Microflora of Cheddar Cheese Made with Sodium Chloride, Potassium Chloride, or Mixtures of Sodium and Potassium Chloride

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ABSTRACT

Cheddar cheese samples from three different split lots of cheese curd were prepared with added NaCl, KCl, or mixtures of NaCl/KCl (2:1, 1:1, 1:2 and 3:4, all on wt/wt basis) to achieve a final salt concentration of 1.5 or 1.75%. Cheeses were stored at 3 ± 1°C and their microbiological characteristics were evaluated over a 36-week ripening period. Populations of aerobic microorganisms, lactic acid bacteria, nonstarter lactic acid bacteria, aerobic spores, coliforms, and yeasts and molds in cheeses made with KCl or NaCl/KCl mixtures were not significantly (P<0.05) different from those of control cheeses made with NaCl. Staphylococcus aureus and Escherichia coli were not detected in any of the test or control cheeses.

Key Words: Cheddar cheese, coliforms, lactic acid bacteria, yeasts, molds, sodium chloride, potassium chloride.

Sodium chloride (NaCl) is a major food additive, and its use in the food processing industry is exceeded only by that of sugar (6). Humans have used NaCl as a preservative throughout history (33).

Consumption of NaCl has become of major concern because of the suspected link between sodium intake and hypertension (56), a condition that afflicts 10% to 20% of the United States population (39). Most Americans consume more sodium than they need (58). The Food and Nutrition Board of the National Academy of Sciences National Research Council (4) considers 1,100 mg to 3,300 mg of sodium per day as safe and adequate for the healthy adult, whereas the current daily sodium intake by individuals is estimated to range from 3,900 mg to 4,700 mg (about 10 g to 12 g of NaCl) (52). The estimated adult minimum daily requirement is 100 mg to 200 mg of sodium (0.25 g to 0.50 g of NaCl) (5,6). Some Americans consume 10 to 35 times as much sodium as they need (50). The Food and Drug Administration has encouraged the food industry to voluntarily reduce the amount of sodium added to processed foods (8). Recommendations to reduce dietary sodium also were made in the U.S. Department of Agriculture Guidelines for Americans (59).

Scientists are seeking ways to reduce the NaCl content of dairy products such as natural and processed cheeses which generally contain high sodium levels when compared to other dairy products. Lindsay, et al (31) used a mixture of NaCl and KCl (1:1 wt/wt) to make Cheddar cheese. Kosikowski (25) and Lindsay, et al (32) also made Cheddar cheeses containing approximately 1% NaCl from milks supplemented with retentate from ultrafiltration of whole milk. Koenig and Marth (23) evaluated the behavior of Staphylococcus aureus in Cheddar cheese prepared with a mixture of NaCl and KCl (1:1, wt/wt).

Fitzgerald and Buckley (15) compared the quality of Cheddar cheese prepared with 1.6% residual NaCl or equivalent amounts (ionic-strength basis) of MgCl2, CaCl2, KCl or a 1:1 mixture of NaCl and the chloride salt of Mg, Ca, or K. Schroeder et al. (49) studied the quality of Cheddar cheese made with reduced levels (0%, 0.37%, 0.73%, 1.12% and 1.44%) of NaCl. Martens, et al (34) used mixtures of NaCl and KCl to prepare Gouda cheese and Leifer et al. (30) made low-sodium Gruyere cheese by replacing NaCl with MgCl2. Others have investigated reduction of the sodium content in cottage cheese (14), American-type process cheese (22), butter (21), buttermilk (14) and ice cream (1).

We completed an extensive study on several attributes of Cheddar cheese made with NaCl, KCl or mixtures of NaCl/KCl to reduce the sodium content of the cheese. Reported elsewhere are our findings on proteolysis (45), lipolysis (44), composition (43), and sensory properties (47) of the experimental cheeses. This communication describes the microflora of the experimental cheeses; information on kinds of lactic acid bacteria isolated from the cheeses and fermentation patterns of the bacteria is presented in a companion publication (46).

MATERIALS AND METHODS

Cheddar cheese manufacture.

