FACTORS AFFECTING SURVIVAL OF *SALMONELLA* IN CHEDDAR AND COLBY CHEESE

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ABSTRACT

Factors affecting the growth and survival of salmonellae in the experimental manufacture of 7 lots of Colby and 65 lots of Cheddar cheese were studied. Milk was artificially contaminated just prior to making of cheese. Manufacturing variables tested included effects of milk pasteurization, size of starter inoculum, titratable acidity and cheese pH, type of lactic culture, chemical additives, salt and moisture content, supplemental cheese microorganisms, and curing temperatures. The rate and amount of acid produced during making, the pH of the cheese, and the type and size of starter inoculum had significant effect in suppressing the growth and survival of salmonellae. Salt, moisture, chemical additives, and pasteurization of the milk, had little or no effect on the salmonellae. Addition of large numbers of *Propionibacterium* and *Leuconostoc* seemed to favor survival of salmonellae. Lactobacilli and enterococci tested had no effect.

Salmonellosis is a continuing health problem and its apparent increase can be attributed to an increasing awareness and more extensive surveillance. Salmonellae contamination in manufactured dairy products has become of increasing concern since 1966 when 11 serotypes were isolated from nonfat dry milk (NDM) from nine states *(8)*. Consequently, the Food and Drug Administration has indicated that it plans to screen all dairy products. In anticipation, these laboratories began a survey of a variety of dairy products to determine which manufacturing variables contribute to growth and dissemination of salmonellae and how procedures could be modified to insure safety of the product. Reports for Cottage cheese *(5)* and NDM *(6)* have been published.

A review of cheese-borne infections up to 1947 is given by Fabian *(1)*. More recently Price et al. *(7)* reported an outbreak from infected Cup cheese. In all instances for which background information is available, the cheeses were made from raw milk or from milk which was recontaminated after pasteurization. Pasteurization of cheese milk is not required providing the cheese is aged for at least 60 days prior to selling. Tucker et al. *(9)* reported survival of *Salmonella typhimurium* in Colby cheese for 302 days at 43 to 48 F. Goepfert et al. *(2)* reported survival of the same species in stirred curd Cheddar cheese for at least 12 weeks at 7.5 to 13 C.

Information is generally lacking concerning the effects of the many variables on salmonellae survival during manufacture and curing of Cheddar and Colby cheese. This paper reports the results of a study to determine such effects.

MATERIALS AND METHODS

Cultures

Four test species of *Salmonella* were selected on the basis of their heat resistance and frequency of occurrence in dairy products. They were *Salmonella senftenberg* 775W, *Salmonella typhimurium* TM1, *Salmonella new brunswick* 1608, and *Salmonella newport*. In most trials, 18-hr cultures of each, grown in trypticase soy broth, were added to the cheese milk 1 hr prior to manufacture. The inoculum ranged from 300 to 5000 per ml of milk.

Commercial mixed-strain lactic cultures were used in most instances as the starter. Additional cultures, used singly or as supplementals, included one or more species of *Leuconostoc, Streptococcus, Lactobacillus, Micrococcus, Pseudomonas,* and *Propionibacterium*. All lactic cultures were subcultured in sterile 10% NDM prior to use. The *Propionibacterium* was grown in lactose broth and the *Micrococcus* and *Pseudomonas* in nutrient broth.

Salmonellae determinations

Analyses for salmonellae were made on the following: *(a)* cheese milk at time of addition of salmonellae and lactic starter, *(b)* milk at setting (addition of coagulating enzyme), *(c)* curd and whey at cutting, *(d)* whey at cooking, *(e)* whey at draining, *(f)* curd at draining, *(g)* curd at salting, *(h)* curd at hooping (packing curd in molds), and *(i)* the 21-hr cheese. The cheeses were subsequently tested in two weeks and at monthly intervals thereafter.

