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Growth of *Listeria monocytogenes, Salmonella* spp., *Escherichia coli* O157:H7, and *Staphylococcus aureus* on Cheese during Extended Storage at 25°C

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ABSTRACT

Potentially hazardous foods require time/temperature control for safety. According to the U.S. Food and Drug Administration Food Code, most cheeses are potentially hazardous foods based on pH and water activity, and a product assessment is required to evaluate safety of storage >6 h at 21°C. We tested the ability of 67 market cheeses to support growth of *Listeria monocytogenes* (LM), *Salmonella* spp. (SALM), *Escherichia coli* O157:H7 (EC), and *Staphylococcus aureus* (SA) over 15 days at 25°C. Hard (Asiago and Cheddar), semi-hard (Colby and Havarti), and soft cheeses (mozzarella and Mexican-style), and reduced-sodium or reduced-fat types were tested. Single-pathogen cocktails were prepared and individually inoculated onto cheese slices (~10^5 CFU/g). Cocktails were 10 strains of *L. monocytogenes,* 6 of *Salmonella* spp., or 5 of *E. coli* O157:H7 or *S. aureus*. Inoculated slices were vacuum packaged and stored at 25°C for ≤15 days, with surviving inocula enumerated every 3 days. Percent salt-in-the-moisture phase, percent titratable acidity, pH, water activity, and levels of indigenous/starter bacteria were measured. Pathogens did not grow on 53 cheeses, while 14 cheeses supported growth of SA, 6 of SALM, 4 of LM, and 3 of EC. Of the cheeses supporting pathogen growth, all supported growth of SA, ranging from 0.57 to 3.08 log CFU/g (average 1.70 log CFU/g). Growth of SALM, LM, and EC ranged from 1.01 to 3.02 log CFU/g (average 2.05 log CFU/g), 0.60 to 2.68 log CFU/g (average 1.60 log CFU/g), and 0.41 to 2.90 log CFU/g (average 1.69 log CFU/g), respectively. Pathogen growth varied within cheese types or lots. Pathogen growth was influenced by pH and percent salt-in-the-moisture phase, and these two factors were used to establish growth/no-growth boundary conditions for safe, extended storage (≤25°C) of pasteurized milk cheeses. Pathogen growth/no-growth could not be predicted for Swiss-style cheeses, mold-ripened or bacterial surface–ripened cheeses, and cheeses made with nonbovine milk, as insufficient data were gathered. This challenge study data can support science-based decision making in a regulatory framework.

Temperature-dependent storage of most cheeses has three major roles—to allow for curing/ripening of cheeses that contain added or indigenous bacteria and enzymes, to prevent quality defects, and to control pathogen growth *(3)*. The U.S. Food and Drug Administration (FDA) Food Code *(41)* defines a potentially hazardous food as one that requires time/temperature control to limit the growth of pathogenic microorganisms or toxin formation. According to the Food Code, foods with a pH of <4.2 and any water activity (a_w) or a_w of <0.88 and any pH are not considered potentially hazardous. Foods considered potentially hazardous, also known as time/temperature control for safety (TCS) foods, fall into one of the following categories: $a_w \geq 0.88$ and pH > 5.0, $a_w > 0.90$ to 0.92 and pH > 4.6, or $a_w > 0.92$ and pH > 4.2. The Food Code indicates that TCS foods must be maintained at ≤5°C, or, if placed outside refrigeration, can be stored for up to 6 h at a temperature no greater than 21°C, after which the product must be discarded *(45)*.

The composition of many cheeses, when evaluated using the Food Code criteria, places them into the category of TCS foods, thus limiting the ability of retailers to market the cheeses under room-temperature conditions that could enhance cheese flavor and aroma *(12)*. The Food Code–mandated time and temperature control may also limit industry flexibility in the transportation, handling, and storage of cheeses. It has, however, been suggested that the biochemical changes that occur during cheese ripening create an environment hostile for pathogen growth and that time/temperature control of some cheese is primarily needed to maintain the organoleptic quality of cheese, not to maintain safety *(3)*. Bishop and Smukowski *(3)* conducted a thorough review of the literature available up until 2006 and recommended that cheeses meeting certain criteria, e.g., cheeses manufactured in the United States with pasteurized

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or heat-treated milk (≥63°C for ≥16 s), cheeses manufactured following good manufacturing practices and under the principles of hazard analysis and critical control points (HACCP), and cheeses manufactured meeting standards of identity outlined in 21 Code of Federal Regulations part 133 *(43),* should be exempted from refrigeration requirements during ripening, storage, shipping, and display. Bishop and Smukowski recommended that the following cheeses could meet these criteria: Asiago (medium and old), Cheddar, Colby, Feta, Monterey Jack, Muenster, Parmesan, Pasteurized process, Provolone, Romano, and Swiss/Emmentaler.

To establish whether a particular food, e.g., cheese, can be exempt from TCS requirements, the Food Code allows processors or retailers to conduct a microbial challenge study to assess the ability of a food product to inhibit pathogenic bacterial growth or inactivate these microorganisms. The FDA has outlined parameters for conducting such challenge studies *(44).*

When experts consider the major microbiological hazards across the food supply, the risk of bacterial illness from dairy products, such as milk and cheese, can be attributed primarily to *Listeria monocytogenes, Yersinia entercolitica, Campylobacter* spp., and nontyphoidal *Salmonella* spp. *(2).* Between 1990 and 2011, there were 105 reported foodborne illness outbreaks in the United States, with over 2,000 illnesses, linked to cheese/cheese products *(7).* Major pathogens linked to these cheese-related outbreaks included *Salmonella* spp. (37 outbreaks), *L. monocytogenes* (16 outbreaks), pathogenic *Escherichia coli* (6 outbreaks), *Staphylococcus aureus* (4 outbreaks), norovirus (21 outbreaks), *Campylobacter* spp. (9 outbreaks), and *Brucella* spp. (5 outbreaks) *(7).* Among the 105 outbreaks, 17 were linked to cheeses made with pasteurized milk, 30 were linked to cheese made with raw milk, and the pasteurization status of cheeses involved in the remaining 58 outbreaks was unspecified. The pathogenic bacteria primarily responsible for foodborne illness outbreaks linked to cheese manufactured with pasteurized milk were *L. monocytogenes, Salmonella* spp., and *E. coli* O157:H7. Cheeses implicated in these outbreaks included processed cheese and Mexican-style cheeses *(7, 9).* The low incidence of *S. aureus*–linked outbreaks related to cheese is presumed to be due to the low incidence of this pathogen in pasteurized milk and the growth characteristics of this bacterium *(21).* However, *S. aureus* is commonly carried by humans and thus could contaminate cheese during post-pasteurization handling *(16). S. aureus* is also the bacterial pathogen considered to have the highest tolerance to reduced-moisture conditions or increased salt concentration *(22)* and therefore could be considered a target pathogen in determining the safety of cheese contaminated postprocessing and stored for extended periods of time at room temperature.

The goal of this project was to evaluate survival of strains of *L. monocytogenes, Salmonella* spp., *E. coli* O157:H7, and *S. aureus* on natural market cheeses during extended storage at 25°C and to determine the effect of cheese compositional factors such as pH, a_w, and salt on pathogen survival. Pathogen survival data from laboratory research and data from published literature were then combined to establish the boundary conditions for pathogen growth/no-growth during storage of cheese at room temperature.

MATERIALS AND METHODS

Cheeses. Sixty-seven cheeses were purchased from local retail establishments or obtained directly from the manufacturer and stored at 4°C. Cheeses studied were Asiago (aged, young), Brick (two brands), Cheddar (mild, regular, and sharp), Cheddar-mozzarella, Colby, Colby Jack, Farmer's, Feta, Gouda, Gruyere, Havarti (two brands), Jack (goat's milk), Monterey Jack, Muenster (two brands), Parmesan, Pepper Jack (two brands), Provolone (mild and regular; two brands of sharp), Provolone-mozzarella, Queso Blanco, Queso Fresco, Queso Quesadilla, String cheese (two brands), Swiss (Baby, two brands; Lacey, regular), reduced-fat cheeses (Cheddar, Colby Jack, and Provolone), and reduced-sodium cheeses (Colby Jack and Provolone). Where a type of cheese was tested more than once, replicates were from different brands and/or from different production dates of the same brand. All cheeses were manufactured in the United States from pasteurized milk (Tables 1 and 2, Cheese).

