as the starting inoculum in this study. A more likely scenario is that a few
cells of *L. monocytogenes* would contaminate the curd or cheese surface. Lin *et al.* (2006) showed that inoculation of the surface of retail QF with *L. monocytogenes* at levels of $10^6$ and $10^7$ cfu/g resulted in counts of $3.07 \pm 0.52$ and $3.37 \pm 1.05$ log$_{10}$ cfu/g, respectively, after 2 weeks of storage at 4°C. Counts for the QF of this study were approximately 2.0 log$_{10}$ higher at the same storage time and temperature (Fig. 1B). After 4 weeks of storage, counts for the two inoculation levels were $3.28 \pm 1.02$ and $3.86 \pm 1.19$ log$_{10}$ cfu/g, respectively, while those of this study were approximately 4.0 log$_{10}$ cfu/g higher. Even after 12 weeks of storage at 4°C, the *L. monocytogenes* counts (Lin *et al.* 2006) did not approach those of this study, possibly because of the competition with the native microflora load, which was reported as 5.0 log$_{10}$ cfu/g initially and as high as 9.0 log$_{10}$ cfu/g by the end of the study. However, the initial microflora load of about 3.0 log$_{10}$ cfu/g observed in this study did not appear to inhibit the growth of *L. monocytogenes*, suggesting that the bacterial species present within the indigenous microflora of Lin *et al.* (2006) for cheese obtained from Mexico may have affected the growth of *L. monocytogenes*. Thus, additional research to identify the specific species present may explain this observation. The combined results of these studies and other studies (Genigeorgis *et al.* 1991; Uhlich *et al.* 2006; Soni *et al.* 2010), underscore the fact that regardless of the inoculation level or the presence of the indigenous microflora, *L. monocytogenes* will increase significantly within the shelf life of QF at refrigeration temperatures and pose a public health threat.

**Gompertz Model**

Following the approach given by Gibson *et al.* (1988), the Gompertz growth curve given by Eq. (1) was fitted to the data collected for the *L. monocytogenes* inoculated within the cheese curds IN (Fig. 1A) and on the cheese slices ON (Fig. 1B) as a function of storage at 4 and 10°C, respectively, to determine the model parameters *A*, *C*, *B* and *M* and to establish a primary level model for these data.

The values of the parameters for the four different treatments are shown in Table 1. The GR, LT and GT were calculated using the parameters of Table 1 and Eqs. (2)–(4), respectively, and are shown in Table 2.

The small values of the root mean square error, the square root of the average of the squares the differences between the data points and the Gompertz model, indicated a good fit of the data to the model. The values of GR for the IN and ON samples were not different (*P > 0.05*) at either 4 or 10°C, respectively (Table 2), and are similar in value to those reported for a queso blanco (Uhlich *et al.* 2006) made without the use of starter cultures, pH of 6.8 and salt content of 2.32%. The values of GT for the IN and ON samples stored at either 4 or 10°C, respectively, were not significantly different (*P > 0.05*). The calculated values of LT for the IN (1.64 days) and ON (0.46 days) samples stored at 4°C were different (*P < 0.05*), which may be due to the empirical nature of the Gompertz equation (Huang 2011), but were not different (*P > 0.05*) for the IN (0.0 day) or ON (0.25 day) samples stored at 10°C. LT decreases with increasing temperature and its duration is a function of the time needed for the bacterium to acclimate to its new environment before multiplication and growth begin (Uhlich *et al.* 2006). Longer LT (*P < 0.05*) was also noted for queso blanco stored at 5C (65 h) than at 10, 15, 20 or 25°C with an average length ranging from (2.14 ± 1.18 h) to (19.93 ± 15.62 h), which showed high variation but were not significantly different (*P > 0.05*). LT is also a function of inoculum level (Francois *et al.* 2005). When smaller inoculum levels on the order of $10^6$ or $10^7$ cfu/g, respectively, were used, *L. monocytogenes* was detected on the surface and in the interior of QF within 2 weeks at 4°C and 1 week at 12°C (Lin *et al.* 2006). The relatively short LT under refrigeration (4°C) conditions and moderate abuse conditions (10C) of this study and magnitude of inoculation level demonstrate that the moderate pH and salt levels of QF are no barrier to *L. monocytogenes* growth.

