The Behavior of Selected Microorganisms During the Manufacture of High Moisture Jack Cheeses from Ultrafiltered Milk

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ABSTRACT

Whole milk was pasteurized and concentrated two times by ultrafiltration. Starter cultures, *Lactococcus lactis* ssp. *cremoris* and *Lactococcus lactis* ssp. *lactis*, were propagated in either reconstituted skim milk, two times UF retentate, or UF permeate, or a direct vat system was used for the starter culture. The cheese milk was simultaneously inoculated with starter culture and *Pseudomonas fragi* 4973, *Staphylococcus aureus* 196E, and *Salmonella typhimurium* var. Hillfarm. Control whole milk, UF control milk, inoculated whole milk, and inoculated UF milk were made into Monterey Jack cheese using traditional procedures. The process of cheese manufacture was followed by determination of pH, titratable acidity, and microbial population levels. The cheeses were stored for 6 mo and analyzed every month for percentage solids and microbial population levels. Generally, numbers of contaminant microbes increased at a similar rate during manufacture in all cheeses. During the 6-mo ripening period, bacterial starter culture population levels remained high, psychrotrophs declined slowly, *Staphylococcus* levels remained stable, and *Salmonella* populations decreased. No *Staphylococcus* enterotoxin was detected by reverse passive latex agglutination assay.

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(Key words: pathogenic microbes, Monterey Jack cheese, ultrafiltered milk)

Abbreviation key: RSM = reconstituted skim milk, 2x = concentrated twofold by volume.

INTRODUCTION

Ultrafiltration technology has made rapid inroads into the dairy industry. Because of its benefits, it is employed for cheese manufacture (19), especially the Maubois, Mocquot, and Vassal process (23). The benefits are increased cheese yields, greater efficiency in plant space utilization, less starter culture required, less coagulant needed, reduced whey, and facilitated use of continuous production (1, 19).

Pathogenic and spoilage microbes can proliferate during cheese manufacture if they are contaminants of the cheese milk. This has been demonstrated for *Staphylococcus aureus* (35, 36, 38, 40), as well as for *Salmonella typhimurium* (12, 15, 24, 25). Similarly, the behavior of psychrotrophic spoilage microbes has been well documented (6), although interest has centered more on growth in milk, flavor and enzymatic alterations of milk, and cheese yield than on growth during actual cheese manufacture.

More recently, there has been interest in survival of microbes during the UF or reverse osmosis concentration, processing, and storage (4, 7, 8, 16, 17, 26, 27, 39) and in the retentates (8, 9, 14, 29, 37). These retentates have a different component balance compared with milk or milk equivalents (10, 13). Furthermore, the cheeses produced from such milks may require different manufacturing conditions and can possess different physicochemical properties (21, 28, 34). This could affect the behavior of contaminating microbes during cheese manufacture. The increased solids content of retentate versus whole milk means that
the lactic acid starter culture bacteria must produce large quantities of acid to counteract the greater buffering capacity of the retentate. If acid production is insufficient, the possibility exists for undesired microbes to reproduce in the retentates without the inhibition afforded by normal acid development (28).

Thus, the object of this study was to compare the behavior of spoilage and pathogenic microbes in high moisture Monterey Jack cheese made from whole milk with high moisture Monterey Jack cheese made from UF milk concentrated twofold by volume (2x).

MATERIALS AND METHODS

Pasteurization

Three sets of experiments were performed. For the first set of 12 vats, whole milk was HTST pasteurized (Cherry Burrell Co., Superplate SAS-75; Louisville, KY) at 72.8°C for 16 s. The milk for the following two sets of 8 and 12 vats was low temperature, long time pasteurized at 62.8°C for 30 min.

Ultrafiltration

Ultrafiltration equipment consisted of an Osmonics unit (SHA-41x-1SS316-SAN; Osmonics, Inc., Minnetonka, MN) equipped with a polysulfone spirally wound membrane possessing a molecular weight cutoff of 20,000. The milk was concentrated twofold by volume at 50°C. The inlet pressure was .59 MPa (85 pounds per square inch gauge), and the outlet was .41 MPa (60 pounds per square inch gauge).