Two procedures were employed to detect and enumerate salmonellae. When counts exceeded 10/g, direct plating on a selective, modified, lysine iron agar (Difco) was used. The antibiotic novobiocin[2] (5-10 μg/ml) was added aseptically to the cooled lysine agar just prior to plating to suppress the growth of many gram-positive microorgansims and select strains of *Proteus* and *Escherichia coli*. A very thin underlayment and overlayer were used in conjunction with conventional pour techniques. Plates were incubated at 37 C for 24 to 48 hr. All salmonellae used in this study grew readily in the agar, producing hydrogen sulfide and developing into large black lens-shaped colonies. Samples were prepared for plating by blending 11 g of cheese with 99 ml w/v of a sterile 0.2% sodium citrate solution. When counts were lower than 10/g, quantities of the blended samples were preenriched in a selective lysine-iron broth

[1]Agricultural Research Service, U. S. Department of Agriculture.

[2]Novobiocin, sodium (Albamycin UpJohn Co.)

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(3) and identified by standard cultural procedure.

Make procedure

Seven lots of Colby cheese and 65 lots of Cheddar cheese were made in small steam-jacketed vats, each having a capacity of 50 lb of milk. Manufacturing variables studied included the effects of: milk pasteurization, size of starter inoculum, titratable acidity (TA) and cheese pH, type of lactic culture, chemical additives, salt and moisture content, supplemental cheese microorganisms, and curing temperatures. The manufacturing methods were essentially those prescribed by Lochry et al. (4) for Cheddar cheese and Wilster (10) for Colby cheese. Variations in the "make" procedure were necessary to compensate for some of the test variables.

Fresh whole milk, standardized to 3.3% fat, was usually pasteurized at 163 F for 15 sec and cooled to 90 F before inoculation with the *Salmonella* test organisms. Two vats were made with raw milk as the variable.

Both mixed and single strain cultures of *Streptococcus lactis* and *Streptococcus cremoris* were compared. Inoculum size was varied from 0.1 to 3%. In an attempt to maintain comparable pH values and TA in the cheese during manufacture and curing, the make time-schedule and other variables had to be adjusted. For example, the ripening time for the milk with 3% starter was reduced from 1 hr to 5 min, the time between cooking and draining was shortened to 15 min, cheddaring time was reduced to 30 min, the pressing temperature was lowered to 40 F rather than 70 F and the curing temperature during the first month was 32 F rather than 40 F.

In an attempt to pinpoint the role of pH, various methods for controlling acid were utilized. These included variations in schedule to mill at TA from 0.25 to 0.55%, buffering the cheese milk with phosphates, the use of age-attenuated lactic starters and direct acidification of cold cheese milk with edible acids.

The role of salt in the finished cheeses was studied by varying the salt content from 1.4 to 2.7%. The curd was usually dry salted, but in two experiments salt was added directly to the cheese milk at the rate of 1 and 1.5% w/v.

Make procedures were altered to control moisture levels within a range of 35 to 48%. Other variables included the use of inhibiting agents such as diacetyl, potassium sorbate, nisin, and sodium lactate.

Goepfert et al. (2) reported evidence that the production of volatile fatty acids during curing, especially acetic, may be a major factor in elimination of salmonellae from cheese. To explore this theory, cheeses were made by direct acidification using lactic and acetic acids. Sufficient acid was added to the cold cheese milk (40 F) to lower the pH to 5.35. Rennet and 0.1% starter were also added while cold. The temperature was then slowly raised to 80 F without stirring by circulating 100 F water through the jacket of the vats. After coagulation, in about 0.5 hr, the coagulum was cut and cooked to 100 F. The curd was then drained, cheddared for 1 hr, and salted.

Most cheeses were hooped and pressed at 75 F for 21 hr and were sealed by dipping in melted cheese wax. Most were stored at 40 F, but some were divided so that portions could be stored at 32, 40, and 50 F.

The pH of the cheese was determined after 21 hr in the press, after two weeks curing, and at monthly intervals thereafter.

