THE FATE OF *SALMONELLA TYPHIMURIUM* IN THE MANUFACTURE AND RIPENING OF LOW-ACID CHEDDAR CHEESE[1]

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Abstract

Cheddar cheese was made by the stirred-curd procedure from pasteurized milk inoculated with *Salmonella typhimurium* and with a slow acid-producing strain of *Streptococcus lactis*. The Most Probable Number technique was used to enumerate salmonellae in milk and in cheese during its manufacture and ripening. Salmonellae grew rapidly during manufacture and limited additional growth occurred in cheese during the first week of ripening at 13 C after which there was a gradual decline in population. Salmonellae survived during ripening for up to approximately 7 months at 13 C and 10 months at 7 C. Cheese made in 2 of 5 trials exhibited a limited increase in number of salmonellae during the first 2 weeks at 7 C followed by a decline in population of these bacteria. Other cheeses held at 7 C exhibited a reduction in number of viable salmonellae without the initial increase. Growth of salmonellae during the early stages of ripening and subsequent extended survival of these organisms may, in part, be attributable to high moisture (average 43.2%) and high pH (5.75 after overnight pressing) of the cheese which resulted from use of a slow acid-producing starter culture.

The increase in food-borne disease outbreaks in the United States during the past 30 years has been attributed both to mass production and distribution of convenience foods and to an improved reporting system. Even though milk and milk products were among the first of the convenience foods to achieve mass distribution, disease outbreaks involving these foods declined sharply after widespread acceptance of pasteurization by the dairy industry and after adoption of the grade-A milk ordinance many years ago.

Salmonellosis is one of the major food-borne diseases and recently it has received a great deal of attention. Concern about this disease in the dairy industry was prompted by the recovery, in 1966, of *Salmonella newbrunswick* from nonfat dry milk *(6)*.

According to a recent review by Marth *(6)*, the incidence of salmonellosis caused by consumption of contaminated cheese is quite low. Nevertheless, some outbreaks, mainly of typhoid fever, have been reported. Gauthier and Foley *(3)* described an epidemic of typhoid fever which occurred in Canada in 1941 and resulted in 40 cases and six deaths. The source of infection was 10-day old Cheddar cheese made from raw milk which had been handled by a typhoid carrier. Another outbreak of typhoid fever involving Cheddar cheese was described by Foley and Poisson *(2)*. In this instance it was believed that the cheesemaker's wife, who had an active case of typhoid fever, was responsible for contaminating the cheese. Menzies *(7)* observed that 111 of 507 cases of typhoid fever in Alberta between 1936 and 1944 resulted from consumption of infected Cheddar cheese.

Survivial of *Salmonella typhi* in Cheddar cheese was studied by Ranta and Dolman *(9)*. They mixed the organism with the cheese and were able to recover viable salmonellae after storage for one month at 20 C. When the surface of cheese was inoculated and the product then held at room temperature, survival of S. *typhi* was similar to that observed with the cheese-organism mixture. It was further noted that storage at a refrigeration temperature was accompanied by extended survival of salmonellae and that the bacteria penetrated into the cheese to a depth of 4-5 cm in 17 days.

Campbell and Gibbard *(1)* inoculated milk with S. *typhi* and used it to make Cheddar cheese. All cheeses were ripened for two weeks at 14.4 to 15.6 C, after which one cheese from each duplicate set was transferred to storage at 4.4 to 5.6 C. At the lower temperature seven out of 10 cheeses contained viable S. *typhi* cells for more than 10 months, whereas at the higher temperature the organism generally disappeared after three months of ripening.

More recently Goepfert et al. *(4)* reported that *Salmonella typhimurium* grew rapidly during the manufacture of stirred-curd Cheddar cheese until salt was added to the curd. They observed that

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10 to 12 weeks of ripening at 13 C or 14 to 16 weeks at 7.5 C were required before viable salmonellae in cheese dropped to essentially undetectable levels. Hargrove et al. *(5)* inoculated cheese milk with several serotypes of salmonellae and were able to recover these organisms from the resulting Cheddar cheese for a period of 3 to 7 months. They concluded that pH was the principal factor which governed survival of salmonellae in cheese.

It is well recognized that insufficient production of acid during cheese manufacture can permit staphylococci, if present, to grow and produce enterotoxin thus rendering the product unsafe for consumption *(10)*. Common reasons for inadequate formation of acid include: presence of antibiotics in milk, a starter culture infected with bacteriophage, or a slow acid-producing strain of lactic streptococcus. Since no reports have appeared on the survival of salmonellae in Cheddar cheese made in a manner to preclude development of sufficient acid, this work was undertaken. A preliminary report on some of the results has been presented *(8)*.