Milled-curd Cheddar cheese was manufactured at a commercial cheese factory, Fairview Cheese Factory, Pulaski, WI. The procedure for cheese making was as described by Kosikowski (24). Whole milk (3.6% milkfat) was pasteurized (72°C, 17 s),
cooled to 32°C, and inoculated with a lactic starter culture (the cultures used, M30, M29, and KH were obtained from the Marshall Division, Rhone Poulenc, Madison, WI). Cultures M30 and M29 were of the bulk-set type and were used in trials 1 and 2. Culture KH was for direct-to-the-vat use (360 ml/2,268 kg of milk) and was used in trial 3. Culture M30 was a blend of Lactococcus lactis subsp. lactis and L. lactis subsp. cremoris; culture M29 consisted of L. lactis subsp. lactis; and culture KH consisted of L. lactis subsp. cremoris. Bulk-set cultures were incubated in previously steamed (100°C, 45 min) reconstituted nonfat dry milk (Marstar Funnel Grade starter medium, Marshall Division, Rhone Poulenc, Madison, WI) at 21°C for 16-18 h before inoculating into cheese milk at the rate of 0.75%. Double-strength rennet extract (Marshall Division, Rhone Poulenc, Madison, WI) served as the clotting agent. Calcium chloride and anatto color were not added. Cheddar curd was milked at pH 5.3 (0.55-0.60% titratable acid).

The milked curd was divided into eight lots, 22.7 kg each. Lots of curd were then salted with NaCl (Diamond Crystal, St. Clair, MI), KCl (Mallinckrodt, St. Louis, MO), or a mixture of NaCl/KCl, all on a wt/wt basis. The eight treatments (designated as A through H) were replicated thrice over a 2-week period. Quantities of NaCl, KCl or NaCl/KCl mixtures were added to obtain final salt concentrations in cheese of 1.5% or 1.75% for NaCl or NaC!/KCl and 1.5% for KCl. For Cheddar cheese, 1.04 kg of NaCl per 45.4 kg of curd is recommended to obtain 1.5% NaCl in the finished product (25). The salting rate is increased depending upon the amount of salt lost in whey, which can be as much as 50% (24).

Curd in the eight treatments of trial 1 was salted as follows, all based on weight of salt per 45.4 kg of curd: treatment A, no added salt of any type; B, 2.3% NaCl; C, 2.7% NaCl; D, 2.3% KCl; E, 2.3% NaCl/KCl (2:1, wt/wt); F, 2.3% NaCl/KCl (1:1, wt/wt); G, 2.3% NaCl/KCl (1:2, wt/wt); and H, 2.7% NaCl/KCl (3:4, wt/wt). Since the actual concentrations of NaCl, KCl, or NaC!/KCl in finished cheeses of trial 1 were less than the expected 1.5% or 1.75%, the salting rates for treatments in trials 2 and 3 were increased to 2.5% and 2.9% rather than 2.3% and 2.7%. Ratios of NaCl/KCl in mixtures for trials 2 and 3 were identical to those of trial 1. NaCl, KCl or NaC!/KCl was added to curd at three intervals (each 3 min apart in trial 1 and 5 min apart in trials 2 and 3) with thorough mixing to obtain uniform distribution. The salted curd was hooped and pressed overnight at ambient temperature as 2.3 kg rectangular loaves which were waxed, sealed into barrier film pouches (Cryovac, Division of W. R. Grace Co., Cedar Rapids, IA), and stored at 3±1°C for ripening. Ripening was continued at this temperature until samples were removed at appropriate times for analysis.

Compositional analysis.

A grated composite cheese sample was prepared for each treatment by taking representative samples as described in Standard Methods for the Examination of Dairy Products (48). Analyses were done on 3-day-old cheeses.

Duplicate moisture determinations were made on 3.0±0.5 g cheese samples by calculating the percentage of weight lost following drying in a vacuum oven at 100±2°C for 5 h as described in Standard Methods (48).

The pH of samples was determined as described in Standard Methods (48). A pH meter (Corning Model 125, Corning Medical, Medfield, MA) equipped with a standard combination electrode (Ross electrode No. 810400, Orion Research Inc., Boston, MA) was used.

Duplicate milkfat determinations were made on 9 g of cheese by using the modified Babcock test for natural cheeses as described in Standard Methods (48).

The chloride analyzer (Corning Model 926) method was used to measure salt concentrations in cheese samples. The chloride analyzer method gives results comparable to those obtained by the AOAC Volhard method (60), as demonstrated by Johnson and Olson (20). Sodium chloride results were calculated as outlined in the manufacturer's instructions (3). The concentrations of KCl or NaCl/KCl in cheese were calculated as described by Reddy (42).

