ROLE OF ENTEROCOCCI IN CHEDDAR CHEESE: GROWTH OF ENTEROCOCCI DURING MANUFACTURE AND CURING[1]

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ABSTRACT

Eight lots of Cheddar cheese were manufactured to determine the microbiological response of two strains each of *Streptococcus faecalis* and *Streptococcus durans* when used as supplemental starters in combination with a commercial lactic culture. Each lot consisted of a control vat of cheese manufactured with the lactic starter only, and an experimental vat of cheese containing the lactic starter and one of the enterococcus strains. Combinations of two curing temperatures (7.2 and 12.8 C) and two early cooling treatments (air vs. brine cooling) were used for cheeses from each vat to determine environmentally-induced variability.

Growth patterns were monitored throughout the manufacture period up to the end of pressing, and during curing up to 6 months. Enterococcus populations showed little or no decrease when the cheeses were being pressed, whereas populations in control cheeses decreased over the same period. During curing, control cheeses cured at 7.2 C showed marked population decreases over the 6 months; those cured at 12.8 C showed a rapid decrease followed by an upsurge in population. Populations of S. *faecalis* in the experimental cheeses decreased only slightly, and S. *durans* showed almost no decrease. Generally, cheeses cured at 7.2 C showed the greatest numerical survival and there appeared to be no population differences caused by early cooling treatment.

Any industry that relies on viable organisms for product manufacture must cope with the inherent metabolic variability of these organisms. The manufacture of Cheddar cheese is as closely controlled as practicable for a bulk fermentative procedure which is monitored, in most instances, largely by human judgment. Culture inconsistency with regard to flavor and acid production, subtle changes in temperature of manufacture and curing and differences in cool-off time caused by various stacking methods provide opportunities for cheese variability from batch to batch. These factors may have a significant effect on the metabolic activities of both starter culture organisms and adventitious flora.

An organism less susceptible to environmentally-induced variability was added to cheese milk in an attempt to achieve greater uniformity among blocks from the same batch when exposed to selected variables. The Enterococcus group of the genus *Streptococcus* was selected because of its relative durability, including heat resistance *(24)*, and because it occurs in large numbers as adventitious cheese flora *(4)*. Similar studies have been conducted by a number of other workers *(2, 3, 5-9, 11, 12, 13, 15, 17, 18, 22, 26, 27, 29)*. There is conflict among their results, and generally microbiological, chemical, and organoleptic analyses were not integrated.

It was assumed that enterococci, when used as supplemental starters, would resist change to a greater degree than normal starter organisms in number and metabolic activity with corresponding changes in the microenvironment of the cheese. To test this hypothesis, chemical, microbiological, and organoleptic analyses were done. This paper, the first of a series, discusses the microbiological aspects of the use of enterococci as supplemental starters in Cheddar cheese.

MATERIALS AND METHODS

Manufacture of cheese

Eight lots of Cheddar cheese were manufactured in the Dairy Products Laboratory, Food Technology Department, Iowa State University. Each lot consisted of a control and an experimental vat of cheese each manufactured from the same bulk milk on the same day in two 2270-kg vats. Milk used for cheese manufacture was treated by heating to 62.8 C with no hold, followed by immediate cooling. The milk was stored at 4.5 C, usually overnight, before use. Cheeses were manufactured according to the schedule and procedure outlined by Wilson and Reinbold *(28)*.

Starters used

The enterococci used as starters for cheesemaking had been previously isolated from young Cheddar cheese by Clark and Reinbold *(4)* and screened for lipolytic and proteolytic activity by Dovat et al. *(10)*. These strains were concentrated and canned by a commercial culture manufacturer. Until use, the concentrated cultures had been maintained for several months at −196 C in liquid nitrogen. Each can contained 75 ml of concentrate with viable counts of appoximately 10^{10} cells/ml. Two strains of *Streptococcus faecalis* and two of *Streptococcus durans* were used throughout the experiment.