MATERIALS AND METHODS

Bacterial cultures

A 24 hr old nutrient broth culture of S. *typhimurium* (Department of Bacteriology, University of Wisconsin) was added to pasteurized milk to result in an initial level of approximately 100 salmonellae per milliliter of milk. The culture of S. *typhimurium* was maintained by daily transfer in nutrient broth.

A slow acid-producing strain of *Streptococcus lactis* (Dr. G. W. Reinbold, Department of Food Technology, Iowa State University, Ames) was used as the starter culture and was added to cheese milk at a level of 1%. This culture was maintained by transfer in sterile 10% reconstituted nonfat dry milk at 48 hr intervals.

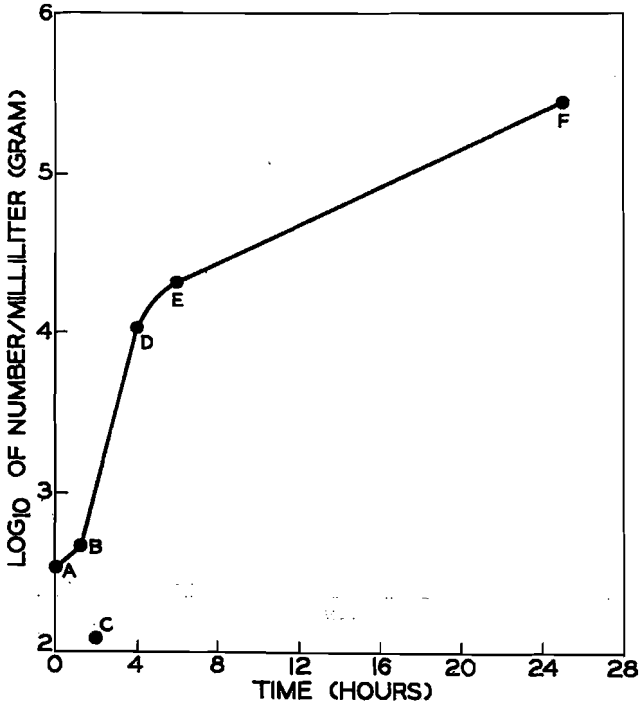

Figure 1. Growth curve of *Salmonella typhimurium* during the manufacture of low-acid stirred-curd Cheddar cheese. Each point represents the average value for five vats of cheese. (A) Milk after (5 min) addition of starter and *Salmonella*, (B) Curd prior to cutting, (C) Whey after cooking, (D) Curd after draining, (E) Curd prior to hooping, (F) Cheese after pressing overnight.

TABLE 1. TYPICAL MANUFACTURING SCHEDULE OF LOW-ACID STIRRED-CURD CHEDDAR CHEESE MADE FROM 440 LB LOTS OF PASTEURIZED MILK INOCULATED WITH *Salmonella typhimurium*.

Steps	Time	Temperature	Titratable acid (%)	pH
Add starter	8:30 a.m.	31.2 C (88 F)	0.155	
Add rennet (39.6 ml/440 lb milk)	9:15	31.2 C (88 F)	0.16	
Cutting curd	9:50	31.2 C (88 F)	0.09	
Steam on[a]	10:05	31.2 C (88 F)		
Steam off	10:35	40.6 C (105 F)		
Drain[b]	12:25 p.m.	40.6 C (105 F)	0.10	6.39
Stir and salt (5.87 oz)[c]	1:00			
Stir and salt (5.87 oz)	1:20			
Stir and salt (5.87 oz)	1:40.			
Hoop[d]	2:25		0.15	6.18
Press	2:40			
Vacuum on (25 in. vacuum)	9:30 a.m. (next morning)			
Vacuum off	10:20			

[a]To this point, 40 min additional time required over normal procedure.

[b]To this point, 55 min additional time required over normal procedure.

[c]To this point, 70 min additional time required over normal procedure.

[d]To this point, 90 min additional time required over normal procedure.

Enumeration of salmonellae

The methods described by Goepfert et al. *(4)* were employed to enumerate salmonellae in samples obtained during the manufacture and ripening of cheese.

Measurement of moisture and pH

The moisture content of cheese was determined by placing 3 g of cheese in a 50 ml beaker and then drying the cheese at 110 C for 16 hr in a forced draft oven. The pH of cheese was measured with a saturated calomel half-cell, gold electrode, and a Leeds and Northrup portable potentiometer.