Experimental Conditions

Set 1. The first set of experiments consisted of 12 vats of Monterey Jack cheese. Each vat contained 227.3 kg (500 lb) of whole milk or 227.3 kg (500 lb) of 2x UF milk. The starter culture used was Miles D70 and D30 (Chr. Hansen’s Laboratories, Inc., Milwaukee, WI). The coagulant was calf chymosin (Pfizer Hi-C, Milwaukee, WI). The experimental vats were inoculated with Pseudomonas fragi 4973, Staphylococcus aureus 196E, and Salmonella typhimurium var. Hillfarm at levels approximately $1 \times 10^6$ cfu/ml of milk.

Set 2. The second set of experiments consisted of 8 vats of Monterey Jack cheese. Each vat held 6 L of whole milk or 2x UF milk. The starter culture was a 14-h culture of Lactococcus lactis ssp. lactis Cg and Lactococcus lactis ssp. cremoris EB1 propagated in reconstituted skim milk (RSM) or 2x UF retentate. The coagulant was calf chymosin (Pfizer Hi-C, Milwaukee, WI). The vats were inoculated with Staph. aureus 196E and S. typhimurium var. Hillfarm at levels approximating $1 \times 10^4$ cfu/ml of milk. Both Staph. aureus 196E and S. typhimurium var. Hillfarm were propagated in sterilized RSM overnight at 37°C.

Set 3. The third set of experiments consisted of 12 vats of Monterey Jack cheese. Vat capacity was 6 L. Whole milk or 2x UF milk was used to make cheese. The starter culture was a 14-h culture of L. lactis ssp. lactis Cg and L. lactis ssp. cremoris TR propagated in RSM or 2x UF permeate. The coagulant was a chymosin-rennet mixture (90% chymosin calf rennet; New Zealand Milk Products, Petuluma, CA). The experimental vats were inoculated with Staph. aureus 196E and P. fragi 4973 at levels approximating $1 \times 10^4$ cfu/ml of milk. Both Staph. aureus 196E and P. fragi 4973 were propagated in sterilized RSM overnight at 37 and 30°C, respectively.

Cheese Manufacture

Monterey Jack cheese was manufactured according to a make schedule given by Kosikowski (18). Samples were taken at pasteurization, starter addition, inoculation with contaminants, renneting, cutting (both curds and whey), end of cooking (both curds and whey), washing, salting, hooping, and at 1, 7, 30, 61, 92, 122, 152, and 183 d of storage at 4.5°C.

Analyses

Microbial Analyses. Microbial levels for total population and psychrotrophic populations were determined by spread plating on standard plate count agar, incubating at 32°C for 48 h and at 7°C for 10 d, respectively, and counting colonies (32).

The Staph. aureus and S. typhimurium population levels were determined by spread plating on Baird-Parker and xylose-lysine desoxycholate agar, incubating at 35°C for 48 h and at
37°C for 24 h, respectively, and counting colonies (32). Difco (Detroit, MI) dehydrated culture media were used for all microbial evaluation analyses.

Reverse passive latex agglutination assay, sensitive to .5 mg/g, was utilized to detect staphylococcal enterotoxins (SET-RLPA; Denka Seiken Ltd., Japan for Oxoid Diagnostic Reagents, Hampshire, Engl.)

Physicochemical Analyses

The cheese-making process was monitored and controlled according to pH measurements (Orion 901 or SA 520, Boston, MA) and titratable acidity measurements by back titration to pH 8.20 to 8.30 (20, 22). This method was chosen over the phenolphthalein method for consistency (elimination of color judgment) and because different vats were colored with small amounts (10 ml) of a nontoxic vegetable dye (McConnick and Co., Inc., Baltimore, MD) for identification purposes. Percentage solids for milks, retentates, and permeates were determined by oven-drying according to AOAC (2) and by Brabender analysis. Samples were taken as previously described. Protein was determined by Kjeldahl (2), and fat was determined by the Mojonnier method (3).

Statistical Analysis

Statistical analysis of the data was performed using Statistical Analytical System (SAS®, 1986, Version 5.18, Cary, NC). General linear models appropriate to the data were used to determine the effects of month of manufacture, contaminant, level of contaminant concentration of the milk, process step, and starter. An unbalancing of data occurred due to the experimental design, degrees of freedom, and limitations placed on use of pathogens in the dairy processing facilities. Because starter culture and month of manufacture were confounded, they were combined and used as a block to eliminate their influence. After blocking, each remaining variable was sorted and analyzed using an analysis of variance, a general means statement, and a Tukey’s mean comparison and a Scheffé’s mean comparison (5, 31).