Proximate analysis. Cheeses were characterized by percentage of moisture, percentage of salt, and a_w at the beginning of each trial. The pH was measured at each sampling time on pathogen-inoculated cheeses (days 0, 3, 6, 9, 12, and 15). To discern the impact of acid production by indigenous or starter bacteria on the microenvironment, the percentage of titratable acidity (%TA) was measured on uninoculated cheeses on days 0, 6, and 15. Uninoculated cheeses were handled the same as inoculated cheeses (see the following), except that 0.1 ml of Butterfield's phosphate diluent (Nelson Jameson, Marshfield, WI) replaced the inoculum. Duplicate trials were performed for each compositional analysis, and average values were reported.

The percentage of moisture was determined using a standard method *(4)* by drying a representative 3-g sample at 100°C for 5 h in a vacuum oven maintained at −98 kPa throughout the drying process (M.D.O. Vacuum Oven, Model 3623, Lab-Line Instrument Inc., Melrose Park, IL). The percentage of salt was determined by titration of chloride using the silver titration standard method *(4).* For each trial, a representative 5-g sample was diluted with distilled water 1:20 (wt/vol) and the percentage of chloride was determined according to the standard method using a Model M926 Chloride Analyzer (Nelson Jameson). The percent chloride content was automatically calculated by the analyzer and expressed as milligrams percent of sodium chloride per liter, which was converted to percent salt by multiplying the appropriate dilution and conversion factors. Salt (percent) and moisture (percent) of an individual cheese sample were used to calculate percent salt-in-the-moisture phase (%SMP) using equation 1:

$$\%\mathrm{SMP} = (\%\,\mathrm{salt} \times 100)/(\%\,\mathrm{salt} + \%\,\mathrm{moisture}) \qquad (1)$$

The a_w was determined for each cheese at the beginning of each trial using an AquaLab LITE water activity meter (Decagon Devices Inc., Pullman, WA) according to a standard method *(1).* Titratable acidity (%) was monitored during storage (days 0, 6, and 15) according to a standard method *(4).* Briefly, for each cheese/trial, one sample (10.0 ± 0.5 g) that had been manually crumbled was automatically blended with 50 ml of distilled water and titrated using a Model DL22 Automatic Titrator (Mettler Toledo, Schwerzenbach, Switzerland), which was set to calculate %TA using the molecular weight of lactic acid. To determine the impact,

if any, of the presence of inoculum bacteria or growth of indigenous bacteria on cheese pH, the surface pH was measured for individual inoculated cheese slices at each sampling time (days 0, 3, 6, 9, 12, and 15) using an Accumet AB15 pH Meter equipped with a flat surface combination electrode (Fisher Scientific, Itasca, IL).

Inoculum preparation. Ten strains of *L. monocytogenes,* six strains of *Salmonella* spp., five strains of *E. coli* O157:H7, and five strains of *S. aureus,* representing a wide variety of sources and serotypes, were used in this study (Table 3). Stock cultures were maintained at −20°C in brain heart infusion broth (BHIB; Difco, BD, Sparks, MD) with 10% (wt/vol) added glycerol (Fisher Scientific). Fresh working cultures were prepared monthly by thawing stock cultures and streaking for isolation as follows: *L. monocytogenes* on Listeria selective agar (Oxoid, Ogdensburg, NY) with added Listeria selective supplement (Oxford formulation, Oxoid); *Salmonella* and *E. coli* O157:H7 on modified Levine's eosin methylene blue agar (m-LEMB), prepared from lactose-free LEMB agar (Difco) with the addition of 10 g/liter D-sorbitol (Fisher Scientific) and 5 g/liter NaCl (Fisher Scientific); and *S. aureus* on Baird-Parker agar (Difco) with added egg yolk tellurite enrichment (Difco). Working culture plates were incubated for 24 h at 35°C for *Salmonella* spp. and *E. coli* O157:H7 and 48 h at 35°C for *L. monocytogenes* and *S. aureus,* whereupon all cultures were observed for consistent colony morphology and stored at 4°C for <40 days. Inoculation cultures were prepared for individual strains by transferring a single colony of each strain into a separate tube containing 9 ml of nutrient broth (Difco) for *L. monocytogenes* or BHIB for *Salmonella* spp., *E. coli* O157:H7, and *S. aureus*. Preliminary studies showed better survival of *L. monocytogenes* over 15 days at 25°C on Cheddar and Swiss cheeses when inocula had been grown in nutrient broth, while the other three pathogens survived better on cheeses when inocula had been grown in BHIB ($n = 2$, data not shown). Following incubation for 20 to 24 h at 35°C, 1 ml of stationary-phase culture of each strain for a designated pathogen (10^8 CFU/ml for *L. monocytogenes* and 10^9 CFU/ml for *Salmonella* spp., *E. coli* O157:H7, and *S. aureus*) was transferred to a sterile 9-ml tube to produce a single-pathogen, multistrain cocktail. Each pathogen cocktail was mixed by vortexing and diluted, as necessary, to produce a starting inoculum cocktail of 10^7 CFU/ml. Pathogen levels in the cocktails were estimated by plating the inocula on brain heart infusion agar (Difco) and incubating at 35°C for 24 h.

Sample inoculation. The working surface of a biosafety cabinet was sterilized with 70% (vol/vol) ethanol and covered with aluminum foil prior to cheese inoculation. Cheese slices (approximately 25 to 30 g and approximately 70 to 80 cm^2) were placed on the aluminum foil aseptically, six cheese slices per trial. An aliquot (0.1 ml) of a single-pathogen cocktail (10^7 CFU/ml) was pipetted onto each of the six cheese slices. An L-shaped spreader was used to evenly distribute the inoculum over the surface of the six slices, then samples were left to air dry under the hood for 15 min to allow bacterial attachment and evaporation of excess liquid. The a_w values of control and air-dried inoculated samples were not significantly different ($n = 3$; $P > 0.05$; data not shown). Inoculated cheese slices were folded into half with the inoculated cheese surfaces facing inward to avoid inaccuracies due to pathogen adherence to packaging film. Folded cheese samples were weighed, then individually vacuum packaged in standard retail barrier bags (B-2175, Cryovac Food Packaging and Food Solutions, Duncan, SC) and stored at 25°C for up to 15 days. Oxygen transmission rate for the bags was 3 to 6 cm^3/m^2 at 40°F in 24 h. The initial inoculum level on each cheese slice was ~10^5 CFU/g.

Sampling and enumeration. Packaged cheese samples were analyzed following inoculation (time 0) and throughout storage for up to 15 days. Every 3 days, one cheese slice per pathogen was removed from incubation, the storage/barrier bag was aseptically opened, and Butterfield's phosphate diluent was added to create a 1:10 (wt/wt) dilution. The cheese-diluent mixture was stomached in the bag (AES Smasher, AES Chemunex, Bruz, France) for 2 min at high speed. Stomached samples were serially diluted in Butterfield's phosphate diluent, and 0.1-ml portions were spread plated onto Listeria selective agar, m-LEMB, m-LEMB, and Baird-Parker agar for cheeses inoculated with *L. monocytogenes, Salmonella* spp., *E. coli* O157:H7, and *S. aureus,* respectively. A preliminary trial confirmed better recovery of *Salmonella* spp. by plating on m-LEMB rather than on xylose lysine desoxycholate agar (Difco) and better recovery of *E. coli* O157:H7 by plating on m-LEMB agar rather than on sorbitol MacConkey agar (Difco). Inoculated samples were also spread plated on deMan Rogosa Sharpe agar (MRS; Difco) at 0, 6, and 15 days to monitor changes in lactic acid bacteria (LAB) populations during storage and to thereby investigate the impact, if any, of indigenous, starter, or adjunct bacterial growth on inoculum survival. The m-LEMB spread plates were incubated 24 h at 35°C, Listeria selective agar and Baird-Parker agar plates 48 h at 35°C, and MRS plates 72 h at 35°C, after which time counts were recorded for each plate, with countable plate converted to log CFU per gram. On m-LEMB agar, typical colonies of *E. coli* O157:H7 appear colorless to pink, while colonies of *Salmonella* spp. are dark red-black with a metallic green sheen. Colonies of *S. aureus* are typically shiny black and surrounded with clear zone on Baird-Parker agar. *L. monocytogenes* colonies are normally grey in color surrounded by a black halo on Listeria selective agar. On MRS agar, lactobacilli appear as medium to large white colonies. Data were used to calculate Δlog CFU per gram, relative to time 0, over the 15-day storage period for each bacterium-cheese combination.