Microbiological analysis.

Sampling of cheeses to enumerate bacteria was done as described in Standard Methods for the Examination of Dairy Products (48). An 11 g cheese sample was obtained from a composite sample that was prepared from aseptically taken representative portions of a 2.3 kg loaf. The 11 g cheese sample was weighed into a sterile blender jar and 99 ml of a 2% sterile sodium citrate solution at 40-45°C was added. The sample was then blended for 2 min at low speed. Further serial dilutions as needed were made using sterile phosphate-buffered dilution water.

Aerobic plate counts (APC) were made using Plate Count Agar (Difco) and incubation at 32°C for 48 h (48). Coliform counts were determined with the Violet Red Bile Agar method (48) and with a tube method (single-tube dilution series) employing lauryl sulfate tryptose broth, followed by confirmation of positive tubes using 2% brilliant-green lactose bile broth (2).

*Escherichia coli* determinations were made according to the procedure outlined in the *Bacteriological Analytical Manual* (2). Confirmatory biochemical tests employed for *E. coli* consisted of the simplified 48 h indole-methyl red-Voges Proskaucer-citrate (IMViC) test as described by Powers and Latt (40), but with modifications in Kovacs reagent and methyl red-Voges-Proskauer (MR-VP) agar as follows. Kovacs reagent was prepared by dissolving 5 g of p-dimethylaminobenzaldehyde in 75 ml of n-amy1 alcohol warmed to 50-60°C in a water bath. The mixture was cooled and 25 ml of concentrated HCl was added. The reagent, in an amber bottle with a glass stopper, was stored at 5°C. When the color of the reagent darkened, it was discarded and fresh reagent was prepared. The MR-VP agar was prepared by suspending 7 g of proteose peptone, 5 g of glucose, 5 g of sodium chloride and 10 g of agar in 1,000 ml of distilled water. The mixture was dissolved by heating and was sterilized at 121°C for 15 min. Yeast and mold counts were done as described in Standard Methods (48).

Numbers of *S. aureus* were determined by the direct plating method using Baird-Parker medium (Difco) as described by Mi- nor and Marth (37). Plates were examined for presence of black, shiny, convex colonies with or without clear surrounding zones after 48 h of incubation at 35°C. Three colonies thought to be *S. aureus* were randomly selected from plates with such colonies and were confirmed using the catalase (37) and tube coagulase tests (2).

The aerobic spore count (ASC) was determined on a heated (80°C for 10 min) and cooled suspension of cheese which was plated using Plate Count Agar (Difco); plates were incubated aerobically at 30°C for 48 h, as described by Thompson and Marth (54).

The lactic acid bacteria (LAB) count was obtained by using All Purpose Tween (APT) agar (Difco), as described by Meilinger et al. (36), but with the sodium azide concentration in the APT agar reduced to 0.0075% (wt/v), since the suggested level of 0.04% inhibited growth of lactic acid bacteria present in Cheddar cheese. Poured plates were incubated at 30°C for 48 h.

The nonstarter lactic acid bacteria (NSLAB) count was obtained as described by Turner and Thomas (57). Appropriate serial dilutions were plated using Lactobacillus Selection (LBS) Agar (Becton Dickinson Microbiology Systems, Cockeysville, MD). Lactobacillus Selection Agar is used for selective isolation and enumeration of lactobacilli in meats and other foods including dairy products (10). Plates were incubated anaerobically (BBL Gas Pak, Becton Dickinson Microbiology Systems, Cockeysville, MD) for 5 days at 30°C, and colonies were counted.
**RESULTS AND DISCUSSION**

**Composition of cheese**

The mean values of pH, moisture, salt, salt in moisture (S/M) content and fat in dry matter (FDM) are in Table 1. The moisture content in all salted cheeses of the three trials was less than the 39% maximum allowed for U.S. Cheddar cheese (9). The FDM content of all cheeses also met the U.S. legal specification for a minimum of 50% in Cheddar cheese (9). There were no marked differences in the moisture content of cheeses prepared with NaCl, KCl or mixtures of the two salts. As expected, the moisture content of unsalted cheeses was higher than that of salted cheeses, and in two of three trials exceeded 39%. Similar results wherein the moisture content of unsalted cheese exceeded 39% were reported by Thakur, et al (33) and Ibrahim et al. (18).