The statistical analyses were chosen to test the effect of these variables: bacterial strain, level of contamination, ultrafiltration-concentration of the milk, and process step. In the following discussion, results from the Tukey’s mean comparison and Scheffé’s mean comparison are used, but there was essentially no difference in results obtained other than a slightly greater confidence interval around the means for the Scheffé analysis. The Tukey test attempts to minimize Type 1 error. The Scheffé test is the most conservative mean comparison test (5) that also tries to minimize Type 1 error (33), but it may have a higher Type 2 error rate. Because there was no difference, the statistical results reported are from the Tukey analyses. All determinations of statistical difference were set at .05.

RESULTS AND DISCUSSION

High moisture Monterey Jack cheese was chosen for the experimental system because the combination of low acid development coupled with high moisture body creates conditions more conducive to microbial survival. Park et al. (24) indicated that high moisture, high pH, and pressing at room temperature were factors allowing high population levels of microbes and an increase in numbers of S. typhimurium during manufacture and ripening of Cheddar cheese. High moisture Jack cheese meets these requirements of high moisture and high pH because the standards of identity (11) allow moisture content not less than 44% and not more than 50%, which should enhance the growth of undesirable microbes.

Process Step. The data from all sets of experiments indicated a general increase in total microbial numbers over time during manufacture of cheese for both starter culture and contaminants. This was consistent with previously published data (24). During ripening (steps 11 to 18), the numbers of total microbes remained relatively stable and high at $1 \times 10^8$ cfu/g (Figure 1). Population levels of psychrotrophs, as represented by P. fragi 4973, indicated a gradual decrease over 6 mo (Figure 2). The Staph. aureus was better able to survive cheese ripening, and population levels remained stable or declined slowly during ripening (Figure 3). The S. typhimurium appeared more sensitive to acid development and salt during ripening because numbers declined during the 6-mo ripening period (Figure 4). This
PATHOGENIC MICROBES IN MONTEREY JACK CHEESE

Figure 1. Total population of microbes in high moisture Monterey Jack cheese made from whole milk or twice ultrafiltered retentate during manufacture and storage.

The process steps were 1) pasteurized milk, 2) retentate, 3) starter addition, 4) contaminant addition, 5) renneting, 6) cutting (curds and whey), 7) draining, 8) washing, 9) salting, 10) hooping, 11) 1 d, 12) 7 d, 13) 30 d, 14) 61 d, 15) 92 d, 16) 122 d, 17) 152 d, 18) 183 d.

Symbols: whole milk, no contamination (A); whole milk, low contamination (O); whole milk, high contamination (©); retentate, no contamination (A); retentate, low contamination (O); retentate, high contamination (©).

was similar to the cases reported by Goepfert et al. (12). The general behavior for all microbes was strongly dependent on proper acid development by the starter culture and addition of salt.

These microbiological results were not unexpected. The pH of cheese drops only slightly during Monterey Jack cheese manufacture because there is no acid ripening step as in the manufacture of Cheddar or Mozzarella cheese. The minimum curd pH is usually attained around 1 d to 1 wk, after which it slowly increases. This decrease and then increase in pH were also seen in this study (data not shown). Thus, there was a decrease in S. typhimurium and P. fragi populations over time but not during manufacture itself. The lactic acid bacteria used as starter cultures are more resistant to acid, as are the Gram-positive Staph. aureus. Thus, Staph. aureus demonstrated better survival (Figures 1 to 4).

Statistically, differences in bacterial response among the process steps were found (Table 1). For the total population levels (Figure 1) without inoculation with contaminants, differences in means were observed during storage. The mean population levels in the UF milk cheeses were significantly higher at 30 through 152 d of storage compared with salting and hooping. This was not the case with whole milk cheeses, which implies that the salt and acid were not as effective in controlling numbers of bacteria as in whole milk. With whole milk, population numbers attained a maximum mean after washing and did not increase significantly after this processing step.