Literature data search and selection. To provide additional data to augment our product assessment, data from published literature were combined with data from this study. In searching for relevant published studies, keywords including, but not limited to, ''pathogen, survival, cheeses, temperature, pH, salt'' were entered into online scientific databases. Reference lists of publications were also screened for relevant studies with appropriate data. Published challenge studies that met the following criteria were selected: (i) the inoculated cheeses were made with pasteurized cow's milk, (ii) the cheeses were inoculated with at least one of the pathogens, *L. monocytogenes, Salmonella* spp., *E. coli* O157:H7, or *S. aureus,* (iii) the pathogen(s) was inoculated on the finished cheese, not into the milk, and (iv) inoculated cheeses were stored at 20 to 30°C. Studies with surface-ripened, mold-ripened, Swiss, or processed cheeses or cheese made with nonbovine milk were excluded. Of 155 studies published between 1959 and 2012 and which investigated pathogen behavior in or on cheeses, six published studies met the criteria *(14, 24, 25, 33, 34, 39).* From each publication, the following information was extracted (Table 4): type of cheese, temperature and length of storage, type and number of pathogen strains, composition (all available information for pH, a_w, percentage of moisture, %SMP, %TA) of cheeses and behavior (growth versus no growth) of pathogen(s).

Evaluating compositional characteristics affecting pathogen growth. The relationship between compositional factors and behavior of pathogens on cheeses was explored. Compositional factors of cheese, the percentage of moisture, initial (day 0) pH, %SMP, a_w, and initial %TA, were paired, i.e., one compositional

TABLE 1. *Composition of natural cheeses that did not support growth of* L. monocytogenes, Salmonella *spp.*, E. coli *O157:H7, and* S. aureus *and pathogen survival during storage for 15 days at 25°C*

Cheese[a]	Brand	% moisture[b]	% salt[c]	%SMP[d]	a_w[e]	pH[f]		%TA[g]	LAB count[h]		Pathogen survival (Δlog CFU/g)[i]			
						Day 0	Day 15	Day 0	Day 0	Day 15	LM	SALM	EC	SA
Asiago (young)	1	36.00	1.71	4.53	0.96	5.36	5.21	2.16	7.36	6.87	−2.05	−3.74	−2.12	−1.13
Asiago (young)	1	38.63	1.83	4.52	0.96	5.12	5.01	2.82	7.79	7.40	−2.26	−2.12	−0.68	−1.07
Asiago (aged)	10	38.84	0.96	2.41	0.97	5.15	4.98	1.78	6.22	7.16	−2.92	−2.79	−3.70	−3.53
Asiago (aged)	10	43.30	1.94	4.29	0.97	5.09	5.06	2.02	6.02	6.94	−3.84	−3.63	−1.59	−2.67
Baby Swiss	5	38.36	0.61	1.57	0.98	5.77	6.28	ND[j]	7.07	7.50	−0.71	−1.38	−1.98	−0.62
Baby Swiss	5	36.25	0.69	1.87	0.98	5.79	6.32	ND	6.94	8.05	−0.67	−0.76	−0.75	−1.15
Baby Swiss	9	37.21	0.62	1.64	0.99	5.55	6.04	ND	7.19	7.72	−1.00	−2.43	−1.27	−0.79
Baby Swiss	9	35.58	0.65	1.79	0.99	5.71	6.27	ND	7.35	7.72	−0.39	−1.45	−0.61	−1.02
Brick	11	40.39	1.52	3.63	0.96	5.43	4.90	1.29	7.23	7.66	−0.40	−0.71	−0.38	−0.74
Brick	11	41.21	1.95	4.52	0.97	5.30	4.98	0.90	7.19	7.82	−0.32	−0.70	−0.40	−0.98
Brick	2	38.28	1.52	3.82	ND	5.25	5.37	1.07	6.33	8.08	−0.09	−0.22	−0.42	−0.79
Cheddar (mild)	3	37.34	1.57	4.04	0.96	5.09	5.00	1.89	7.41	6.78	−0.70	−0.88	−0.30	−0.43
Cheddar (mild)	3	36.59	1.77	4.61	0.97	5.09	5.06	1.44	7.39	6.81	−0.76	−1.00	−0.80	−0.17
Cheddar (reduced fat)	6	40.26	1.60	3.82	0.97	5.19	5.11	1.15	5.35	6.21	−0.13	−0.65	−0.43	−1.28
Cheddar (reduced fat)	6	44.00	1.66	3.64	0.98	4.99	5.27	0.90	5.52	5.79	−0.69	−0.57	−0.55	−0.97
Cheddar (sharp)	3	36.34	1.78	4.67	0.96	5.27	5.27	1.69	4.30	6.39	−0.35	−0.75	−0.96	−1.19
Cheddar (sharp)	3	36.57	1.32	3.48	0.97	5.19	5.28	1.71	4.63	5.84	0.00	−1.03	−0.59	−1.34
Cheddar-mozzarella	6	40.09	1.62	3.88	ND	5.19	5.33	1.42	6.99	6.24	−0.09	−0.27	−0.31	−0.48
Colby	4	35.96	1.61	4.28	0.96	5.45	5.61	1.09	5.76	7.39	−0.39	−0.50	−0.21	−0.57
Colby	4	40.14	1.60	3.83	0.97	5.30	5.47	1.78	5.91	6.38	−0.11	−0.63	−0.24	−0.39
Colby Jack	5	36.13	1.42	3.78	0.96	5.17	5.10	1.26	7.19	7.19	−0.20	−0.97	−0.80	−0.46
Colby Jack	5	36.85	1.35	3.53	0.98	5.01	5.40	1.37	7.70	7.38	−0.44	−0.59	−0.08	−0.46
Colby Jack (reduced fat)	6	43.96	1.64	3.60	0.97	5.29	5.00	1.09	5.79	7.68	0.02	−0.90	−0.76	−1.12
Colby Jack (reduced fat)	6	46.00	1.76	3.69	0.97	5.08	5.11	1.39	4.52	6.91	−0.56	−0.74	−0.73	−1.05
Colby Jack (reduced Na)	6	36.30	1.26	3.35	0.97	5.11	5.03	1.48	4.52	6.91	−0.17	−0.46	−1.03	−1.09
Colby Jack (reduced Na)	6	36.45	1.13	3.01	0.98	5.09	5.17	0.89	4.12	5.40	−0.69	−0.96	−0.39	−0.64
Feta	3	57.10	2.35	3.95	0.99	4.29	4.60	2.80	4.80	6.57	−4.58	−4.71	−4.60	−2.93
Feta	3	57.64	1.72	2.90	0.98	4.38	4.53	2.86	3.30	3.40	−4.89	−4.94	−4.07	−4.74
Gouda	6	41.15	1.62	3.79	0.97	5.28	5.25	0.88	7.29	7.38	−0.51	−0.32	−0.23	−0.83
Gouda	6	41.08	1.39	3.27	0.97	5.30	5.28	1.24	7.40	7.48	−0.44	−0.46	−0.34	−0.79
Havarti	3	37.79	1.33	3.40	0.97	5.49	5.52	1.08	6.88	7.26	−0.25	−0.61	−0.21	−0.73
Havarti	3	38.17	1.20	3.05	0.98	5.34	5.59	0.66	6.88	7.20	−0.51	+0.21[k]	−0.29	+0.01[k]
Havarti	6	41.32	1.27	2.98	ND	5.11	5.26	1.40	8.28	7.75	−0.16	−0.61	−0.37	−0.70
Monterey Jack	5	45.10	1.87	3.98	0.98	5.15	5.20	2.41	8.08	8.16	−1.03	−0.91	−0.33	−0.37
Monterey Jack	5	35.45	1.64	4.42	0.97	5.08	5.11	2.28	8.06	7.98	−2.63	−1.17	−0.91	−0.66
Muenster	3	42.20	1.63	3.72	0.97	5.20	5.28	1.27	6.90	7.80	−0.49	−0.25	−0.24	0.00
Muenster	3	41.94	1.75	4.01	0.98	5.29	5.12	0.74	7.11	6.26	−0.10	−0.75	−0.45	−0.46
Parmesan	8	32.44	2.52	7.21	0.93	5.41	5.36	1.40	6.92	4.00	−0.88	−1.45	−1.25	−0.59