The mean values for S/M content were in the range of 3.9% to 4.7%. An S/M content of 4% to 6% is typical for Cheddar cheese (17,57), whereas the optimum is 4% to 5% (38). Lawrence, et al (29) reported that in New Zealand the S/M values for premium and first grade Cheddar were 4% to 6% and 2.5% to 6%, respectively.

Table 1 gives mean pH values for 3-day-old experimental cheeses. The pH values for unsalted cheeses were 0.07-0.14 unit less than those for salted cheeses. The higher numbers observed for the APC (Fig. 1) and the LAB (Fig. 2) of unsalted cheese, when compared to cheeses made with NaCl, KCl, or mixtures of the two (Fig. 1 and 2), probably caused the low pH values of unsalted cheeses since without added salt, bacteria in cheese continue to grow and ferment lactose to lactic acid which lowers the pH. The low pH values observed in this study for unsalted cheese agree with those reported by other investigators (49,53). The mean pH values (Table 1) of cheese salted with NaCl/KCl or KCl were 0.04 to 0.07 unit lower than of cheese salted with NaCl. These results agree with those of Koenig and Marth (23), who reported that Cheddar cheese prepared with NaCl/KCl mixtures had a pH value 0.07 + 0.02 unit lower than cheese salted with NaCl.

**Changes in the bacterial flora of experimental Cheddar cheese during ripening.**

**Aerobic bacteria.** The APC is generally indicative of overall changes in the microflora of cheese during ripening (54). Figure 1 gives the mean APC values for cheese of each salt treatment throughout the 36-week ripening period. Fresh cheese contained about $10^9$ bacteria/g regardless of the type and amount or ratio of salt mixture added to the cheese. These results are similar to those of Thompson and Marth (54) who reported $10^9$-g and $10^9$-g aerobic bacteria in fresh Parmesan cheese curd. The initial population of $10^9$-g remained relatively constant through the first 4-week period and then decreased by about 0.5, 1.0 and 2.0 orders of magnitude.
magnitude after 12, 24 and 36 weeks, respectively. There were no significant differences (P>0.05) in the APC of cheese at any particular age caused by various salt treatments. However, the overall mean APC of cheese salted with NaCl was 0.09 and 0.02 order of magnitude lower than of cheese salted with KCl or NaCl/KCl, respectively (Fig. 1). The APC of unsalted cheese was not significantly different (P>0.05) from that of cheese given the other treatments. However, values were consistently 0.12 to 0.26 order of magnitude higher than for salted cheeses through the 2- to 36-week period of ripening.

**Lactic acid bacteria.** Mean changes in numbers of LAB for cheese given various salt treatments are in Fig. 2.

![Figure 2. Lactic acid bacteria count of experimental Cheddar cheese ripened for 36 weeks and made with various levels of NaCl, KCl, or mixtures of the two salts. Each set of data represents the average of three trials. A, unsalted cheese; B, cheese with 1.42% NaCl; C, 1.57% NaCl; D, 1.68% KCl; E, 1.54% (1.03% NaCl + 0.51% KCl); F, 1.54% (0.77% NaCl + 0.77% KCl); G, 1.54% (0.51% NaCl + 1.03% KCl); H, 1.65% (0.69% NaCl + 0.96% KCl).](image)

Changes in LAB counts during ripening were similar to those for APC (Fig. 1). Fresh cheese contained 10^8 LAB/g, and this level was maintained through 4 weeks of ripening, after which the number decreased by about 0.5, 1.3 to 1.4, and 2.0 orders of magnitude at 12, 24, and 36 weeks of ripening, respectively. There were no significant (P>0.05) differences in LAB numbers in cheese given various salt treatments at any particular age. Unsalted cheese throughout the ripening period consistently yielded LAB numbers ranging from 0.10 to 0.20 log cycle higher than those of salted cheeses. The overall mean value was significantly different (P>0.05) when compared to that of cheeses made with NaCl, KCl, or their mixtures.