The microbial responses were very similar in both cheeses inoculated at the 1 x 10^6 cfu/ml level, except for the point in time when a decline in numbers occurred. A decrease
The process steps were 1) pasteurized milk, 2) retentate, 3) starter addition, 4) contaminant addition, 5) renneting, 6) cutting (curds and whey), 7) draining, 8) washing, 9) salting, 10) hooping, 11) 1 d, 12) 7 d, 13) 30 d, 14) 61 d, 15) 92 d, 16) 122 d, 17) 152 d, 18) 183 d.

Symbols: whole milk, no contamination (Δ); whole milk, low contamination (O); whole milk, high contamination (O); retentate, no contamination (Δ); retentate, low contamination (O); retentate, high contamination (O).

total numbers after salting and hooping was observed compared with 1 to 7 d of storage with whole milk. However, this decline was noted later when comparing the mean levels in UF cheese at 30 d of storage with those at 61 through 183 d of storage.

Total microbial populations declined significantly in the whole milk cheeses inoculated with contaminants at $2 \times 10^6$ cfu/ml of milk when comparing the salting through the 30-d steps with 122 through 183 d of storage (Figure 1). The populations in UF cheese achieved a maximum mean at washing, and populations did not significantly change throughout the rest of processing and storage.

Although P. fragi 4973 was not inoculated into control vats, naturally occurring psychrotrophs were present in the cheeses (Figure 2). In UF milk cheeses, means were significant from hooping to 122 d of storage compared with renneting through salting. Population levels in these cheeses declined in the later phases of storage, d 122 to 183, compared with mean numbers at d 1. On the other hand, whole milk cheeses showed increases after washing compared with the starter step but with many fewer steps, indicating that acid and salt control numbers of psychrotrophs in whole milk cheese versus UF milk cheeses. This was further supported by the greater frequency of significant declines in mean population levels seen over storage from d 122 through 183 compared with mean population numbers in UF cheeses at washing through 91 d of storage.

The differences in the uninoculated psychrotroph data are very strongly reinforced by the P. fragi inoculated at $1 \times 10^6$ cfu/ml of
milk. Here, the decrease in significant mean numbers was observed from salting through 183 d of storage compared with washing through 91 d of storage. This decrease in mean numbers was delayed to 122 to 183 d of storage compared with draining through 91 d of storage. The whole milk cheeses exhibited an earlier and longer lasting increase in mean numbers than the UF cheeses at this inoculation level.

At the high contamination rate (1 x 10^6 cfu/ml), the numbers of contaminating *P. fragi* overcame the barriers of salt and pH (Figure 2). Mean population levels were at high levels in both cheeses at the beginning and sporadically increased for whole milk cheese. However, for UF cheeses, there was a wave of significantly higher mean numbers at 91 to 152 d of storage compared with mean numbers at contamination through 1 d of storage, which implied that numbers increased in UF cheese over storage but that this was not true with whole milk cheeses.

The very low levels of *Staph. aureus* (Figure 3) contamination in the uninoculated control vats were included in the analyses. A likely source of the contamination, other than human contact with the product, is the UF unit, which correlated with higher numbers in UF cheeses versus whole milk cheeses. The significant increase in mean numbers appeared earlier in processing and was of shorter duration for the whole milk cheeses, hooping through 7 d of storage compared with pasteurization through salting, than it was for the UF cheese. Interestingly, the UF cheese exhibited a delayed response in significantly higher mean numbers, but this response extended longer, that is 1 d through 122 d, compared with pasteurization through salting. Furthermore, significant decreases in numbers were seen for storage times of 71 to 183 d compared with mean numbers at 1 d for whole milk cheeses.

At the 1 x 10^4 cfu/ml level of inoculation for *Staph. aureus*, no significant differences were found in mean analyses results from hooping through 183 d of storage compared with washing through 183 d of storage. Means were significantly greater for the whole milk cheeses for draining through 183 d compared with contamination through draining. This differed from the UF cheese, for which the significantly higher means were seen only between 1 d and 61 d of storage compared with contamination through draining. Thus, *Staph. aureus* in whole milk cheeses attained higher levels sooner, and these levels held longer than in UF milk cheeses.

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The converse was true in the cheeses made from milks or retentates inoculated with *Staph. aureus* at the high level (1 × 10⁶ cfu/ml). In this case, the means were significantly greater for the whole milk cheeses from draining through 183 d of storage compared with contamination and renneting. In contrast, the UF cheeses had significantly higher means from draining through 183 d of storage compared with contamination through salting. This indicated that numbers were significantly lower for longer time with regard to process step for the UF cheeses.