TABLE 1. *Continued*

Cheese[a]	Brand	% moisture[b]	% salt[c]	%SMP[d]	a_w[e]	pH[f]		%TA[g]	LAB count[h]		Pathogen survival (Δlog CFU/g)[i]			
						Day 0	Day 15	Day 0	Day 0	Day 15	LM	SALM	EC	SA
Parmesan	8	32.70	2.35	6.70	0.92	5.45	5.40	1.48	5.31	7.23	−1.51	−1.66	−1.86	−1.80
Pepper Jack	4	36.13	1.58	4.19	0.98	5.11	4.76	2.12	2.00	7.66	−0.85	−3.87	−0.81	−1.09
Pepper Jack	4	38.69	1.60	3.97	0.97	4.93	5.12	1.94	4.69	7.14	−2.86	−3.40	−3.25	−3.58
Pepper Jack	3	40.42	1.64	3.90	0.97	5.14	5.12	1.53	4.65	7.30	−2.39	−2.32	−2.10	−0.72
Pepper Jack	3	38.27	1.54	3.87	0.97	5.21	5.15	1.45	8.25	7.39	−0.62	−0.73	−0.35	−0.30
Provolone	4	42.15	1.38	3.17	ND	5.24	4.97	1.81	7.70	8.70	−1.34	−0.97	−0.16	−0.72
Provolone (mild)	8	43.05	2.08	4.61	ND	5.18	5.22	1.80	5.53	6.70	−0.50	−1.84	−0.57	−0.71
Provolone (sharp)	10	40.02	1.72	4.12	ND	5.09	5.17	2.20	6.43	7.45	−1.59	−2.83	−1.27	−1.73
Provolone (reduced fat)	3	48.98	1.43	2.84	0.97	4.97	4.67	1.83	6.95	7.98	−2.80	−2.23	−0.62	−1.55
Provolone (reduced fat)	3	52.71	1.35	2.50	0.98	4.98	4.94	ND	3.70	7.94	−0.56	−0.95	−0.24	−0.97
Provolone-mozzarella	6	42.26	1.68	3.82	ND	5.38	5.33	1.61	7.67	7.28	−0.25	−0.19	−0.17	−0.68
Swiss	6	38.57	0.52	1.33	0.98	5.36	5.50	ND	5.95	6.59	−1.20	−1.11	−0.73	−2.32
Swiss	6	36.91	0.64	1.70	0.99	5.50	5.80	ND	5.28	6.19	−0.93	−1.30	−0.36	−1.20
Swiss (Lacey)	5	45.17	0.33	0.73	0.99	6.02	5.87	ND	7.00	8.18	−0.43	−1.19	−0.46	−1.02
Swiss (Lacey)	5	45.92	0.37	0.80	0.99	5.65	5.94	ND	7.92	5.70	−1.83	−1.21	−0.31	−1.06

[a] Cheeses were national brands obtained from local retail outlets or directly from manufacturers. Qualifying descriptive information, e.g., mild and sharp are reproduced where provided on the package.
[b] Moisture content (%) of cheese sample on day 0, $n = 2$.
[c] Percentage of salt of cheese sample on day 0, $n = 2$.
[d] Percentage of salt-in-the-moisture phase (%SMP) of cheese sample on day 0. Calculated from percentage of moisture and percentage of salt of the same cheese.
[e] The a_w of cheese sample on day 0.
[f] The pH of cheese slice surface on days 0 and 15, $n = 2$.
[g] The percentage of titratable acidity (%TA) of cheese sample on day 0, $n = 2$.
[h] MRS agar count for LAB on days 0 and 15 (log CFU per gram), $n = 2$.
[i] Survival of pathogen (LM, *L. monocytogenes*; SALM, *Salmonella* spp.; EC, *E. coli* O157:H7; SA, *S. aureus*): Δlog count (CFU per gram) day 15 minus day 0. +, growth; −, no growth.
[j] ND, not determined.
[k] Growth of pathogen did not exceed plating variability: 0.39, 0.41, 0.27, 0.25 log CFU/g for LM, SALM, EC, and SA, respectively.

TABLE 2. *Composition of natural cheeses that supported growth of* L. monocytogenes, Salmonella *spp.,* E. coli *O157:H7, and/or* S. aureus *and pathogen survival during storage for 15 days at 25°C*

Cheese[a]	Brand	% moisture[b]	% salt[c]	% SMP[d]	a_w[e]	pH[f]		%TA[g]	LAB count[h]		Pathogen survival (Δlog CFU/g)[i]			
						Day 0	Day 15	Day 0	Day 0	Day 15	LM	SALM	EC	SA
Farmer's	12	39.85	1.71	4.11	ND[j]	5.46	4.99	1.14	4.63	9.10	−0.41	−0.10	−0.39	**+1.48**[l]
Gruyere	7	34.25	1.01	2.86	0.97	5.68	5.74	1.04	5.70	7.40	**+1.01**	**+1.01**	−0.40	**+3.08**
Gruyere	7	32.07	1.41	4.21	0.98	6.28	5.78	1.55	5.04	6.70	−0.54	**+2.13**	−0.67	**+2.19**
Jack (goat's milk)	13	45.20	2.33	4.90	ND	5.41	5.24	1.44	7.74	6.88	−0.40	**+2.50**	−0.62	**+1.62**
Muenster	6	41.58	1.21	2.83	ND	5.48	5.53	0.66	4.85	7.67	+0.17[k]	**+1.65**	**+0.41**	**+1.77**
Provolone	3	43.17	1.03	2.33	0.97	5.29	4.78	1.36	2.70	5.40	−1.10	−0.40	−0.88	**+0.80**
Provolone	3	44.08	1.58	3.46	0.98	5.15	5.19	1.55	3.78	7.19	−0.40	−0.80	−0.52	**+0.81**
Provolone (reduced Na)	6	42.93	1.05	2.39	0.98	5.15	4.95	1.24	6.25	7.79	−1.20	−0.31	−0.30	**+0.62**
Provolone (reduced Na)	6	44.09	1.02	2.26	0.98	5.28	5.12	1.62	5.73	7.39	−0.29	−0.27	−0.63	**+1.59**
Queso Blanco	7	47.02	3.30	6.56	0.96	6.37	6.11	0.26	4.38	6.78	**+2.68**	−1.07	−2.11	**+2.57**
Queso Fresco	7	51.19	1.85	3.49	0.98	6.49	5.05	0.31	4.86	8.68	**+2.09**	**+3.02**	**+2.90**	**+1.55**
String	14	47.91	1.98	3.97	ND	5.44	4.96	1.59	4.87	8.86	**+0.60**	**+2.00**	**+1.75**	**+2.39**
String	6	47.07	2.18	4.43	ND	5.33	5.02	1.67	4.85	8.65	+0.22[k]	+0.39[k]	−0.38	**+1.58**
Queso Quesadilla	7	43.10	2.18	4.81	0.97	5.35	5.39	1.21	4.57	6.29	−0.01	−0.57	−0.48	**−0.40**[m]

[a] Cheeses were national brands obtained from local retail outlets or directly from manufacturers. Qualifying descriptive information, e.g., mild or sharp is reproduced where provided on the package.
[b] Moisture content (%) of cheese sample on day 0, $n = 2$.
[c] Percentage of salt of cheese sample on day 0, $n = 2$.
[d] Percentage of salt-in-the-moisture phase (%SMP) of cheese sample on day 0. Calculated from percentage of moisture and percentage salt of the same cheese.
[e] The a_w of cheese sample on day 0.
[f] The pH of cheese slice surface on days 0 and 15, $n = 2$.
[g] Percentage of titratable acidity (%TA) of cheese sample on day 0, $n = 2$.
[h] MRS agar count for LAB on days 0 and 15 (log CFU per gram), $n = 2$.
[i] Survival of pathogen (LM, *L. monocytogenes*; SALM, *Salmonella* spp.; EC, *E. coli* O157:H7; SA, *S. aureus*): Δlog count (CFU per gram) day 15 minus day 0. +, growth; −, no growth.
[j] ND, not determined.
[k] Growth of pathogen did not exceed plating variability: 0.39, 0.41, 0.27, and 0.25 log CFU/g for LM, SALM, EC, and SA, respectively.
[l] Bold numbers indicate growth beyond the pathogen-plating variability.
[m] Growth (+0.57 log CFU/g) at day 6 sampling; no net growth over 15-day storage period.