There were no marked differences between values of APC and LAB counts. This was expected because the lactococci, although fastidious in their growth requirements readily grow on Plate Count Agar. Dawson and Feagan (13) used tryptose glucose extract agar (7) with added skim milk, an early *Standard Methods* medium, to examine population trends in Cheddar cheese made with individual strains of *L. lactis* subsp. *lactis*, *L. lactis* subsp. *cremoris* and *L. lactis* subsp. *lactis* biovar *diacetilactis*. They found that almost the entire APC consisted of starter organisms up to 21 days after the cheese was made. Upon further aging of the cheese, numbers of *L. lactis* subsp. *cremoris* decreased rapidly but those of *L. lactis* subsp. *lactis* and *L. lactis* subsp. *lactis* biovar *diacetilactis* continued to constitute a high proportion of the APC until the later stage of ripening. In this study, we observed the overall mean populations of LAB (Fig. 2) were 0.08 to 0.26 order of magnitude higher than the APC (Fig. 1) at any given interval of testing. Perhaps the APT agar provided nutrients more suitable for recovery of LAB than did PCA.

**Nonstarter lactic acid bacteria.** The nonstarter lactic acid bacteria in Cheddar cheese made from pasteurized milk came from the factory environment, including air and equipment, since few, if any, survive milk pasteurization. These contaminants, considered as adventitious organisms, contribute to development of flavor during Cheddar cheese ripening. The nonstarter flora that affects Cheddar cheese ripening consists principally of lactobacilli (12,19,27,35), micrococci (13,35) and pediococci (16,26,57). In this study, we noted that lactobacilli predominated in the nonstarter lactic flora; this was independent of the amount and type of salt or salt mixture used to make the cheese (46). Unsalted cheeses also contained lactobacilli as the predominant component of the NSLAB flora (46). No heterofermentative lactobacilli were observed in unsalted cheese or in cheeses made with NaCl, KCl, or mixtures of the two salts (46).

The mean changes in NSLAB during cheese ripening are summarized in Fig. 3. Our results are similar to those of Law and Sharpe (26), Prentice and Brown (41), and Chapman and Sharpe (11) who reported that fresh cheese curd contains from 10^7 to 10^8 lactobacilli/g, and that they multiply in cheese, reaching 10^9/g to 10^10/g in 10 to 60 days. These numbers are maintained for 4 to 6 months and then decrease. In this study, lactobacilli were present in fresh cheeses at about 10^6/g (Fig. 3). Their numbers increased to
ripening (24 and 36 weeks) numbers of NSLAB were comparable for cheeses of the three trials, and ranged from 4.5 log CFU/g to 5.3 log CFU/g.

Aerobic spore count. Mean ASC values are in Fig. 4. The initial ASC of each treatment was about 3.7 log CFU/g. A small decrease occurred in numbers of spores during cheese ripening; 0.7 and 0.2 log CFU/g for all cheeses tested after the second and twelfth week, respectively. However, the spore number, about 3.7 log CFU/g, of cheeses ripened for 24 and 36 weeks was about the same as initially. Spore counts were not significantly different (P>0.05) within any given ripening period for cheeses made without salt or with NaCl, KCl or mixtures of the two salts. Our data (Fig. 4) also suggest there was no growth of aerobic spore formers in Cheddar cheese during its ripening. Thompson and Marth (54) noted aerobic spores at about 10^5/g in freshly made Parmesan cheese and the numbers remained relatively constant throughout the 14-month ripening period.

Coliform bacteria. Numbers of coliform bacteria in the cheese during ripening are reported in Tables 3 and 4. Coliform determinations were made with the Violet Red Bile Agar plate method (hereafter called plate method) and the tube method which employs selective liquid media and permits recovery of injured or stressed cells, if any are present. Cheese is one of the dairy products believed to contain stressed coliform bacteria (48). Coliforms were absent and <10/g by tube and plate methods, respectively.

![Figure 4](https://example.com/figure4.png)

**TABLE 2.** Mean levels of nonstarter lactic acid bacteria in experimental Cheddar cheese in relation to starter culture used.