The results for the *Salmonella* (Figure 4) in the uncontaminated, control cheeses were all the same. There were no differences among process steps because none of the cheeses had *Salmonella* contamination.

At a *Salmonella* contamination rate of 1 × 10⁴ cfu/ml of milk, the UF cheeses exhibited a significant drop in mean population of *Salmonella* from 7 d through 183 d of storage compared with contamination through 30 d of storage. Apparently, *S. typhimurium* var. Hillfarm was more sensitive to salt and acid than *P. fragi*, judging from the differences in decline observed between the two species.

At the high *Salmonella* contamination level of 1 × 10⁶ cfu/ml, a significant increase in mean numbers during processing through cutting was observed. A leveling off in numbers of *Salmonella* was indicated by an area of no significant differences in means between draining through 1 d of storage versus salting through 61 d of storage. This was followed by a general decline in mean numbers from 91 to 183 d of storage compared with draining through 61 d of storage. Whole milk cheeses showed a significant decline starting at 91 d of storage compared with UF cheeses, which started to significantly decline in mean numbers at 122 d of storage; otherwise, the response was the same.

Ultrafiltration. The main objective of the investigation was to determine any alterations in the response of the spoilage and pathogenic microbes during cheese manufacture based on the UF milk variable. Analyses of the data indicated that UF concentration had little effect on total population levels. However, it did appear to play a role in the response of *Staph. aureus, P. fragi* 4973, and *S. typhimurium* (Table 1).

It was postulated that the increased buffering capacity of the UF milk would inhibit the pH drop during processing and allow for greater proliferation of contaminants than in the control whole milk. The variable of UF milk or whole milk appeared to influence the decrease in pH for only a short time, because all pH were in the range of 4.9 to 5.4 within 1 wk except for the 2 vats in the first set of experiments with inactive starter culture. A slight difference in final pH was noted. The UF milk cheeses had a slightly higher pH.

Statistical analyses indicated that UF played a significant role in the general linear model (Table 1) and ANOVA (data not shown). When evaluating the bacterial population levels as a function of UF, the regression yielded generally low R² values of .2 to .6 (data not shown). Starting with total counts of the means deemed significantly different by both Tukey and Scheffé mean comparisons, a general trend in the high level inoculated cases was observed (data not shown). After salting through 6 mo, the UF cheeses had higher mean bacterial levels than the whole milk cheeses. This could be the result of several factors. The whole milk may allow attainment of higher populations during manufacture. The UF retentates may be a suboptimal growth medium. Buffering capacity due to higher solids in the UF retentates may not allow for a pH drop as rapid as in whole milk cheeses, which may allow more bacteria to survive upon ripening. The UF cheeses have higher moisture, a result of the buffering capacity and acidity development affecting syneresis. This would in effect dilute the salt concentration, which in turn may be less inhibitory than in the whole milk cheeses. The *P. fragi* levels were higher through manufacture in the whole milk cheeses, but later during storage the population levels of *P. fragi* in UF cheeses were higher than those in whole milk cheeses. This was more evident in the cheeses made with a 1 × 10⁴ cfu/ml contaminant level than at the higher 1 × 10⁶ cfu/ml level. This finding was paralleled by the uncontaminated cheeses. In this case, the psychrotrophs present as natural contaminants increased to levels of 1 to 2 log cycles higher than those of the UF milk during manufacture through 3-mo storage. Subsequently, the levels of naturally present psychrotrophs in the UF milk cheeses exceeded the levels in the whole
milk cheeses. A possible explanation for this finding is the additional thermal treatment of the milk afforded by UF concentration of the milk at 50 to 52°C. Other researchers have found that UF at 50°C eliminates or reduces microbial populations (7, 8, 9, 16, 17, 26, 27). This could delay the attainment of the higher levels in UF cheeses versus whole milk cheeses seen in the experiments using milk deliberately contaminated with *P. fragi* 4973.