Bolton and Frank (1999) defined the growth/no growth interface for *L. monocytogenes* in QF manufactured using both lactic acid and rennet to produce the curd. QF formulations were defined that would have less than a 5% probability of *L. monocytogenes* growth, with recommendations for pH of QF ranging from pH 5.0 to 6.0 and brine content ranging from 8.17 to 16%, which is equivalent to salt contents ranging from approximately 5 to 11%. Clearly, these extreme conditions would impact the quality and sensory properties of QF.

The results of this study confirm that QF made according to a procedure used in the U.S.A. supports *L. monocytogenes* growth under storage conditions of 4 and 10°C. QF manufacture must be conducted using Good Manufacturing Practices and stringent hygienic conditions to prevent contamination by *L. monocytogenes*. D’Amico *et al.* (2008) suggested that producers of soft cheese using only sanitation measures to prevent *L. monocytogenes* contamination include the U.S. Department

### Table 1. Gompertz Equation Parameters*

<table>
<thead>
<tr>
<th>Storage temperature (C) and treatment</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>M</th>
</tr>
</thead>
<tbody>
<tr>
<td>4C IN</td>
<td>3.307</td>
<td>0.1253</td>
<td>4.6542</td>
<td>9.6233</td>
</tr>
<tr>
<td>4C ON</td>
<td>2.3097</td>
<td>0.1113</td>
<td>5.7560</td>
<td>9.4325</td>
</tr>
<tr>
<td>10C IN</td>
<td>2.8823</td>
<td>0.4760</td>
<td>4.7898</td>
<td>1.6207</td>
</tr>
<tr>
<td>10C ON</td>
<td>4.2041</td>
<td>0.4128</td>
<td>5.1430</td>
<td>2.6725</td>
</tr>
</tbody>
</table>

* From Gibson *et al.* (1988). A, asymptotic log count as time (t) decreases indefinitely; B, relative growth rate at M; C, the asymptotic amount of growth that occurs as t increases indefinitely; M, t at which the absolute growth rate is at its maximum value; IN, *L. monocytogenes* was inoculated into the salted, milled curds prior to hooping and slicing; ON, *L. monocytogenes* was inoculated onto the cheese slices.
of Agriculture (USDA), Food Safety Inspection Service (2010) rule, Alternate 3 for facilities producing postlethality exposed ready-to-eat (RTE) food products, in their Hazard Analysis and Critical Control Point plans. Alternative 3 recommends testing of the food contact surfaces in the environment following postprocessing and testing of the final food products.

Newer, larger plants used to manufacture QF and other similar Hispanic-style cheese conducted many of the cheese-making operations in closed vats in which the cheese curds have little contact with the environment, contaminated surfaces or risk of cross-contamination from utensils or workers until they are hooped. The hoops are packed by hand. After dehooping, the cheese may or may not be pressed and is then immediately refrigerated after packaging. In smaller processing plants, cheese-making operations are frequently conducted in open vats and operations such as trenching and cutting of the curds, salting, milling, hooping and pressing involve significant hand manipulation and utilization of utensils. Van Hekken et al. (2012) demonstrated that the milling step may be eliminated in the QF procedure with minimal effects on the crumbliness, which is a characteristic of this cheese. Removal of this step should increase the safety of this cheese.

While improvements in process operations will reduce the risk of L. monocytogenes contamination, the use of antimicrobials (Soni et al. 2010) and/or postprocessing interventions may be necessary to prevent the growth of L. monocytogenes in soft cheese. Implementation of these measures would be in accordance with the recommendations of Alternatives 1 and 2 of the USDA-FSIS (2010) rules for RTE products. Alternative 1 recommends the use of a postlethality treatment and an antimicrobial agent or process and Alternative 2 recommends the use of a postlethality treatment or an antimicrobial agent or process. Alternative processing technologies, such as high pressure processing, that can be used as a postprocessing or postlethality intervention directly on the packaged product, with or without added antimicrobials, may be necessary to reduce L. monocytogenes growth throughout QF.

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