Experimental cheese cultures consisted of 1% commercial mixed-strain Cheddar cheese starter culture in combination with 75 or 150 ml of one of the frozen enterococcus concentrates per 2270 kg of milk. The frozen concentrates were thawed quickly and carefully dispersed in a small aliquot

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of cheese milk at 30 C immediately before addition to the vat. Control cheeses were made using 1% of the same commercial mixed-strain lactic culture for all eight lots. Lot designations are given in Table 1.

Treatment of cheese

Forty-pound blocks were pressed overnight (20 h) at approximately 21 C and then wrapped in Marathon foil-cellofoil wrappers (Marathon, Division of American Can Company, Neenah, Wisc.) and sealed with a Flexpress model R.L. 100 (D. L. Manufacturing Company, De Pere, Wisc.). Half of the blocks from each vat (control and experimental) were then cooled rapidly by immersion in 7.5 C brine for 5 days *(19, 20)*, and the other half were immediately placed in curing rooms to air cool. From each of these early cooling treatments, half of the blocks were cured at 7.2 C and the other half at 12.8 C.

Sampling

Samples taken during the manufacturing period were held at 10 C in sterile Whirl-Pak (Nasco Company, Fort Atkinson, Wisc.) containers for bacteriological analysis at the earliest convenient time (always within 2 h). Samples of curing cheese were taken aseptically and plated immediately. For bacterial counts, samples were taken during the manufacture period after starter addition, after rennet addition, after cook, after milling, and after pressing for 20 h. During the curing period, samples were collected at 5, 10, 30, 60, and 90 days and at 6 months.

Micobiological analysis

Total counts and enterococcus counts were made on all samples. The cheeses were prepared for plating as specified in *Standard Methods for the Examination of Dairy Products (1)*. For total bacterial counts, the proper dilution series was plated in duplicate in Eugonagar (Baltimore Biological Laboratories, Baltimore, Md.) and incubated at 32 C for 7 days. For enumeration of enterococci, similar dilutions of enterococcus cheeses and their corresponding controls were plated in Eugonagar and incubated at 45 C for 3 days. When control cheeses were plated at 45 C, there were no colonies evident at the same dilutions used to enumerate enterococci in the experimental cheeses. Consequently, it was assumed that colonies growing at 45 C resulted from the deliberate addition of enterococci.

RESULTS

Growth during manufacture

All eight lots exhibited similar growth and survival patterns during the make period. Figure 1 shows trends in total bacterial counts for each negative control cheese to the end of the pressing period. The heavy line is a composite representation of the overall response. In all instances, the greatest increase in numbers occurred between the addition of the rennet and the end of the cook period. After reaching their maximum, usually following cooking, numbers declined steadily to the end of the press period. The degree of population decline between milling and the end of the press period for all lots was from 35% to 94%, with an average decline of 73%.

Figure 2 shows growth and survival patterns of enterococci in the eight lots of cheeses made with a supplemental enterococcus starter in combination with