RESULTS AND DISCUSSION

A typical pattern of salmonellae growth during manufacture is shown in Table 1. There was very little growth up to the time of cutting. Then a thirtyfold increase occurred during the 2-hr interval between cutting and draining. Since one would expect an approximate tenfold increase from cell concentration in the curd, the difference indicates a moderate growth rate of about two generations during the 2-hr period. Multiplication continued, resulting in a sevenfold increase (approximately three generations) during the 3-hr interval between draining and hooping. Following hooping, active growth stopped sometime during the pressing operation as evidenced by an 80% decline between 6-1/2 and 21 hr. These data are in good agreement with those of Goepfert et al. (2) who reported approximately 4.5 generations between cutting and hooping, followed by a 75% decline during pressing.

An assumption that salt initiated the rapid initial decline between hooping and 21 hr was generally disapproved by the fact that subsequent vats of cheese having salt contents from 1.4 to 2.7% showed no difference in decline during the same period. Additional vats of cheese were made in which 1.5% salt was added to the cheese milk. Resulting inhibition of the lactic starter necessitated increasing the inoculum to 3% or lengthening the cheddaring time by about 15% before milling. No inhibition of salmonellae was observed. Additional salt was then added to the curd to raise the final level to normal values. Figure 1 compares salmonellae survival during curing as affected by differences in salt content. Here again, no differences were observed. It can be concluded that salt plays little or no part in hastening the death of salmonellae during cheese manufacturing and curing.

Survival of salmonellae during curing varied from 2 to 9 months with an average of 6.5 months for all vats. The variables significantly affecting survival were pH and type and amount of starter. These data are presented in the accompanying figures.

Figure 2 shows the effect of cheese pH on survival of salmonellae throughout curing. Zero time represents the salmonellae count of the cheese after 21 hr in the press. The pH range given indicates the pH of the cheese from 21 hr to final sampling. Cheeses with an abnormally high pH, caused by starter failure (dead vats) obviously have little or no inhibitory effect on the salmonellae. The most rapid decline was associated with pH 5.0. Such cheese made from pasteurized milk would probably be criticized for acidity and bitterness. However, raw milk cheeses with pH values from 5.0 to 5.1 are common and are usually acceptable in flavor and acidity.

Figure 3 shows dramatic differences in effects of amount of starter on survival during storage. Zero time in the figure represents the salmonellae count

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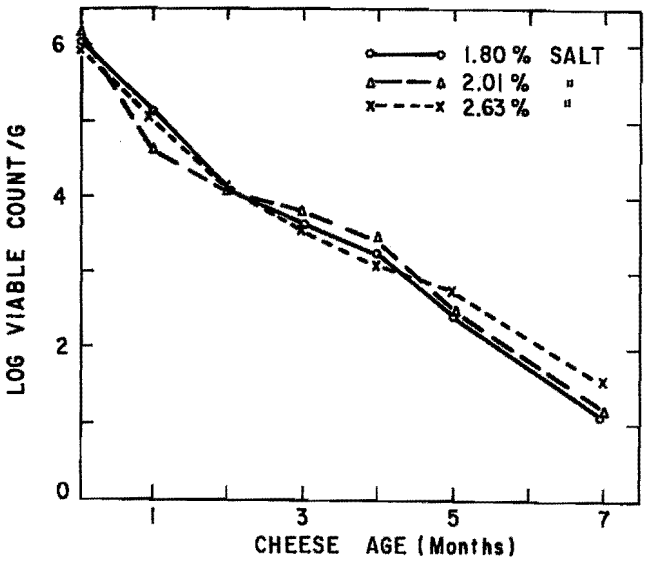

Figure 1. Effect of salt on *Salmonella* survival in Colby cheese.