TABLE 3. *Pathogen strains used in laboratory cheese challenge studies*

Inoculum	Serotype	Strain[a]	Collection[b]	Source[c]
Listeria monocytogenes	4b	LM 101	FRI	Hard salami
	4b	LM 310	FRI	Goat cheese
	4b	ATCC 43256	ATCC	Mexican-style cheese, California (1985 outbreak strain)
	4b	ATCC 43257	ATCC	Mexican-style cheese, California (1985 outbreak strain)
	4b	ATCC 51414	ATCC	Raw milk, Massachusetts
	4b	ATCC 51776	ATCC	Cheese, Belgium
	4b	ATCC 51777	ATCC	Cheese, Belgium
	4b	ATCC 51778	ATCC	Cheese, Belgium
	4b	Scott A	FRI	Clinical
	1/2a	V7	FRI	Raw milk
Salmonella spp.	Cerro	FSL R8-370	FSL	Bovine
	Typhimurium	FSL S5-433	FSL	Bovine
	Newport	FSL S5-436	FSL	Bovine
	Agona	FSL S5-517	FSL	Human
	Typhimurium	FSL S5-536	FSL	Human
	Newport	FSL S5-639	FSL	Human
Escherichia coli O157:H7	O157:H7	FRIK 22	FRI	Unknown
	O157:H7	FRIK 2000	FRI	Bovine
	O157:H7	F5854	FRI	Cheese curds (1998 outbreak strain)
	O157:H7	039732	NMDH	Gouda cheese (2010 outbreak strain)
	O157:H7	CWD EC1	VT	Farmstead goat cheese
Staphylococcus aureus		I	FPL	Raw milk
		J	FPL	Raw milk
		FRI 100	FRI	Cake
		FRI 1007	FRI	Genoa sausage
		ATCC 25923	ATCC	Clinical

[a] Strain designation provided by collection.
[b] Collection: FRI, Food Research Institute, University of Wisconsin–Madison; ATCC, American Type Culture Collection, Manassas, VA; FSL, Food Safety Laboratory, Dr. Katherine Boor, Cornell University, Ithaca, NY; NMDH, New Mexico Department of Health, Santa Fe; VT, Vermont Institute for Artisan Cheese, Dr. D. J. D'Amico, University of Vermont, Burlington; FPL, Food Pathogen Laboratory, Dr. Barbara Ingham, University of Wisconsin–Madison.
[c] Source provided by collection.

factor as x and one as y and a growth versus no-growth outcome was plotted for each cheese as a function of the x and y values to analyze the influences of the paired compositional factors on pathogen growth. Values of compositional factors were normalized to a 100-point scale before plotting as follows: for each compositional factor, the minimum value of the data set was subtracted from the observed value and the total was divided by the range of the values and multiplied by 100 to obtain the normalized value, as shown in equation 2.

$$\text{Normalized value} = [(\text{value} - \text{minimum value})/\text{range}] \times 100 \quad (2)$$

In this analysis, a growth result was indicated for a cheese when the Δlog CFU/g for any cheese-pathogen combination over the 15-day storage period was a positive value that exceeded the pathogen-specific plating variability: 0.39, 0.41, 0.27, and 0.25 log CFU/g for *L. monocytogenes, Salmonella* spp., *E. coli* O157:H7, and *S. aureus,* respectively. The growth/no-growth outcome plot from each pair of compositional factors was inspected and compared with predictions from a logistic regression equation (SAS 9.2, SAS Institute, Cary, NC). A model at $P = 0.05$ based on the variables initial pH and %SMP was generated, according to the method of McMeekin et al. *(29)* (Fig. 1).

RESULTS AND DISCUSSION

In this study, 67 cheese samples, representing a variety of national brands, were tested for their ability to support pathogen growth during extended storage at 25°C (Tables 1 and 2). Cheeses were manufactured using pasteurized milk in facilities meeting applicable federal and state food safety regulatory requirements. Cheeses met a standard of identity, where applicable. Among the 67 cheese samples tested, 52 were duplicate samples of cheeses from different lots or production dates of the same brand. The majority of cheeses that were tested in this study would be labeled as hard or semi-hard cheeses, according to FDA classification *(43),* and were expected to be safe for extended room-temperature storage due to reduced moisture level and low pH. Soft cheeses with higher moisture were also included to clarify compositional differences affecting pathogen growth/no-growth outcomes. Inoculated cheeses were vacuum packaged to prevent moisture loss, delay mold growth, and to allow for pathogen growth, if any.

Salmonella spp., *L. monocytogenes,* and *E. coli* O157:H7 have, in recent years, been implicated in foodborne illness outbreaks linked to cheeses made with pasteurized milk *(7, 9). S. aureus* has not often been associated with foodborne illness outbreaks linked to cheese, even though this pathogen is generally linked to foods, such as cheese, which are often hand manipulated during processing and packaging *(8, 40).* We included *S. aureus* in the study design not only because of its link to

TABLE 4. *Data from published research selected to augment laboratory product assessment*

Reference	Pathogen	No. of strains	Cheese	Storage (days)	Temp (°C)	pH[a]	%SMP[b]	a_w	Growth/death[c]
24	*Salmonella*	9	Queso Fresco	—[d]	20	6.60	1.64	—	LT: 2.5–3.5 h; GT: 1.65–2.17 h
25	*E. coli* O157:H7	2	Queso Fresco	—	20	6.60	1.61	—	LT: 3–3.45 h; GT: 2.33–2.56 h
33	*L. monocytogenes*	5	Cheddar	30	21	5.06	1.70	0.98	−1.11
						5.30	1.80	0.97	−0.48
						5.66	5.00	0.95	−0.14
						5.28	4.80	0.95	−0.96
34	*Salmonella* spp.	5	Cheddar	30	21	5.06	1.70	0.98	−3.2
						5.30	1.80	0.97	−3.9
						5.66	5.00	0.95	−3.8
						5.28	4.80	0.95	−3.5
39	*L. monocytogenes*	5	Queso Blanco	6.25	25	6.80	4.53	0.97	>5.00
14	*L. monocytogenes*	5	Queso Fresco	3	30	6.60	6.60	—	+0.39
			Queso Fresco	6	30	6.60	4.50	—	+0.95
			Queso Fresco	3	30	6.50	6.15	—	+0.74
			Queso Ranchero	1	30	6.20	4.10	—	+2.60
			Queso Panela	3	30	6.20	2.50	—	+1.81
			Queso Panela	1	30	6.70	3.95	—	+3.18
			Queso Panela	3	30	6.60	3.48	—	+0.79
			Cotija	8	30	5.60	9.60	—	>−2.00
			Cotija	6	30	5.50	12.50	—	>−2.00
			Monterey Jack	4	30	5.00	3.00	—	>−1.40
			Monterey Jack	13	30	5.20	2.72	—	>−2.09
			Mild Cheddar	4	30	4.90	2.60	—	>−1.26
			Mild Cheddar	7	30	5.20	4.49	—	>−2.09
			Colby	9	30	5.50	4.93	—	>−2.36
			String cheese	9	30	5.50	4.24	—	>−2.36
			Provolone	9	30	5.60	4.62	—	>−2.36
			Muenster	9	30	5.50	3.80	—	>−2.36
			Domestic Feta	4	30	4.30	7.50	—	>−2.04
			Domestic Feta	4	30	4.30	2.20	—	>−2.04

[a] pH values of cheeses at initial sampling point of experiment.
[b] Certain publications stated %SMP as percentage of brine, which was calculated using the same equation as in this study (equation 1). For publications that included both percentage of moisture and percentage of salt, %SMP was calculated using equation 1.
[c] Behavior of pathogen over storage, expressed as Δlog CFU per gram or lag time (LT) and generation time (GT).
[d] —, not specified.