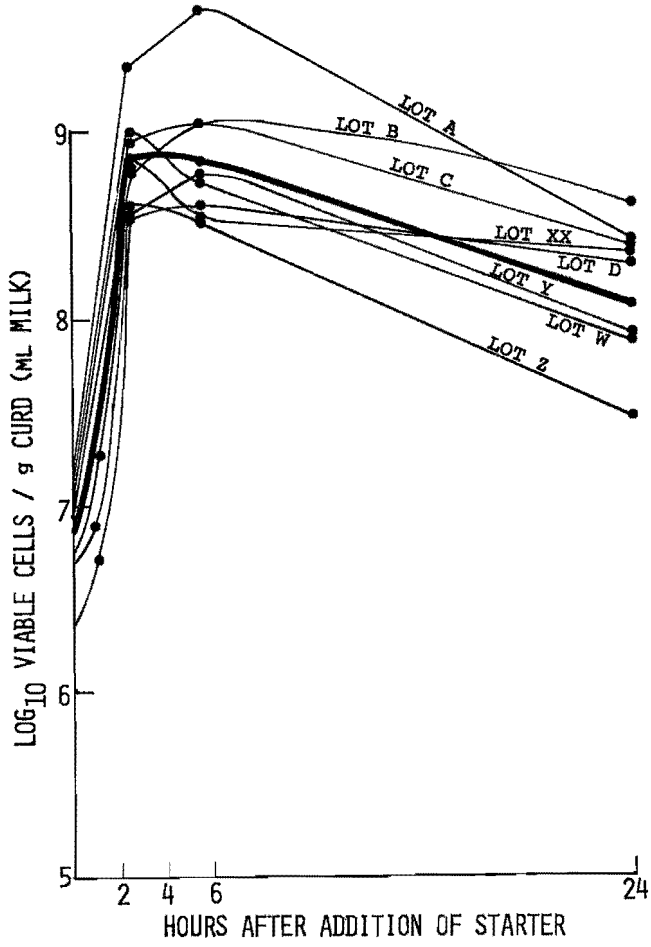

Figure 1. Total bacterial counts of control cheeses during manufacture.

a commercial lactic starter. The increase in population during the ripening period was not as great as that of the lactic streptococci in the negative control vats. In all lots, the maximum population of enterococci was reached at milling, and with the exception of Lot A, this level was either maintained or increased slightly until the end of the press. Two lots (C and W) decreased by 13.3% and 14.2%, respectively, in this period, and in the other six lots, enterococcus populations increased from 3.6% to 36.3%.

Total bacterial counts in cheeses made with enterococci (representing numbers of both lactic starters and enterococci) exhibited much the same pattern as the total counts in the control cheeses, with the exception of a less pronounced decline in numbers after milling.

Growth during curing

Survival patterns during curing at 7.2 C for all eight lots of control cheese are given in Figure 3. For simplicity, only the air-cooled cheeses are represented in the figure because the brine-cooled cheeses followed a nearly identical pattern. The heavy line is a composite representation of the overall response.

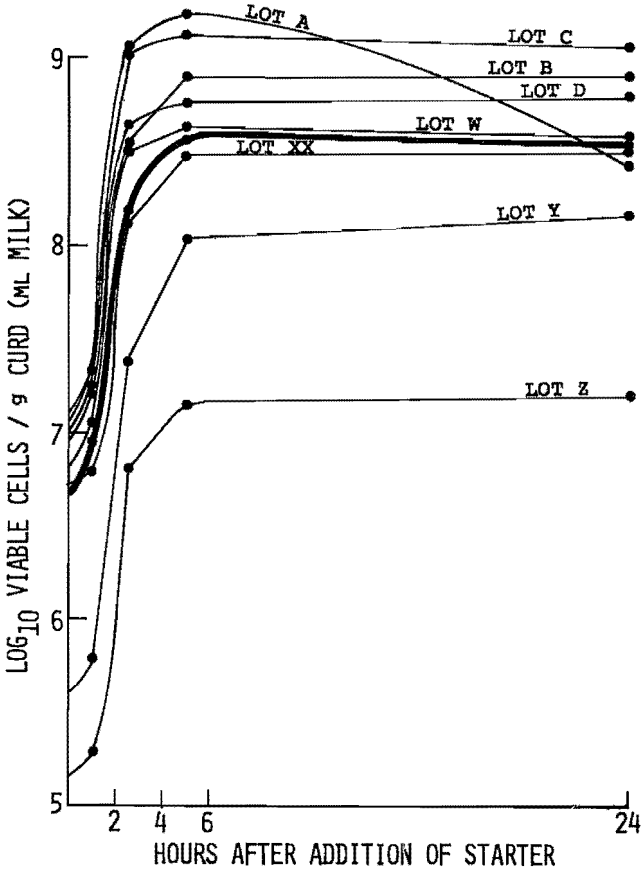

Figure 2. Enterococcus counts of experimental cheeses during manufacture.

There was a rapid decline in numbers initially, especially during the first 5 days of curing. Although some cheeses showed total bacterial count increases between 10 and 30 days, the population continued to decrease gradually. In all instances, the total population of the control cheeses decreased as much as 3.5-4.0 log units. Lots W and XX, however, increased in count after 90 days of curing.