<table>
<thead>
<tr>
<th>Trial</th>
<th>0</th>
<th>2</th>
<th>4</th>
<th>12</th>
<th>24</th>
<th>36</th>
</tr>
</thead>
<tbody>
<tr>
<td>1^-</td>
<td>4.44 ± 0.22</td>
<td>4.53 ± 0.22</td>
<td>7.75 ± 0.18</td>
<td>7.35 ± 0.22</td>
<td>4.47 ± 0.35</td>
<td>5.29 ± 0.45</td>
</tr>
<tr>
<td>2^-</td>
<td>4.26 ± 0.25</td>
<td>4.29 ± 0.30</td>
<td>7.47 ± 0.16</td>
<td>8.35 ± 0.24</td>
<td>5.23 ± 0.73</td>
<td>5.26 ± 0.29</td>
</tr>
<tr>
<td>3^-</td>
<td>4.73 ± 0.18</td>
<td>7.10 ± 0.41</td>
<td>8.06 ± 0.20</td>
<td>5.30 ± 0.55</td>
<td>5.19 ± 0.49</td>
<td>5.07 ± 0.27</td>
</tr>
</tbody>
</table>

^1Values are the averages of 7 cheeses salted with NaCl, KCl, or mixture.
^2Starter culture, a blend of *L. lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris*.
^3Starter culture, *L. lactis* subsp. *lactis*.
^4Starter culture, *L. lactis* subsp. *cremoris*.

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Growth of NSLAB in experimental cheeses was examined in relation to the S/M content. Lawrence and Gilles (28) reported that a low S/M content will not inhibit growth of NSLAB and that these bacteria can grow by using cheese constituents other than lactose, provided the temperature and other environmental conditions are suitable. In our study, cheeses with a low S/M content (<4%), including unsalted cheeses, did not have significantly higher (P>0.05) NSLAB counts than did cheeses with S/M in the range of 4% to 5%. The growth rate and the die-off of these organisms was similar among the various cheeses during the ripening process. The low temperature (3 ±1°C) at which experimental cheeses were stored may have played a role in controlling growth of NSLAB. Although not significant (P>0.05), the unsalted cheeses yielded slightly higher values for NSLAB than did salted cheeses. The overall mean value of NSLAB for unsalted cheeses was 9.3 x 10^4/g, whereas that for the salted cheeses was 5.8 x 10^4/g.

The mean changes in NSLAB in relation to starter culture used in the three trials are shown in Table 2. Cheeses of trial 2 were made with *L. lactis* subsp. *lactis* and had NSLAB counts of 1.8 x 10^4 and 3.0 x 10^4/g at 0 and 4 weeks, respectively, whereas the corresponding counts for trial 1 cheeses made with a blend of *L. lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris* were 2.8 x 10^4 and 5.6 x 10^4/g. In contrast, trial 3 cheeses made with *L. lactis* subsp. *cremoris* had counts of 5.4 x 10^4 and 1.1 x 10^5/g at 0 and 4 weeks, respectively. Thus, between 0 and 4 weeks of ripening, trial 3 cheeses prepared with *L. lactis* subsp. *cremoris* had higher NSLAB counts than did trial 1 cheeses prepared with a blend of *L. lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris* or trial 2 cheeses prepared *L. lactis* subsp. *cremoris*. The rapid die-off of *L. lactis* subsp. *cremoris* in cheeses of trials 1 and 3 and the possible persistence of *L. lactis* subsp. *lactis* in trial 2 cheeses must have produced several different environments for growth of NSLAB. At 12 weeks of ripening, numbers of NSLAB had decreased by three or more orders of magnitude in trial 3 cheeses, increased by one order of magnitude in trial 2 cheeses and decreased by 0.4 order of magnitude in trial 1 cheeses. At the later stage of ripening (24 and 36 weeks) numbers of NSLAB were comparable for cheeses of the three trials, and ranged from 4.5 log CFU/g to 5.3 log CFU/g.
in all experimental cheeses of trial 1 throughout the 36-week ripening period. In fresh cheeses of trial 2, coliform numbers were <1,000/g and <100/g by the tube and plate methods, respectively (Table 3). Cheese ripened for 12 weeks tested negative and <10/g, respectively, by the tube and plate methods.