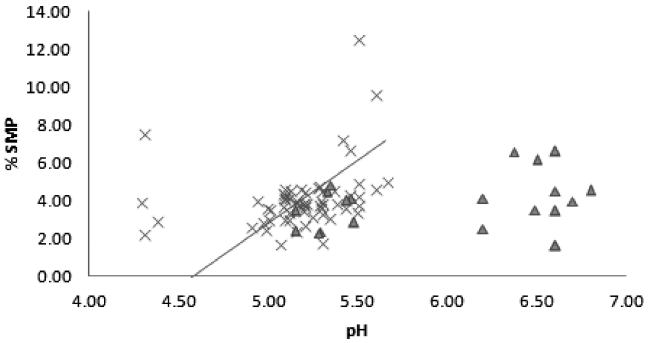

FIGURE 1. *Growth (▲) or no growth (x) of* Listeria monocytogenes, Salmonella *spp.,* Escherichia coli *O157:H7, and* Staphylococcus aureus *on cheeses stored at 20 to 30°C based on cheese pH (day 0) and salt-in-the-moisture phase (%SMP). Data from published research (*n = *26; Table 4) and this study (*n = *55). Solid line represents the growth/no-growth interface (*P = *0.05).*

poor sanitation and postprocessing contamination but also because it is the pathogen most likely to grow in or on foods with reduced moisture and/or low a_w *(21).* For ready-to-eat food products, the FDA has established a zero-tolerance policy for *L. monocytogenes, Salmonella* spp., and *E. coli* O157:H7, due to the potentially low infectious dose of *E. coli* O157:H7 and *Salmonella* spp and the high mortality rate (15 to 30%) associated with *L. monocytogenes* infections *(42).* Although none of these pathogens should be present in finished cheeses made from pasteurized or heat-treated milk, the composition of a cheese supporting growth of any of these bacteria during extended room-temperature storage presents an unacceptable risk. A zero-tolerance policy is not in place for *S. aureus* in ready-to-eat foods because staphylococcal food poisoning occurs as a result of ingestion of a preformed enterotoxin, which is only produced in amounts sufficient to cause illness as a result of extended temperature abuse and growth of the pathogen to a high concentration ($\sim 10^5$ CFU) *(30).* Thus, a cheese with compositional characteristics allowing growth of *S. aureus* during storage is also an unacceptable risk. For these reasons, the growth of four target pathogens, *L. monocytogenes, Salmonella* spp., *E. coli* O157:H7, and *S. aureus,* as postprocessing contaminants on cheeses was investigated.

Pathogen strains used in this study represented a variety of sources and serotypes (Table 3). The strains of *L. monocytogenes* and *Salmonella* spp. had been screened in previous research in our laboratory to confirm tolerance to salt and pH conditions typical of cheese *(13).* Strains of *E. coli* O157:H7, *Salmonella* spp., and *S. aureus* were exposed to some acid during inoculum preparation in BHIB, as a pH drop of ~1 unit was observed during overnight incubation. *L. monocytogenes* was grown in nutrient broth, with no pH drop during inoculum preparation. Where it occurred, the limited exposure to acid during inoculum preparation was unlikely to have led to acid adaptation of strains. Therefore, the key characteristic of strains selected for use in this study was their human or animal–animal product origin, making these strains perhaps representative of organisms to be found in a food processing or handling environment.

The FDA, in its guide to microbial challenge testing, notes that it can be important to evaluate a range of intrinsic factors that can influence the safety of a food during its intended shelf life *(44).* Compositional factors in cheese that could influence pathogen behavior were analyzed: surface pH (days 0, 3, 6, 9, 12, 15); percentage of moisture, percentage of salt, and a_w (day 0); and %TA (days 0, 6, 15). Change in LAB count was determined on days 0, 6, and 15. Across all cheese samples, moisture content ranged from a low of 32.07% to a high of 57.64%, for one lot of Gruyere and Feta cheese, respectively. Salt content ranged from 0.33% for one lot of Lacey Swiss to 3.30% for Queso Blanco. Salt-in-the-moisture phase was calculated from percentage of moisture and percentage of salt (equation 1), with values ranging from 0.73% for one lot of Lacey Swiss to 7.21% for one lot of Parmesan. The a_w varied little across the cheese samples tested, ranging from 0.96 to 0.99, except for Parmesan (average $a_w = 0.93$; Table 1).

Cheese pH on day 0 ranged from 4.33 to 6.49 for Feta (average of two lots) and Queso Fresco, respectively (Tables 1 and 2). Over the 15-day storage period, change in pH ranged from −1.44 to +0.53 pH units for Queso Fresco and Baby Swiss, respectively, with most cheeses exhibiting only slight change in pH. To quantify the amount of lactic acid present in each cheese at the beginning of storage and to determine the effect, if any, of storage on changes in lactic acid level, %TA was measured (Tables 1 and 2). The %TA across the cheeses tested ranged from 0.26 to 2.83% for Queso Blanco and Feta, respectively, at the beginning of storage. Change in %TA over storage was not clearly linked with change in pH and bacterial survival (data not shown). Change in LAB count in cheese samples was estimated during extended storage at 25°C (Tables 1 and 2). LAB count on day 0 across the cheeses ranged from 2.00 to 8.08 log CFU/g for one lot of Pepper Jack and Monterey Jack, respectively. Initial LAB counts on similar cheese samples from different brands, or different lots of the same brand, could vary widely. The day 0 count for LAB on different lots of Provolone (reduced fat; brand 3) varied by 3.25 log CFU/g between purchase dates. Similarly, one sample of Provolone (brand 3) had one of the lowest day 0 LAB counts, 2.70 log CFU/g, while another sample of a different brand of Provolone (brand 4) had one of the highest initial LAB counts, 7.70 log CFU/g. The day 0 LAB counts for the two samples of brand 3 Provolone were 2.70 log CFU/g and 3.78 log CFU/g, and these rose to 5.40 log CFU/g and 7.19 log CFU/g, respectively, equivalent to a Δlog CFU/g of 2.70 and 3.41, respectively. The LAB count for the one lot of brand 4 Provolone increased by one order of magnitude, from 7.70 log CFU/g (day 0) to 8.70 log CFU/g (day 15). Throughout the storage period and across all cheese samples tested, changes in LAB count ranged from −2.92 log CFU/g for one lot of Parmesan to +5.66 log CFU/g for one lot of Pepper Jack (brand 4). Of the 67 cheese samples tested, LAB population increased on storage in 47 cheese samples tested. LAB count was relatively constant ($0 < \Delta\log \leq 0.3$ log CFU/g) in 7 cheese samples tested and declined ($\Delta\log \geq -0.3$ log CFU/g) in 13 other cheese samples during storage.

Pathogens did not grow on 53 cheese samples over the 15 days (Table 1), while 14 cheese samples supported growth of *S. aureus,* 6 of *Salmonella* spp., 4 of *L. monocytogenes,* and 3 of *E. coli* O157:H7 (Table 2). The pattern of pathogen survival for each cheese lot was consistent over storage except for Queso Quesadilla (Table 2). We observed growth of *S. aureus* (+0.57 log CFU/g) on day 6 on Queso Quesadilla; however, by day 15, we noted an overall decrease in pathogen population (of −0.40 log CFU/g). Of the cheese samples that did support pathogen growth, all supported growth of *S. aureus*, ranging from 0.57 to 3.08 log CFU/g (average 1.62 log CFU/g across all 14 cheeses). Growth of *L. monocytogenes, Salmonella* spp., and *E. coli* O157:H7, ranged from 0.60 to 2.68 log CFU/g (average 1.60 log CFU/g), 1.01 to 3.02 log CFU/g (average 2.05 log CFU/g), and 0.41 to 2.90 log CFU/g (average 1.69 log CFU/g), respectively.

Growth of *L. monocytogenes,* which exceeded the plating variability, was observed on four cheese samples: Gruyere (one lot), Queso Blanco, Queso Fresco, and String cheese (brand 14; Table 2). Genigeorgis et al. *(14)* studied the survival of *L. monocytogenes* on market cheeses stored at 30°C and observed pathogen growth only on Hispanic-style cheeses: Queso Fresco, Queso Ranchero, and Queso Panela, ranging from 0.38 to 3.18 log CFU/g. Uhlich et al. *(39)* observed an increase of more than 5 log CFU/g of *L. monocytogenes* on Queso Blanco stored at 25°C for up to 6.25 days. Genigeorgis et al. *(14)* did not observe growth of *L. monocytogenes* on String cheese, instead noting a drop in *L. monocytogenes* population of 2.36 log CFU/g over 9 days at 30°C. The String cheese that Genigeorgis et al. tested had similar pH and %SMP values to the cheese sample that we evaluated but an unknown level of LAB. The String cheese sample in our study that allowed some growth of pathogen simultaneously supported a dramatic increase in LAB population, from 4.87 log CFU/g on day 0 to 8.86 log CFU/g by day 15 (Table 2).