The curing-period growth response of control-cheese flora, air-cooled and cured at 12.8 C is presented in Figure 4. As with cheeses cured at 7.2 C, there was an initial rapid decline in population. Starting from 10 days to as long as 90 days, however, this decline was followed by a rather abrupt increase in numbers. Although the general trend of the eight lots is the same, again there were wide variations in the size of population and in the stage at which the increase occurred.

Figure 5 shows the growth of enterococci during curing in the four lots of cheese made with S. *faecalis* as a supplemental starter. Data presented includes cheeses, air-cooled, cured at both 7.2 C and 12.8 C. There was a gradual decrease in enterococcus count over the 180-day period, with the smallest decrease of 0.7 log units occurring in Lot B when cured at 7.2 C. The greatest decrease of about 1.3 log units was observed in Lot D cured at 12.8 C. When compared with the total counts in control cheeses, it is obvious that S. *faecalis* survives the environment of curing cheese better than normal starter lactic streptococci. Survival at 12.8 C, however, is not quite as great as at 7.2 C.

Enterococcus counts in cheeses made with S. *durans* as the supplemental starter are given in Figure 6. The population decrease was markedly less than that exhibited by S. *faecalis*. Up to the 90-day sampling period, counts in all instances maintained essentially constant numbers. Between 3 and 6 months, there was a slight numerical decrease except in Lot Y at 12.8 C and Lot XX at 7.2 C. In Lots W and XX, which were manufactured with 150 and 75 ml of S. *durans* 15-20 concentrate, respectively, curing temperatures influenced the rate of survival; cheeses cured at 12.8 C had somewhat lower counts than those at 7.2 C after 180 days. The two lots made with S. *durans* 9-20 (Lots Y and Z) exhibited the reverse trends.

When an analysis of variance was done on the enterococci populations in the experimental cheeses, only a slight significant difference in population was

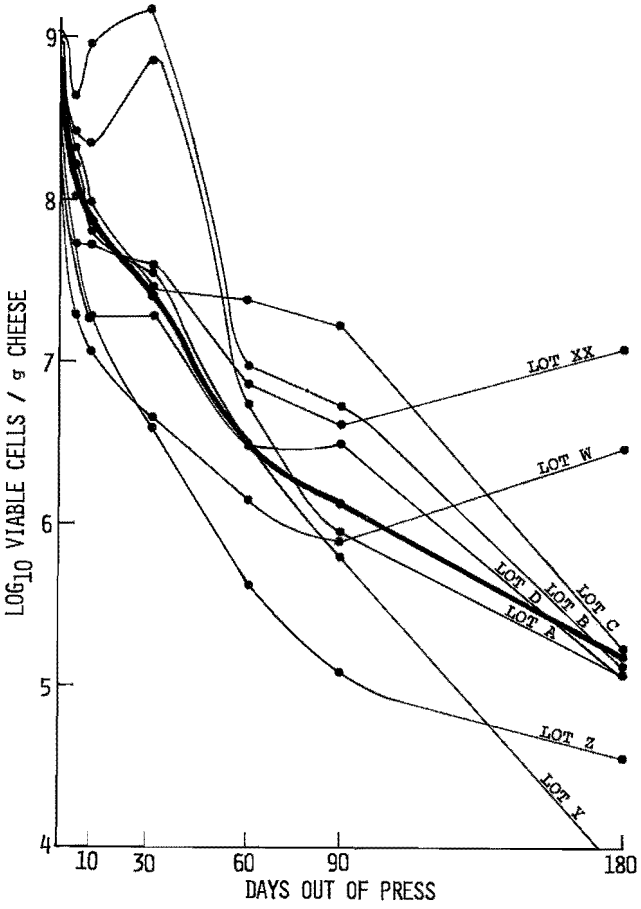

Figure 3. Total bacterial counts of air-cooled control cheeses cured at 7.2 C.