Manufacture of cheese and sampling procedure

The procedure followed for manufacture of cheese and the sampling schedule are outlined in Table 1 and Fig. 1. Five vats of cheese were made, cheeses from each lot were ripened at 7 and 13 C, and they were tested for viable salmonellae weekly during the first month of ripening and monthly thereafter.

RESULTS AND DISCUSSION

Behavior of salmonellae during cheese manufacture

The behavior of S. *typhimurium* during the manufacture of low-acid stirred-curd Cheddar cheese is shown in Fig. 1. There was a slight increase in the number of salmonellae during the interval between inoculation of milk and cutting of the curd. As shown in Table 1, the elapsed time was about 80 min and the temperature was approximately 31 C, both conditions normal for the manufacture of Cheddar cheese. This initial period, or lag phase, during which a slight increase in numbers of viable salmonellae occurred, was probably a time of adjustment by the salmonellae to their new environment.

The lag phase was followed by a rapid increase in number of salmonellae during the interval between cutting the curd and draining the whey. From data in Table 1, it can be seen that approximately 55 min of additional holding time and a 1°C (2°F) elevation in cooking temperature beyond normal were required at this point for cheese manufacture. The increase in salmonellae during this period can be attributed to: *(a)* growth and *(b)* physical entrapment of bacteria by the curd particles. Such entrapment might account for a 10-fold increase in numbers.

After taking into account the entrapment factor, a generation time during the log phase of approximately 36 min was calculated using the formula $td = \log_{10} 2/\alpha$ (α = growth rate constant). The calculated generation time agrees well with the value of 35 min reported by Goepfert et al. *(4)*. Approximately 3.8 multiplications by salmonellae occurred during the 135 min from the beginning to the end of the log phase. This represents an added 0.3 division over that reported by Goepfert et al. *(4)* but this is easily attributable to the extra 25 min of incubation required because the starter culture was inactive.

At the time of cutting, draining, and hooping, subnormal production of acid was observed in all five trials, as determined by titratable acidity and pH measurements. The titratable acid and pH values at two of these stages for stirred-curd Cheddar cheese with more normal acid development are 0.17% and 5.9 at draining, and 0.30% and 5.5 at hooping.

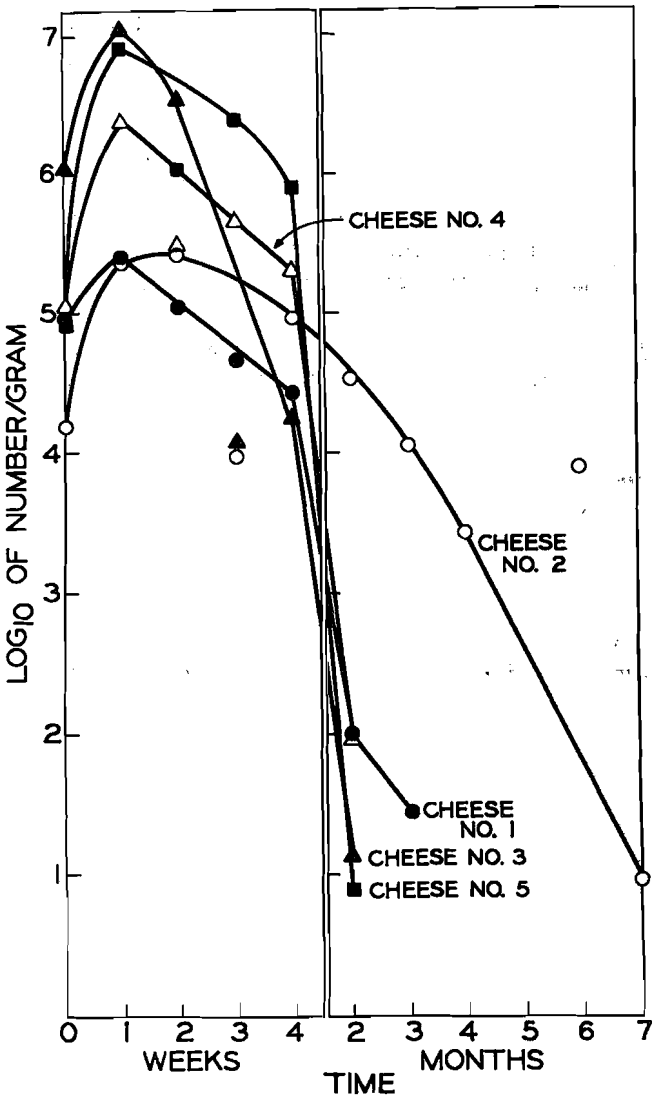

Figure 2. Survival of *Salmonella typhimurium* in low-acid stirred-curd Cheddar cheese ripened at 13 C.