Similarly, *Staph. aureus* followed the pattern of the total population levels and *P. fragi* at high level inoculum, 10⁶ cfu/ml (data not shown). From cutting through 1 d of storage, the levels were higher in the whole milk cheeses. After 30 d of storage, the levels in the UF milk cheeses increased by 2 log cycles. The findings at the lower level inoculum (1 × 10⁶ cfu/ml) differ. Here, in each case from draining through storage, the mean population level was significantly higher by approximately 1 to 2 log cycles in whole milk cheeses compared with their UF milk cheese counterpart. A possible explanation for this reversal of levels found in the two types of cheese could relate to the competitive elimination of *Staph. aureus*. In other studies on growth of pathogens and spoilage microorganisms in UF retentates (9), *Staph. aureus* was not a particularly good competitor, exhibiting slower growth in milk and retentate than other microorganisms. However, *Staph. aureus* persisted for long periods of time and was more resistant to the thermal effects of dairy UF processing. Perhaps at lower inoculum levels and in competition with lactic acid bacteria and two other contaminant microorganisms, the staphylococci can only increase to higher levels when the growth medium is more favorable. Whole milk may be a preferred growth menstrum compared with 2× UF retentate.

Also of interest were the levels of *Staph. aureus* in the uninoculated control vats. Although no *Staph. aureus* was added to these vats, it was still isolated and recovered on Baird-Parker agar. The levels were only slightly higher and only became significantly higher in the UF cheeses after 30 d of storage and then only at very low R² values. This slightly higher level found in UF milk cheeses has two probable bases. First, microorganisms from postpasteurization contamination of the milk would be concentrated during UF. Second, the UF unit and associated connections could be a source of very low numbers of contaminants despite strict adherence to manufacturer’s instructions on cleaning and sanitizing procedures (Monarch Chemical Division of H. B. Fuller Co., Minneapolis, MN).

The data concerning *S. typhimurium* (data not shown) at high inoculum levels are consistent with the behavior of the other test microbes used. Of the differences determined to be statistically significant by the model, the Tukey and Scheffe analyses of the means indicate that whole milk cheese had higher numbers at salting and hooping, whereas UF milk cheeses had higher mean populations during storage. At lower levels, no difference was noted between the UF and whole milk cheeses because fewer repetitions were done.

Inferences about the behavior of *S. typhimurium* could be drawn from research conducted using *Escherichia coli* (14, 29, 30), coliforms (16), or from *P. fragi* in the present research based on growth characteristics in milk retentates (9), but to state that *S. typhimurium* would behave as *P. fragi* at low inoculum would be speculation.

**Inoculum Level.** Inoculum level of the contaminants was significant by the general linear model (Table 1). All tests showed that the milks had the same bacterial levels prior to contamination. The levels differed through manufacture for the contaminants; that is, the control vats were lower than the low inoculum vats, which in turn were lower than the high inoculum vats. The trend of differences in the levels of the inocula became less distinct during storage. For total population levels during storage, sometimes the control would equal the low inoculum or the high inoculum vats or the low and high level vats were the same statistically and both differed from the control. For the *P. fragi* levels in cheeses made with whole milk, a trend was seen when the levels differed over manufacture, but during storage the control and low inoculum cheeses were the same. At the same time, the low and high level inoculum cheese differed. In the UF milk cheeses, the inoculum level showed a similar trend to the whole milk cheeses.

The *Staph. aureus* inoculum levels differed significantly at almost every process step, regardless of whether the milk was ultrafiltered or not. The period between hooping and
3-mo storage was the only time that inoculum levels did not differ significantly for UF and unfiltered milks (other than similar lack of *Staph. aureus* prior to contamination or in the case of whole milk cheeses for which the low and high inoculum trials exhibited the same population levels). Whole milk appeared to be a slightly better growth medium than UF milk because the low level inoculum was able to grow up to the same level as the high level inoculum during manufacture. However, despite the high levels of *Staph. aureus* in the experimental vats, enterotoxin was not detected by a reverse passive latex agglutination assay sensitive to .5 mg/ml or g.

The *S. typhimurium* population levels were the same before contamination. No *Salmonella* was present. Throughout manufacture up to 30 d of storage, the levels in the control vats, low inoculum, and high inoculum vats differed. At 30 d, the control vat levels of zero isolated/100 g equaled the low inoculum vat levels. However, this is statistical equality, not an indication that there was no *Salmonella* in the low inoculum vats. *Salmonella* was recovered from the low level inoculum cheeses up to 4 mo, after which none could be isolated. *Salmonella* levels in the high inoculum cheeses declined by the same amount as in the low level inoculum vats. *Salmonella* was recovered from the low level inoculum cheeses up to 4 mo, after which none could be isolated. *Salmonella* grew to greater numbers during manufacture than *Staphylococcus* when inoculated at the same level, regardless of whether the medium was whole or UF concentrated milk.