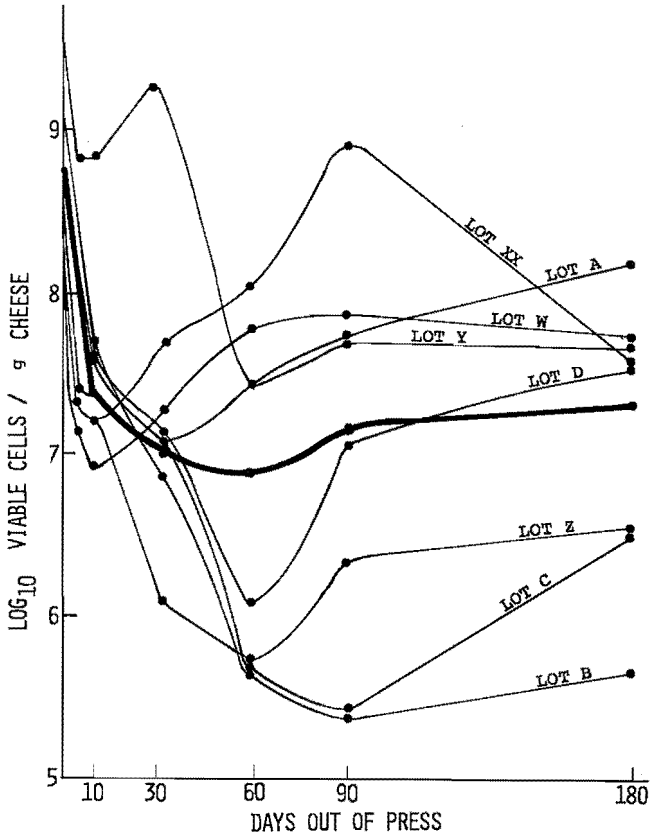

Figure 4. Total bacterial counts of air-cooled control cheeses cured at 12.8 C.

observed when the cheeses were subjected to different curing temperatures. The total counts in the control cheeses exhibited significant differences to much greater degree when the curing temperature was varied. The differences in populations in days of cure (each sampling point) are statistically significant for both control and experimental cheeses, although to a much greater degree for the control cheeses. In control cheeses, the interaction between curing temperature and days of cure is significant whereas it is not in the experimental (enterococci) cheeses. Statistical results are presented in Table 2.

Discussion

Although the supplemental enterococci in the experimental lots did not multiply as rapidly as the starter in the control group during the initial stages of manufacture, the enterococci either maintained constant numbers or slightly increased in number between milling time and the time the cheese was removed from the press. The control starter cheeses exhibited a steady bacterial numerical decrease after milling. This pattern probably resulted because of the capacity of enterococci to tolerate the acidity and brine-salt concentration of Cheddar cheese. Such survival may be desirable since, with a large number of actively metabolizing enterococci, the available carbohydrate will be more quickly utilized than in the control cheeses where the majority of organisms are in a numerically declining phase. This enterococcal survival has a two-fold advantage; first, more rapid depletion of lactose would decrease the likelihood of growth of undesirable microorganisms, and second, more complete fermentation of the carbohydrate would insure the stabilization of the pH of the finished cheese in the desirable range of 5.1-5.3 during curing. Changes of this nature will be reported in a future paper.

Survival patterns during curing of the eight negative control lots are generally similar for each curing temperature, but population variances are evident among the lots. These differences are likely due to the presence in the cheese milk of different types and proportions of adventitious flora (different growth responses under the curing conditions). We did not verify this occurrence but Feagan and Dawson *(14)* have observed that individual cheeses of similar age and from similar milk source showed considerable variability in the types of microorganisms present. Thus, that each control lot would behave identically would be unexpected.