TABLE 2. NUMBERS OF SALMONALLAE IN MILK, CURD, AND CHEESE DURING MANUFACTURE AND MOISTURE CONTENT AND pH OF CHEESE AFTER OVERNIGHT PRESSING

Trial	No. of salmonellae per ml or g						Cheese after pressing	
	Milk	Coagulated milk prior to cutting	Whey after cooking	Curd at draining	Curd prior to hooping	Cheese after pressing	pH	Moisture Content (%)
1	140	550	140	5,600	12,000	91,000	5.82	42.5
2	150	380	60	8,100	3,000	110,000	5.71	43.0
3	600	810	39	5,300	4,900	15,000	5.80	43.9
4	600	270	270	6,600	3,900	82,000	5.78	43.2
5	200	280	89	29,000	81,000	1,100,000	5.65	44.8
Average	340	460	120	11,000	21,000	280,000	5.75	43.2

Salting of the curd reduced the growth rate but it was not accompanied by a decline in numbers of salmonellae as was noted by Goepfert et al. *(4)* when they studied cheese made with a normal starter culture. Instead, an increase in numbers occurred during overnight pressing (Table 2). The failure of salting the curd to bring about a reduction in salmonellae, as observed in these trials, agrees with the data reported by Hargrove et al. *(5)*, although these investigators were studying cheese made by normal procedures.

Factors such as the high pH (average 5.75; Table 2) after pressing, high moisture content (average 43.2%; Table 2), and room temperature (approximately 21 C) storage during pressing were not detrimental to salmonellae and hence the increase in numbers of these bacteria during pressing became possible.

Behavior of salmonellae during ripening of cheese

The behavior of *S. typhimurium* in low-acid Cheddar cheese during ripening at 13 and 7 C is shown in Fig. 2 and 3, respectively. Data in Fig. 2 show that there was an increase in number of salmonellae during the first week of ripening at 13 C followed by a marked decline as ripening proceeded. Neither Goepfert et al. *(4)* nor Hargrove et al. *(5)* observed an increase in salmonellae in normal Cheddar cheese during ripening. The average pH value

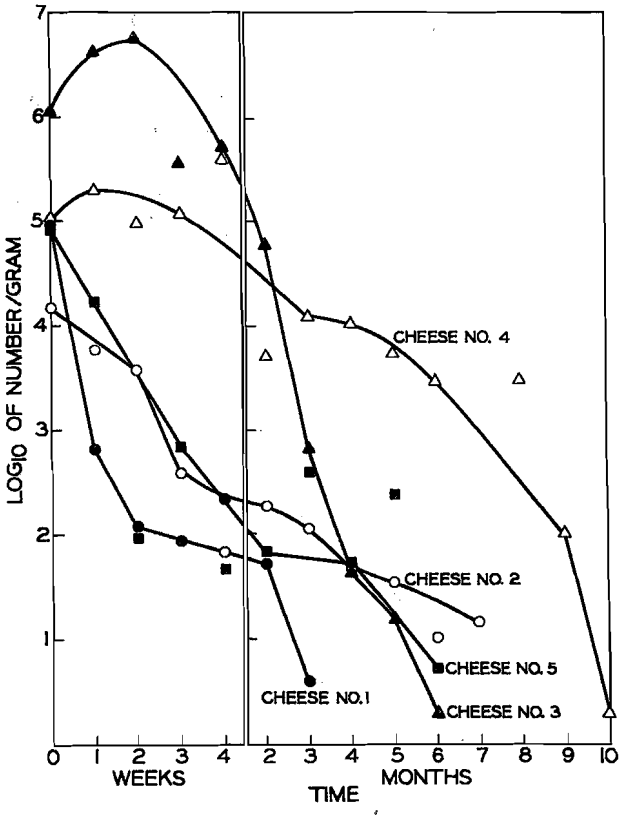

Figure 3. Survival of *Salmonella typhimurium* in low-acid stirred-curd Cheddar cheese ripened at 7 C.