Higher numbers of coliforms were noted in trial 3 cheeses than in those of trial 2. Also, differences in the numbers obtained by the tube and plate methods are more evident for trial 3 cheeses (Table 4). The average numbers of coliforms in fresh cheeses were 5,000-10,000/g and 900/g by the tube and plate methods, respectively. This demonstrates that the tube method employing the selective liquid medium recovered higher numbers than did the plate method. It is likely that the Violet Red Bile Agar medium provided a restrictive environment for growth of injured or stressed coliform bacteria. There was a 100-fold reduction in coliform numbers in unsalted cheese ripened for 12 weeks, as determined by tube and plate methods. The die-off possibly resulted from the more acidic environment (pH 5.01) in unsalted cheese than in cheeses made with NaCl (pH 5.19-5.22), KCI or NaCl/KCI (pH 5.13-5.17). Second, the competitive environment provided by presence of higher populations of aerobic (Fig. 1) and lactic acid bacteria (Fig. 2) in unsalted cheese than in cheeses made with NaCl, KCI or mixtures of the salts (Fig. 1 and 2) probably inhibited growth of coliform bacteria. Reduction of coliform numbers by a factor of about 5 to 10 also was noted for cheeses made with NaCl, KCI or mixtures of the salts and ripened for 12 weeks. Further ripening of cheeses revealed that coliform numbers gradually decreased to an average of 10/g to 99/g and 20/g at 24 weeks when tested by the tube and plate methods, respectively. Numbers in cheeses ripened for 36 weeks decreased further to negative and <10/g by the tube and plate methods, respectively. At any given ripening time, with the exception of unsalted cheese, there were no marked differences in levels of coliform bacteria observed for cheeses made with NaCl, KCI or mixtures of the two salts. Escherichia coli was not detected in any of the cheeses of trials 1, 2 and 3 throughout the 36 weeks of ripening.

Staphylococcus aureus. None of the cheeses contained coagulase-positive staphylococci when tested at the 0, 2, 4, 12, 24 and 36-week intervals during ripening. Koenig and Marth (23) prepared stirred-curd Cheddar cheese from milk artificially contaminated with S. aureus. The curd was salted with either NaCl or a mixture of NaCl/KCI to achieve a final salt concentration of approximately 2.4% or 1.2%. These researchers found that the salt mixture did not enhance the potential for formation of enterotoxin, and, in fact, cheeses salted with NaCl/KCI had a smaller number of S. aureus than did cheeses salted with NaCl.

### TABLE 3. Changes in coliform populations of trial 2 experimental Cheddar cheese during storage.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Actual NaCl, KCl or Mixture Conc. (%)</th>
<th>Coliforms/g (Tube Method)</th>
<th>Coliforms/g (VRB/Plate)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Initial 12 wk*</td>
<td>Initial 12 wk*</td>
</tr>
<tr>
<td>A</td>
<td>Unsalted, 0.14</td>
<td>100-999</td>
<td>&lt;1</td>
</tr>
<tr>
<td>B</td>
<td>NaCl, 1.42</td>
<td>100-999</td>
<td>&lt;1</td>
</tr>
<tr>
<td>C</td>
<td>NaCl, 1.57</td>
<td>10-99</td>
<td>&lt;1</td>
</tr>
<tr>
<td>D</td>
<td>KCl, 1.68</td>
<td>100-999</td>
<td>&lt;1</td>
</tr>
<tr>
<td>E</td>
<td>Mixture, 1.52 [NaCl (1.01) + KCl (0.51)]</td>
<td>100-999</td>
<td>&lt;1</td>
</tr>
<tr>
<td>F</td>
<td>Mixture, 1.58 [NaCl (0.79) + KCl (0.79)]</td>
<td>10-99</td>
<td>&lt;1</td>
</tr>
<tr>
<td>G</td>
<td>Mixture, 1.53 [NaCl (0.51) + KCl (1.02)]</td>
<td>10-99</td>
<td>&lt;1</td>
</tr>
<tr>
<td>H</td>
<td>Mixture, 1.73 [NaCl (0.70) + KCl (1.03)]</td>
<td>10-99</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

*Coliform count at 24 and 36 weeks of ripening was <1 and <10/g by tube and VRB plate method, respectively.