The increase in population in normal starter (nega-

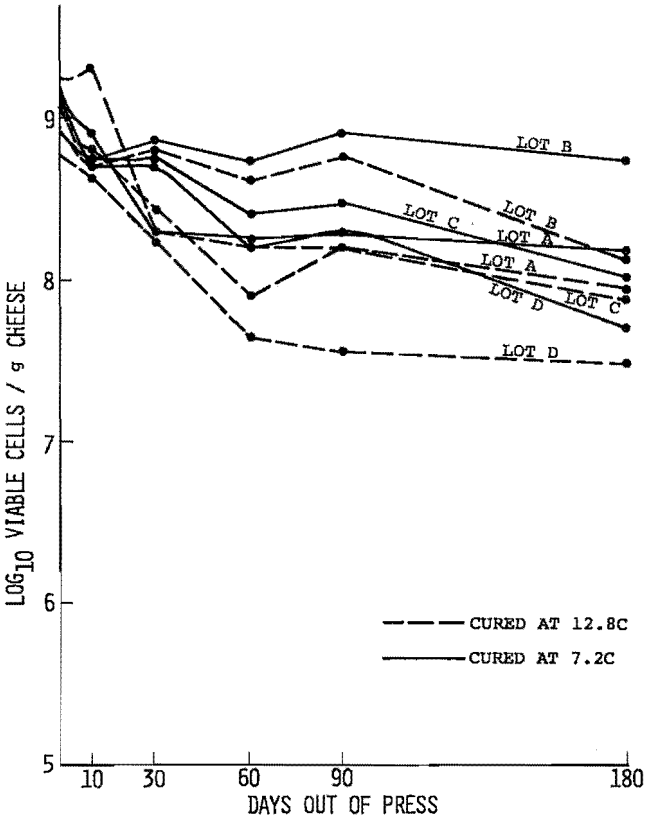

Figure 5. Enterococcus counts during cuing of air-cooled experimental (S. *faecalis*) cheeses.

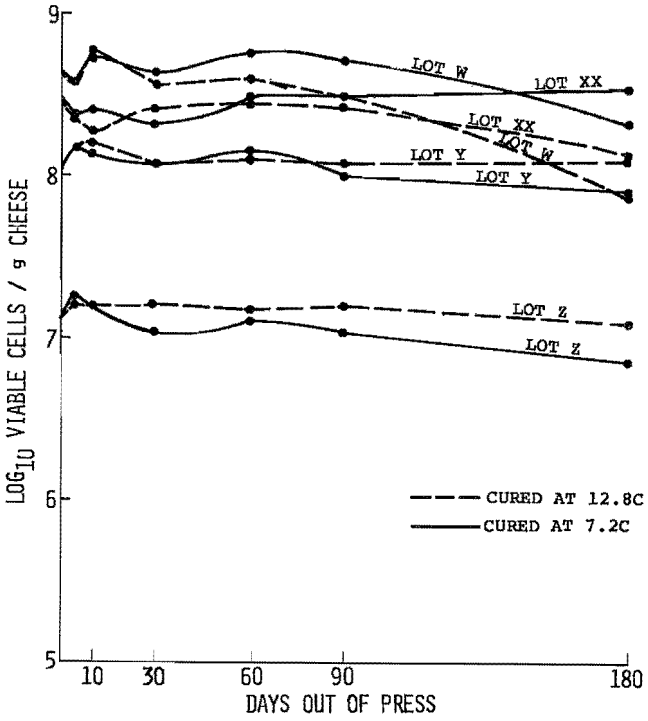

Figure 6. Enterococcus counts during curing of air-cooled experimental (S. *durans*) cheeses.

tive control) cheeses when cured at 12.8 C was observed as long as 78 years ago by Russell *(23)*, who reported that the numbers of bacteria in fresh curd decline for a day or so, followed by an increase. At the time that report was made the accepted curing temperature was rather high (> 12.8 C) and thus compares with the present results. Several workers have indicated that lactobacilli predominate after the starter organisms have died. Feagan and Dawson *(14)* stated that lactobacilli constitute 80% of the non-starter population, and Johns and Cole *(16)* claimed that lactobacilli multiplied rapidly even during the first 1 or 2 days of curing, reaching their maximum numbers in 3 to 6 months. These reports indicate that the increase in population in negative control cheeses cured at 12.8 C may be due to a proliferation of lactobacilli at this temperature.