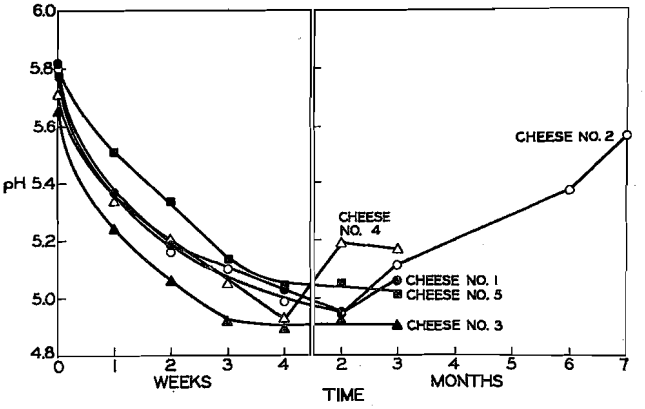

Figure 4. The pH of low-acid stirred-curd Cheddar cheese during ripening at 13 C.

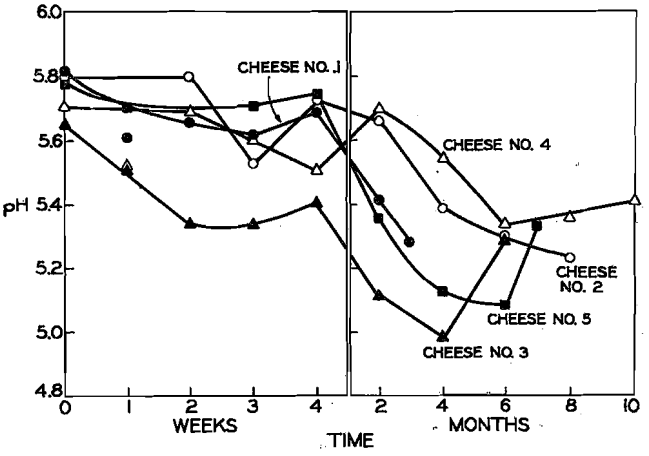

Figure 5. The pH of low-acid stirred-curd Cheddar cheese during ripening at 7 C.

of cheese in the five trials at the end of the first week at 13 C was approximately 5.4 but in one cheese the pH exceeded 5.5 (Fig. 4). This drop in pH from 6.18 at the time of hooping and from 5.75 after pressing may have contributed to the decline in numbers of salmonellae at 13 C even though the cheese contained an average of >43% moisture. Survival of salmonellae in cheese ripened at 13 C ranged from approximately 3 to 7 months, apparently depending on the rate at which the pH of the cheese dropped. A comparison of data in Fig. 2 and 4 reveal that the pH of one cheese (No. 2) began to increase when it was two months old and continued to do so until it approached 5.6 at the end of 7 months. The same cheese, according to data in Fig. 2, also exhibited extended survival of the salmonellae.

Figure 3 records the behavior of *S. typhimurium* in the five cheeses ripened at 7 C. Results of these trials were not as consistent as those observed when cheeses were ripened at 13 C. In cheese No. 1, there was a rapid decline in numbers and apparent loss of viability by salmonellae after approximately three months of ripening. The pH of this cheese

remained fairly constant during the first month of ripening and then dropped to approximately 5.3 after three months of storage (Fig. 5).

Salmonellae in cheese No. 2 (Fig. 3) behaved in a fashion somewhat similar to that of cheese No. 1, except that viable cells remained for at least seven months. The pH of this cheese remained elevated for a longer time than that of cheese No. 1. This may serve to explain the extended survival of salmonellae observed in cheese No. 2.

Two of the cheeses, No. 3 and 4 (Fig. 3), exhibited a limited increase in number of salmonellae during the first two weeks of ripening, followed by a sharp decline in viable salmonellae in cheese No. 3 and a slow decline in cheese No. 4. The pH of cheese No. 3 dropped more rapidly than that of cheese No. 4 (Fig. 5), which may account for the six months of survival by salmonellae in the former and 10 months in the latter cheese.

Results obtained with a fifth cheese (No. 5) were irregular but tended to approximate those observed with cheese No. 2. The drop in pH of cheese No. 5 also was similar to that noted with cheese No. 2 except that it occurred earlier in the ripening period.

Manufacture of Cheddar cheese with an inactive starter culture results in a product which may undergo any of a series of abnormal fermentations during ripening. Variation during ripening of cheeses made in this study is easily seen by examining data on pH changes presented in Fig. 4 and 5. These differences in fermentations may bring about variations in survival of salmonellae in such cheeses. Consequently, it is difficult to predict how much ripening time is needed before one can be sure that abnormal cheese is free of salmonellae. The extended survival of salmonellae in some of the abnormal cheeses (up to 10 months in these studies) is another reason why the cheesemaker must employ an active starter culture which will continue to produce acid during the entire cheese manufacturing process.

ACKNOWLEDGMENT

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