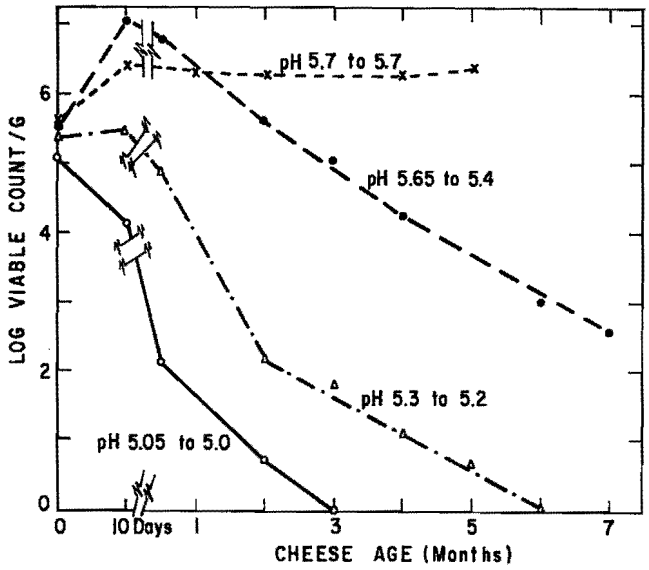

Figure 2. Effect of pH on *Salmonella* survival in Cheddar cheese.

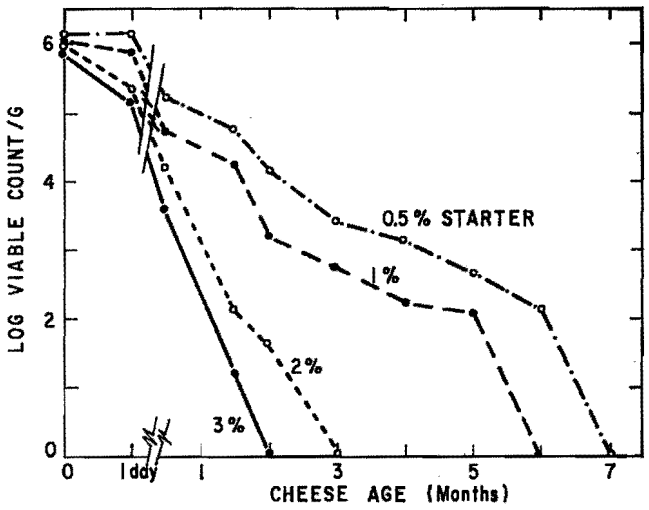

Figure 3. Effect of lactic starter inoculum on *Salmonella* survival in Cheddar cheese.

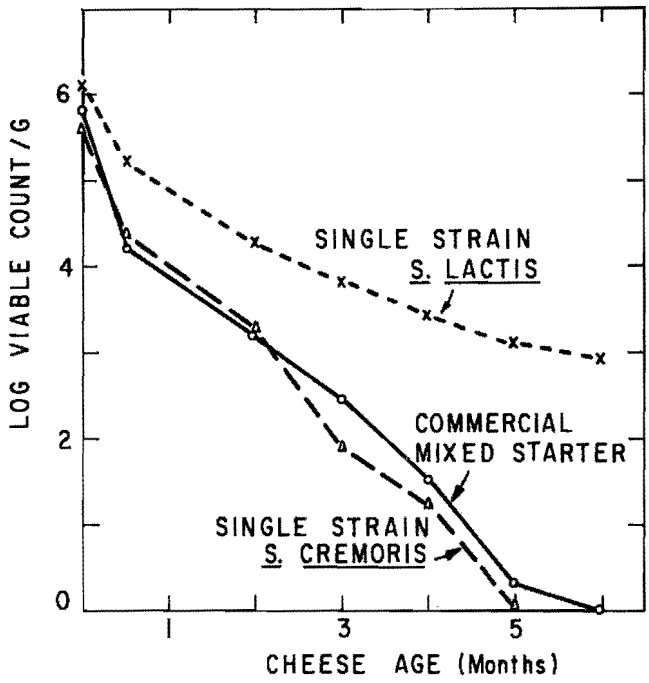

Figure 4. Comparison of cheese starter on *Salmonella* survival in Cheddar cheese.