The high survival rate of S. *faecalis* in curing Cheddar cheese as observed in this study also was noted by Kosikowsky and Dahlberg *(17)*. That S. *faecalis* is able to survive well in curing cheese is probably due to its salt tolerance, acid tolerance, and general resistance to the microenvironment of the cheese. The more rapid decline in population at 12.8 C may result from a more rapid metabolism as compared with activity at 7.2 C. More rapid metabolism would cause a faster depletion of fermentable carbohydrate in the microenvironment. A corollary to this would be the more rapid accumulation of autotoxic end-products resulting in a more rapid rate of cellular death.

The maintenance of an almost unaltered population of S. *durans* in curing cheese leads to speculation on the probably unique nature of the microorganism. It obviously possesses properties that S. *faecalis* does not and that enable sustained survival in the microenvironment of the cheese. Perhaps it metabolizes more slowly than S. *faecalis* almost to the point of dormancy, which would prolong the supply of nutrients and avoid rapid accumulation of toxic by-products. Such behavior, however, would make the presence of S. *durans* unlikely to have much effect on cheese flavor. A second possibility is that S. *durans* accumulates different and less toxic by-products than does S. *faecalis*, which could affect cheese flavor. But such an explanation still leaves the question of depletion of nutrients. There is the possibility that S. *durans* is able to use other components as an energy source, for example citrate, after preferential utilization of lactose, glucose, and galactose. It is well known, however, *(21, 25)* that S. *faecalis* has a broader

TABLE 1. EXPLANATION OF EXPERIMENTAL VARIATION BETWEEN CHEESE LOTS[a, b]

Lot	Species and strain of enterococcus	Amount of concentrate added/2270 kg milk
A	S. *faecalis* 47-13	150 ml
B	S. *faecalis* 47-13	75 ml
C	S. *faecalis* 24-23	150 ml
D	S. *faecalis* 24-23	75 ml
W	S. *durans* 15-20	150 ml
XX	S. *durans* 15-20	75 ml
Y	S. *durans* 9-20	150 ml
Z	S. *durans* 9-20	75 ml

[a]All experimental cheeses were made with 1% commercial mixed-strain lactic starter in addition to enterococcal inoculation.

[b]All control cheeses were manufactured with 1% commercial mixed-strain lactic starter.

TABLE 2. ANALYSIS OF VARIANCE; CONTROL CHEESE POPULATIONS VS. ENTEROCOCCUS POPULATIONS IN EXPERIMENTAL CHEESES[a]

Source of variation	d.f.	F values	
		Control	Experimental
Curing temperature (T)	1	14.66**	5.66*
Cooling procedure (C)	1	0.08	0.25
C × T	1	0.29	0.03
Days of curing (D)	6	65.50**	25.36**
C × D	6	0.19	0.25
T × D	6	13.40**	1.14
C × T × D	6	0.05	0.14
Error[b]	189	0.433	0.071

[a]All cheeses were manufactured with 1% commercial mixed-strain lactic starter. Experimental cheeses contained enterococcus starters as specified in Table 1.

[b]Mean square error term given.

*Significant at $P < 0.05$.

**Significant at $P < 0.01$.

fermentation spectrum than does S. *durans*, and typical S. *faecalis* would be expected to be more versatile in scavenging energy sources. Other possible explanations are that S. *durans* is more salt and acid tolerant, possibly grows anaerobically more easily, and has a metabolic pattern and rate conducive to maintaining a suitable microenvironment longer. Indeed, since certain workers *(21, 25)* claim that S. *faecalis* has more fermentative and reductive powers than S. *durans*, it is likely that S. *faecalis* must produce a greater amount of energy for its more complex systems to operate, thus quickly using the resources of the cheese microenvironment.

Statistical results indicate considerably more population uniformity in enterococcus cheeses regardless of treatment variability as compared with the populations in the negative control cheeses. This indicates that the survival of enterococci when used as a starter is less susceptible to environmental changes, and the overall quality of these cheeses may be more predictable from batch to batch if chemical changes exhibit the same uniformity. Survival of enterococci also may serve to salvage cheese which has lost lactic-starter activity due to bacteriophage infection.

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