### TABLE 4. Changes in coliform populations of trial 3 experimental Cheddar cheese during storage.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Actual NaCl, KCl or Mixture Conc. (%)</th>
<th>Coliforms/g, (Tube Method)</th>
<th>Coliforms/g (VRB/Plate)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Initial 12 wk 24 wk 36 wk</td>
<td>Initial 12 wk 24 wk 36 wk</td>
</tr>
<tr>
<td>A</td>
<td>Unsalted, 0.12</td>
<td>5000-9999 10-99 10-99</td>
<td>720 10 10 10</td>
</tr>
<tr>
<td>B</td>
<td>NaCl, 1.50</td>
<td>5000-9999 100-999 109-99</td>
<td>710 310 10 10</td>
</tr>
<tr>
<td>C</td>
<td>NaCl, 1.72</td>
<td>5000-9999 100-999 10-99</td>
<td>920 220 40 10</td>
</tr>
<tr>
<td>D</td>
<td>KCl, 1.76</td>
<td>5000-9999 100-999 10-99</td>
<td>1100 280 &lt;10 &lt;10</td>
</tr>
<tr>
<td>E</td>
<td>Mixture, 1.56 [NaCl (1.04) + KCl (0.52)]</td>
<td>1000-4999 1000-4999 10-99</td>
<td>700 220 20 &lt;10</td>
</tr>
<tr>
<td>F</td>
<td>Mixture, 1.64 [NaCl (0.82) + KCl (0.82)]</td>
<td>5000-9999 1000-4999 10-99</td>
<td>600 380 20 &lt;10</td>
</tr>
<tr>
<td>G</td>
<td>Mixture, 1.68 [NaCl (0.56) + KCl (1.12)]</td>
<td>5000-9999 1000-4999 10-99</td>
<td>1200 460 60 &lt;10</td>
</tr>
<tr>
<td>H</td>
<td>Mixture, 1.70 [NaCl (0.73) + KCl (0.97)]</td>
<td>5000-9999 1000-4999 10-99</td>
<td>1000 340 20 &lt;10</td>
</tr>
</tbody>
</table>
TABLE 5. Mean changes in yeast and maida counts of experimental Cheddar cheese during ripening.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Actual NaCl, KCl or mixture in cheese (%)</th>
<th>S/M (%)</th>
<th>pH</th>
<th>0</th>
<th>Yeast/g at Weeks of Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Unsalted, 0.1</td>
<td>0.27</td>
<td>4.99</td>
<td>20</td>
<td>90 230 100 10</td>
</tr>
<tr>
<td>B</td>
<td>NaCl, 1.42</td>
<td>3.91</td>
<td>5.13</td>
<td>&lt;10</td>
<td>20 30 30 20</td>
</tr>
<tr>
<td>C</td>
<td>NaCl, 1.57</td>
<td>4.35</td>
<td>5.13</td>
<td>&lt;10</td>
<td>&lt;10 10 &lt;10 &lt;10</td>
</tr>
<tr>
<td>D</td>
<td>KCl, 1.68</td>
<td>4.64</td>
<td>5.09</td>
<td>20</td>
<td>50 40 20 10</td>
</tr>
<tr>
<td>E</td>
<td>1.54 [NaCl (1.03) + KCl (0.57)]</td>
<td>4.27</td>
<td>5.08</td>
<td>&lt;10</td>
<td>30 &lt;10 &lt;10 &lt;10</td>
</tr>
<tr>
<td>F</td>
<td>1.57 [NaCl (0.77) + KCl (0.77)]</td>
<td>4.21</td>
<td>5.07</td>
<td>&lt;10</td>
<td>&lt;10 10 &lt;10 10</td>
</tr>
<tr>
<td>G</td>
<td>1.54 [NaCl (0.51) + KCl (1.03)]</td>
<td>4.25</td>
<td>5.06</td>
<td>20</td>
<td>30 30 20 10</td>
</tr>
<tr>
<td>H</td>
<td>1.65 [NaCl (0.69) + KCl (0.96)]</td>
<td>4.62</td>
<td>5.07</td>
<td>&lt;10</td>
<td>30 20 10 &lt;10</td>
</tr>
</tbody>
</table>

*The mean mold count was <10/g for unsalted and salted cheese treatments.

**Values are averages of results from 3 trials.

Yeast and mold. The mean changes in numbers of yeast and mold in experimental Cheddar cheese during ripening are shown in Table 5. Mold populations were <10/g for all cheeses when tested at 0, 4, 12, 24 and 36-week intervals during ripening. Numbers of yeast also were low, about <300/g and 100/g for unsalted and salted cheeses, respectively. Prentice and Brown (41) reported a maximum level of yeast at 5,000/g in Cheddar cheese. Occasionally yeast levels in Cheddar cheese can rise to as high as 10/g without any deleterious effect on the quality of the product (41).

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REFERENCES