of the cheese curd just prior to pressing. The count of the cheese made with 0.5% starter showed no decline during the first day of storage. In contrast, an initial reduction of about 80% occurred in the cheese made with 3% starter. All of this decline was not caused by the acid. The various control techniques mentioned earlier succeeded in maintaining essentially identical pH development through pressing. At milling, the TA of all four lots was 0.53%, and the 21-hr pH values were 5.55, 5.55, 5.45, and 5.45, respectively, for 0.5, 1, 2, and 3% starter. The differences at two months were greater, varying from pH 5.31 (0.5% starter) to 5.00 (3% starter). Thus the control of acid development until the cheeses with less starter could "catch up" to that with 3% starter indicates that the lactic starters were somewhat inhibitory.

In order to study possible differences in natural inhibitory effects of different species of lactic starter, several species of S. *cremoris* and of S. *lactis* were compared to commercial multiple-strain cultures. Efforts were made to develop comparable acidities and to produce cheese of similar pH and composition throughout manufacture and curing, thus eliminating variables other than starter. The pH of these cheeses was within the range of 5.18 to 5.29 after one day and 5.19 to 5.25 after two months of curing. Figure 4 is representative of the results obtained. The species of lactic starter used in cheesemaking appears

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TABLE 1. SURVIVAL OF SALMONELLAE IN CHEDDAR CHEESE MANUFACTURE[1]

Manufacturing operation	Time hr	Salmonellae No/ml - g
Starters added	0	1,400
Milk at setting	1	1,500
Curd + whey at cutting	1 1/2	1,560
Whey at cooking	2	230
Whey at draining	3 1/2	410
Curd at draining	3 1/2	47,100
Curd at salting	5 3/4	310,000
Curd at hooping	6 1/2	318,000
Cheese after pressing	21	60,000

[1]0.75% lactic starter; milling TA 0.40%; 21 hr pH 5.4.

TABLE 2. EFFECT OF TEMPERATURE ON SURVIVAL OF SALMONELLAE DURING STORAGE

Cheese age months	32 F		40 F		50 F	
	No./g	pH	No./g	pH	No./g	pH
0	61×10^4	5.52	61×10^4	5.52	61×10^4	5.52
1	18×10^3	5.38	11×10^3	5.38	16×10^3	5.39
2	39×10^2	5.31	32×10^2	5.23	20×10^2	5.16
3	65×10^1	5.30	60×10^1	5.22	6×10^1	5.15
4	60	5.25	18	5.18	0	5.10
5	30	5.21	2	5.15	0	5.10
6	10	5.22	0	5.15	0	---

to have a marked effect on survival, probably because of the production of an unknown inhibitory factor. Species of S. *cremoris* were much more inhibitory than S. *lactic* species. These results were confirmed by comparing lactic streptococci grown on agar plates seeded with salmonellae. S. *cremoris* strains produced greater zones of inhibition than either S. *lactis* or S. *thermophilus*. The antibiotic "nisin" and nisin-producing strains of S. *lactis* had no effect on salmonellae. Supplemental starter microorganisms, *Lactobacillus bulgaricus, Lactobacillus acidophilus, Lactobacillus casei, Pseudomonas fragi,* and *Micrococcus caseolyticus* had no apparent effect on salmonellae when added to the cheese milk. In contrast, large numbers of *Leuconostoc* bacteria and *Propionibacterium shermanii* added as starters resulted in the survival of salmonellae at higher levels and for longer periods of time than in controls. The pH of these cheeses was slightly higher which may account for this effect.

None of the additional variables tested were of value as a means of salmonellae control. No apparent differences were detected when raw milk cheese was compared with pasteurized milk cheese. Moistures had no direct influence. Potassium sorbate and sodium lactate had no inhibitory effects while high levels of diacetyl slowed the growth of both salmonellae and lactic starter.

Results of storage at 32, 40, and 50 F, shown in Table 2 revealed slight differences with a more rapid decline at higher temperatures. These variations were not considered significant and appeared to be related more to greater acid development at the higher temperatures.