Growth of *L. monocytogenes* was not observed on 63 samples of cheese tested (Tables 1 and 2). Many of the cheeses that did not support pathogen growth would be classified as hard or semi-hard cheeses based on FDA classification *(43)* and may be suitable for extended room temperature storage. Shrestha et al. *(33)* did not observe growth of *L. monocytogenes* on a range of Cheddar-type cheeses stored at 21°C for 30 days, with counts of *L. monocytogenes* dropping by ≤1.1 log CFU/g during storage. We observed a slight decrease in the population of *L. monocytogenes* on mild, reduced-fat, and sharp Cheddar cheeses during storage at 25°C (Table 1). Genigeorgis et al. *(14)* also reported a decrease of *L. monocytogenes* population on mild Cheddar cheeses during storage. Genigeorgis et al. evaluated the growth of *L. monocytogenes* on Monterey Jack, Colby, Provolone, Muenster, and Feta cheeses during storage and observed a decrease in pathogen population of >1 to 2 log CFU/g in all cases. In our study, we noted an average decrease in pathogen population ranging from 0.20 log CFU/g for Colby to 4.74 log CFU/g for Feta (Tables 1 and 2).

Growth of *Salmonella* spp. was observed on six cheeses: Gruyere (two lots), Jack (goat's milk), Muenster (brand 6), Queso Fresco, and String (brand 14). Kasrazadeh and Genigeorgis *(24)* studied the growth of *Salmonella* inoculated onto sliced Queso Fresco stored at 20°C. They noted rapid growth, a lag time of 2.5 to 3.5 h, and a generation time of 1.65 to 2.17 h for *Salmonella* on Queso Fresco. We observed an increase in *Salmonella* spp. concentration of 3.02 log CFU/g on Queso Fresco stored at 25°C over 15 days. This was the highest level of *Salmonella* growth observed over all 67 cheese samples tested.

There were 61 cheeses that did not support the growth of *Salmonella* spp. in this study. Shrestha et al. *(34)* examined the survival of *Salmonella* on a range of Cheddar-type cheeses stored for up to 30 days at 21°C. Cheddar cheese manufactured to standards of pH and salt was comminuted, inoculated with *Salmonella* spp., and stored at 21°C for up to 30 days. *Salmonella* spp. counts decreased significantly at 21°C for all cheese types. We evaluated the survival of *Salmonella* spp. on mild, reduced-fat, and sharp Cheddar cheeses and observed average decreases of 0.3, 1.12, and 1.26 log CFU/g, respectively, for the brands tested.

Three cheeses supported growth of *E. coli* O157:H7, Muenster (brand 6), Queso Fresco, and String (brand 14). Kasrazadeh and Genigeorgis *(25)* reported rapid growth of *E. coli* O157:H7 on Queso Fresco stored at 20 and 30°C, with a lag time of 3 to 3.45 h and a generation time of 2.33 to 2.56 h at 20°C. The authors attributed the fast growth rate of *E. coli* to the lack of starter culture, near neutral pH (6.6), and low %SMP (1.61%). The Queso Fresco that we studied had similar pH (6.49) but higher %SMP (3.49), supporting the assertion that cheese pH has a dominant effect on pathogen growth.

Of the 14 cheeses that supported pathogen growth, all supported the growth of *S. aureus*. Cheese samples that supported growth of *S. aureus* included Farmer's, Gruyere (two lots), Jack (goat's milk), Muenster (brand 6), Provolone (brand 3; two lots), reduced-sodium Provolone (two lots), Queso Blanco, Queso Fresco, Queso Quesadilla, and two brands of String cheese. There are no reports of prior research evaluating the survival of *S. aureus* as a postprocessing contaminant on cheese made from pasteurized milk. Levels of *S. aureus* on Queso Quesadilla increased by 0.57 log CFU/g on day 6 of storage but decreased by 0.40 log CFU/g relative to the time-zero level by day 15. In all other cases, pathogen growth/no growth displayed a consistent increase or decrease over the 15-day storage period.

LAB count increased in 47 of 67 cheeses tested in this study. With one exception, cheeses that supported pathogen growth also supported LAB growth. LAB count decreased in Jack (goat's milk) cheese that supported growth of *Salmonella* spp. and *S. aureus*; otherwise, LAB count increased from 1.54 to 4.47 log CFU/g in cheeses that supported pathogen growth. The level of inoculum on each cheese slice at time 0 averaged 4.7 log CFU/g ($n = 268$). This level allowed for accurate enumeration of growth or death without reaching the limits of research methodology. This inoculum level could have placed pathogens at a level

to effectively compete with active indigeneous organisms. LAB count on day 0 averaged 5.03 log CFU/g for cheeses that supported pathogen growth ($n = 14$, Table 2). Although previous studies have shown that initial inoculum level does not affect the survivability or growth kinetics of pathogens *(6, 26, 46),* a higher proportion of *S. aureus* compared with LAB may aid in the survival or growth of this particular pathogen *(17).* Although growth of *S. aureus* is reported to be weak when a high load of competitive bacteria, e.g., LAB, is present, increasing the proportion of *S. aureus* to LAB has been shown to improve survival of this pathogen *(17, 23).*

The change in pH on storage among cheeses that supported pathogen growth showed no clear trend, remaining the same ($\Delta pH \leq 0.3$ units) in seven samples and increasing in six samples (Table 2). Change in %TA over storage (data not shown) had no apparent relationship with the change of pH and LAB count. Correlation between changes in pH and LAB count in cheeses that supported pathogen growth was weak ($r^2 = 0.15$).

The 14 cheeses that supported pathogen growth were characterized by relatively high pH. When cheese samples were separated into roughly equal groups by initial pH value, 4.29 to 5.20 (29 cheeses), 5.21 to 5.40 (18 cheeses), and 5.41 to 6.50 (20 cheeses), it was apparent that pathogen growth was better supported on higher pH cheeses. With the exception of brand 3 Provolone and reduced-sodium Provolone, cheeses with day 0 pH ranging from 4.8 to 5.2 did not support growth of any pathogen (Table 1). Feta was the most acidic cheese tested (average pH 4.33, $n =$ two lots), and pathogen viability on this cheese type decreased over time more than for any other cheese (Table 1). As pH increased to 5.21 to 5.40, 4 of 18 cheeses supported growth: Provolone (brand 3; one lot), reduced-sodium Provolone (one lot), String cheese (brand 6), and Queso Quesadilla, all supporting the growth of *S. aureus* but no other pathogen (Table 2). In the pH range 5.41 to 6.50, eight cheeses supported pathogen growth: Jack (goat's milk), String (brand 14), Farmer's, Muenster, Gruyere (two lots), Queso Blanco, and Queso Fresco. Pathogen growth on Queso Fresco was the greatest across all cheeses tested; this was also the cheese with the highest initial pH (pH 6.49). Generally, cheeses with an initial pH $\geq$5.46 supported growth of at least one pathogen, with the exception of Swiss-style cheeses (Baby Swiss, Swiss, Lacey Swiss) and one lot of Havarti, which did not support growth. Optimal pH for growth of *S. aureus* is between pH 6.0 and 7.0, with pH 4.0 as the reported minimum for growth *(20).* Minimum pH values that have been reported for growth of *L. monocytogenes, Salmonella* spp., *E. coli* O157:H7 are 4.39, 4.20, and 4.40, respectively *(20).* Only Feta cheese (pH 4.29 and 4.38) was below the reported minimum pH for growth of any of the pathogens tested.

The %SMP for cheeses that supported pathogen growth ranged from 2.26 to 6.56% and from 0.73 to 7.21 %SMP for cheese samples that did not support growth. The greater growth potential that we observed for *S. aureus* on cheeses could be attributed, in part, to the high salt-tolerance of this pathogen. Nunheimer and Fabian *(31)* reported that some strains of *S. aureus* are able to tolerate up to 20% NaCl. Sutherland et al. *(36)* reported growth of *S. aureus* in BHIB with pH 4.48 and 8.5% NaCl at 25°C. Ingham et al. *(19)* reported greater tolerance of *S. aureus* than of *L. monocytogenes* to high salt and low a_w in meat products stored at 21°C.