**Physical Analyses.** Acid development affected the final percentage total solids. Generally, the whole milk cheeses had a higher percentage total solids, i.e., lower moisture, than the UF cheeses (data not shown). This was likely a consequence of decreased syneresis, resulting in more water entrapped in the curd. Although this extra moisture did not appear to alter the response of the bacteria in the cheese, it could affect the legality of the final product and its propensity to attack by molds. After the moisture levels in the first set of experiments were found to be on the high side, the manufacturing conditions were altered slightly. The curd was cut smaller and cooked 10 min longer to improve syneresis by increasing surface area and heat. Even when this was done in the second and third set of experiments, the UF cheeses always had a higher moisture level.

The starter culture propagation media affected the cheese made but not the microbial population levels. The direct vat system cultures used with set 1 gave mixed results. Acid development was sometimes inconsistent, a trait that correlated to UF milk. For set 2, the starter propagated in 2x UF retentate showed marginally better acid development around .1 to .2 pH units compared with RSM. The buffering effect of the 2x UF retentate allowed greater numbers and activity of starter bacteria per unit of starter culture medium. For set 3, RSM-propagated starter yielded better acid development, which could only be countered by lengthening the draining time and holding the curd. This allowed the permeate culture to catch up to the RSM-propagated culture, but at the expense of time. In all cases, though, legal high moisture Monterey Jack cheese was produced (11).

**CONCLUSIONS**

Final analyses indicated that UF cheeses did not show the same characteristics of whole milk cheeses with regard to bacterial contamination. Ultrafiltration delayed bacterial re-
response time. Initially, the increase in population was slightly slower, but after 6 mo of storage the populations tended to be higher. This was true for \textit{P. fragi} and psychrotrophs, \textit{S. typhimurium}, and \textit{Staph. aureus}. The only exception appeared at low levels of inoculated \textit{Staph. aureus}. Here, UF cheeses showed lower population levels than control cheeses. This possibly relates to the delay in increase in populations during processing of UF cheese. The \textit{Staph. aureus} never achieved the level needed to persist at higher levels during storage than the control cheeses as did the other microbial contaminants. For total population, the sum of all bacteria present appeared to override any other factors assessed. No \textit{Staph. aureus} enterotoxin was found in either whole milk or UF milk cheeses using a reverse passive latex agglutination assay for enterotoxins A and D.

The variables of different process steps indicated a general increase in numbers through salting, followed by a stabilization period of slightly varying lengths, depending on the variables of UF concentration, the type of bacterial contaminant, and the level of contamination. At roughly 1 to 3 mo, population levels began to decline.

The type of bacterial contaminant was also important to response in the different cheeses. Both \textit{P. fragi} and \textit{Salmonella} grew better in whole milk and UF retentates during cheese manufacture than did \textit{Staph. aureus}. Although \textit{P. fragi} numbers were decreasing over storage, likely due to salt and acid effects, \textit{S. typhimurium} was affected to a greater extent. The \textit{Staph. aureus} did not grow as well as the other contaminants but was more resistant to the inhibitory effects of acid and salt in the cheeses.

The level of bacterial contaminants also played a role. A high inoculum introduced a level of contamination that overcame barriers of acidity and salinity in cheese. However, at lower levels of contamination, the hurdles could be more difficult to surmount, depending on bacterial species, UF concentration, and process step.

The physical analyses demonstrated that cheese manufacture from 2x UF milk had a slightly higher pH after 6-mo storage than cheese manufactured from whole milk. Furthermore, the cheese manufactured from 2x UF milk had a higher percentage moisture after 6-mo storage than cheese manufactured from whole milk.

With slight adjustments to the make procedures, quality cheese can be made from 2x UF milk. Although pathogenic bacterial and spoilage contaminants respond differently in production and storage of UF concentrated milk cheeses, problems can be minimized by following good manufacturing practices and hazard analysis critical control point monitoring for cheese manufacture.

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