The results obtained from the cheeses acidified with lactic and acetic acids confirm the roles of both pH and starter. Both cheeses were inoculated with only 0.1% starter, much less than would normally be used. The starter organisms grew slowly in the lactic acid cheese to a maximum of 2,000,000/g. This cheese became gassy in three months with a coliform count of 180,000/g. The salmonellae declined slowly during storage but the rate of decline was about one-half that of cheeses with a comparable pH developed by normal amounts of starter. The cheeses acidified with acetic acid gave the same pattern for salmonellae; the decline was about half as fast as expected. This cheese, however, indicated inhibition of lactic starter and coliforms. The starter grew to a maximum of 16,000/g while no coliforms were evident. It appears that a pH of 5.2 to 5.3 was sufficient to cause death of salmonellae during storage, but the lower rate of decline in the absence of normal amounts and development of starter offers further evidence of the natural inhibitory nature of starter organisms. While acetic acid appears to be inhibitory to some organisms, it had no apparent effect on the salmonellae.

The results of this study revealed no variables that could be used to insure freedom of Cheddar or Colby cheese as a source of salmonellosis should these organisms enter cheese milk as post pasteurization contaminants. Only pH and starter significantly influenced salmonellae survival. All other variables had indirect effects; that is, their role was obvious only to the degree that they influenced the development of acid. Thus, factors which contributed to subnormal acid production enhanced growth and survival of salmonellae and those that contributed to normal or excessive acid production were detrimental. It should be pointed out that the degree of salmonellae contamination used in these studies far exceeded that which would likely occur from post-pasteurization contamination. However, it does reflect the need for quality control.

Although the incidence of salmonellosis is apparently increasing, the dairy industry has maintained a good record. United States Public Health officials have found no salmonellae in their survey of Cheddar cheese. In addition, no outbreak of salmonellosis has been traced to Cheddar type cheese

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since 1945. This paper, by pointing out the possibility of salmonellae survival for long periods of time, reemphasizes the need to maintain strict adherence to proper and sanitary procedures.

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REPORT OF IAMFES REPRESENTATIVE TO THE NATIONAL MASTITIS COUNCIL, 1968-1969

As the official representative of the International Association of Milk, Food, and Environmental Sanitarians, Inc., on the Board of Directors of the National Mastitis Council, the following is a brief summary of events to date:

At the annual meeting of the National Mastitis Council held in Chicago in February, 1968, I was appointed to the Board of Directors. I attended only the last day of the Board meetings, mainly to become acquainted with the members.

At the International Association's annual meeting held in St. Louis in August, 1968, I submitted a request to the officers of the Association asking that consultants be appointed to advise me regarding requests and recommendations which I should submit to the National Mastitis Council. This request was granted. Following are the consultants which were appointed from the Farm Methods Committee: M. W. Jefferson, Ben Luce, Leon Townsend, David Monk, and Glenn Cavin.

Attached is a copy of the subjects discussed and submitted by this Committee. These subjects were presented to the proper committees at the National Mastitis Council's annual meeting held in Chicago in January, 1969:

(a) No action. I was advised that this should be presented to the Interstate Milk Shippers Conference. It is the opinion of many that the National Mastitis Council is only interested in research regarding the cause and treatment of mastitis as well as the laboratory procedures and analysis of the number of somatic cells in the milk supply. There are a great many variations in the opinions of the members of the National Mastitis Council on these two subjects.

(b) This request was presented to the Committee on Screening Tests, A. R. Brazis, Chairman. It was accepted and placed in the minutes of the meeting.

(c) I presented this recommendation to the veterinarian's section and asked for more cooperation between the veterinarians and the regulatory sanitarians. There is some question as to the acceptance of this recommendation. The veterinarians feel that they are the only people who are directly involved in a mastitis program and that the regulatory agencies should be interested in the quality of milk only. They could be correct regarding this matter.

At this time, may I request that the Board of Directors of the International Association submit guidelines and advice in regard to the direction we should take with the National Mastitis Council. I hope we can continue to show constructive results for the organization through our combined efforts.

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