Recognizing the potential for compositional variability in cheeses across type, age, and manufacturer, we tested cheeses from different brands or from different lots within the same brand. Along with observed variation in pH and a range of %SMP, the presence of inhibitory compounds in cheese, such as metabolites of LAB and the presence of free fatty acids, may have varied from lot to lot, brand to brand, and between cheese types, resulting in differences in pathogen growth during nonrefrigerated storage. The effect of these factors on microbial survival has been shown to be highly dependent on the concentration of inhibitory compounds and the species and strain of both LAB and pathogen *(11, 15, 17, 35).* The apparent inconsistencies in pathogen growth patterns observed for cheeses of a similar type supports the assertion that compositional characteristics, more than cheese type, determine the likelihood of pathogen growth on a sample of cheese.

The compositional factors of pH, %SMP, a_w, and %TA were paired in all combinations and a pathogen growth/no-growth outcome for each cheese was plotted as a function of each pair of factors. Plotting growth/no-growth outcome as a function of pH and %SMP, combined with logistic regression, created a growth/no-growth interface that could be used to differentiate cheeses that inhibited pathogen growth from those that allowed pathogen growth (Fig. 1). These results are consistent with those of Oh et al. *(32)* who evaluated the effect of compositional factors of low-sodium Cheddar cheeses on the growth of strains of *Salmonella* spp., *L. monocytogenes, S. aureus,* and Shiga toxin–producing *E. coli.* In a model low-sodium Cheddar cheese extract, Shiga toxin–producing *E. coli* survived significantly better than the other three pathogens. Principal component analysis indicated that Shiga toxin–producing *E. coli* survival was primarily determined by pH, and not by percentage of salt or percentage of lactate *(32).*

The eight Swiss-style cheese samples tested did not fit the pattern established by data from the other cheeses tested. These Swiss-style cheeses had the lowest %SMP of all cheeses tested, a relatively high pH, and a high a_w. Despite these compositional factors seeming to be permissive for growth, none of the Swiss-style cheeses supported pathogen growth. Leyer and Johnson *(27)* reported poorer survival of *Salmonella* spp. on Swiss cheeses than on Cheddar and mozzarella. Swiss-style cheeses are unique among the types of cheeses that we tested due to the addition of propionic acid bacteria as an adjunct culture in cheese manufacture. The added propionic acid bacteria can produce metabolites with antimicrobial properties, such as propionic acid, acetic acid, and diacetyl *(10).* Studies have shown greater antimicrobial properties linked to propionic acid ($pK_a = 4.87$) as compared with lactic acid ($pK_a = 3.86$) *(37).* The results of

our study would suggest that target pathogens will not grow on Swiss-style cheeses during extended storage at 25°C, but the safety of such cheeses should be evaluated independently from cheeses that are fermented using only lactic acid–producing bacteria.

Further, our research suggests that the ability of pathogens to grow on bacterial surface–ripened or mold-ripened cheeses should be evaluated independently from cheeses manufactured without these ripening adjuncts. Bacterial surface–ripened and mold-ripened cheeses have added cultures that are capable of growing and altering the environment for pathogen growth. Growth of added bacterial and/or mold cultures can result in the production of antimicrobial compounds (e.g., bacteriocins) that could hinder pathogen growth but can also lead to lactate metabolism, which can subsequently increase cheese pH and enhance pathogen growth *(5)*. Genigeorgis et al. *(14)* found a significant reduction of *L. monocytogenes* (>-2.36 log CFU/g) when inoculated onto Limburger, a bacterial surface–ripened cheese. While the high pH of Limburger (pH 7.2) would suggest that this cheese could support pathogen growth, the growth of smear bacteria results in extensive lipolysis that produces a high concentration of free fatty acids, which are compounds known to have antimicrobial activity *(35)*. Goat's milk cheese may also contain high levels of free fatty acids. Woo et al. *(47)* evaluated the free fatty acid content in a variety of cheeses and concluded that Blue, Swiss, Limburger, and goats' milk cheeses contained high concentrations of free fatty acids. Thus, we conclude that the safety of surface-ripened cheeses, mold-ripened cheeses, and noncow's milk cheeses, along with Swiss-style cheeses, cannot be effectively evaluated using the logistic equation we developed to establish the pathogen growth/no-growth boundary for other cheeses in this study.

The a_w and pH are the two criteria used in the FDA Food Code to determine the shelf stability of food products *(45)*. However, %SMP is an appropriate factor in assessing the likelihood of pathogen survival on cheese. In addition to salt, other solutes in cheese, such as nonprotein nitrogen-containing compounds and products released during proteolysis, could contribute to the reduction of a_w, yet these compounds may not play a role in inhibiting pathogen growth *(28)*. Tapia et al. *(38)* suggested that the usefulness of measured a_w as an indicator of microbial safety or stability is diminished by the ''specific solute effect''; that is, that the solute in the food matrix dramatically alters the minimum a_w for microbial growth. Hilderbrand *(18)* supported %SMP as a more reliable factor than a_w in determining bacterial growth in smoked fish. In addition, %SMP is routinely determined and has historically been used in the cheese industry as a measure of product quality. Our search of published literature indicated that other researchers investigating survival of pathogens as post-processing contaminants on cheese routinely monitored %SMP *(14, 24, 25, 33, 34, 39)*, while only a few studies investigating pathogen survival on cheese considered the impact of product a_w *(33, 34, 39)*. Furthermore we identified that pH and %SMP were the two compositional factors that could be used to differentiate cheeses that supported pathogen growth from those that inhibited growth (Fig. 1). More data are needed to confirm the use of a_w in predicting growth of pathogens in cheese.

Of the 67 market cheeses studied, 53 did not support the growth of *L. monocytogenes, Salmonella* spp., *E. coli* O157:H7, or *S. aureus* and could safely be kept at ≤25°C for an extended period of time. The risk of pathogen growth for those cheeses that supported growth can be characterized as follows: *S. aureus* (growth on 14 of 14 cheeses supporting pathogen growth) >> *Salmonella* spp. (growth on 6 of 14) > *L. monocytogenes* (growth on 4 of 14) > *E. coli* O157:H7 (growth on 3 of 14). None of the cheeses supported growth of *S. aureus* to an extent that would be expected to result in sufficient enterotoxin production to present a food safety hazard. As noted by the National Advisory Committee on Microbiological Criteria for Foods, when growth of *S. aureus* is limited to less than 3 log CFU/g and the initial population of the pathogen does not exceed 3 log CFU/g, production of enterotoxin sufficient to cause illness does not occur *(30)*. The presence of appropriate food safety monitoring programs during cheese manufacture, e.g., HACCP and good manufacturing practices, and sanitation programs in place during postprocessing handling, transportation, and storage would further ensure that the risk associated with *S. aureus* is mitigated. Protection of public health is reinforced by the selection of appropriate cheeses for extended room-temperature storage.

We used data for pH and %SMP from laboratory research and relevant published research from a total of 82 cheeses to establish the boundary conditions for pathogen growth/no-growth during extended room temperature storage of cheese ($P = 0.05$). Based on a search of the literature available at the time, Bishop and Smukowski *(3)* recommended that certain cheeses could be stored for extended periods without refrigeration: Asiago (medium and old), Cheddar, Colby, Feta, Monterey Jack, Muenster, Parmesan, pasteurized process, Provolone, Romano, and Swiss/Emmentaler. Our research does not support this conclusion. Rather, we suggest that cheeses, regardless of type or brand, which meet specific compositional requirements for pH and %SMP may be safely stored at ≤25°C for extended periods of time. Data from Swiss-type cheeses, mold-ripened or bacterial surface–ripened cheeses, or cheeses made with nonbovine milk were excluded from this analysis due to insufficient data or lack of fit. The growth/no-growth interface established by the logistic regression line shows that many common cheese types, if made from pasteurized cow's milk in compliance with U.S. regulatory standards, can safely be considered non-TCS foods. Non-TCS cheeses should be described in terms of pH and %SMP, rather than cheese type or brand. Further research is underway to develop a model that will allow regulators and cheese industry personnel to predict the likelihood of pathogen growth on cheeses prior to extended room temperature storage. The data generated in this research will serve as supporting documentation for science-based decision making for the cheese industry.

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