FATE OF ENTEROPATHOGENIC STRAINS OF *ESCHERICHIA COLI* DURING THE MANUFACTURE AND RIPENING OF CAMEMBERT CHEESE[1]

H. S. Park[2], E. H. Marth, and N. F. Olson
Department of Food Science and the Food Research Institute
University of Wisconsin-Madison, Madison, Wisconsin 53706

(Received for publication September 14, 1973)

Abstract

Camembert cheese was made from pasteurized milk inoculated to contain, per milliliter, approximately 100 cells of enteropathogenic strains of *Echerichia coli*. A Most Probable Number technique was used to enumerate *E. coli* at intervals during manufacture and ripening of the cheese. Identity of isolates obtained was determined serologically. Growth of *E. coli* was minimal until after curd was cut and hooped. Rapid growth ensued and populations in excess of 10^6/g appeared in some cheeses 5 h after the cheesemaking process began. Overnight storage of cheese in hoops was accompanied by a decline in numbers of viable *E. coli*. This coincided with a drop in pH of the cheese to 5.0 or below. Salting of cheese and 1 day of ripening at 15.6 C (60 F) caused a further decline in number of viable *E. coli*. This decline continued during the rest of the week at 15.6 C (60 F) and during storage at 10 C (50 F). From 0 to 9 weeks at 10 C (50 F) were required before cheese was free of viable *E. coli*. Substitution of *Streptococcus cremoris* C_1 for a commercial lactic starter culture favored survival of *E. coli* so that 9-week old cheese contained $>10^6$/g. When cheese was made from milk that contained penicillin, the *E. coli* population was approximately 10^9/g in 24-h old cheese and 10^7/g when the cheese was 9 weeks old.

Certain strains of *Escherichia coli*, designated as enteropathogens, can cause either a *Shigella*-like or *Salmonella*-like illness in adults and children *(12, 16)*. Although for years these bacteria have been known to cause infantile diarrhea, recently there has been increasing concern about the presence of *E. coli* in foods because the enteropathogenic strains can cause a form of foodborne illness. Such illness has been recognized and reported extensively in European countries and Japan *(4, 7)*. Outbreaks of foodborne illness caused by *E. coli* have been associated with consumption of milk, ice cream, kefir, cheese, and other dairy products *(1, 2, 10, 18)*. Enteropathogenic strains of *E. coli* have been found in market milk and market cheese, especially Camembert cheese from France, Sweden, and Denmark *(3, 19, 22)*.

Between November 12 and December 8, 1971 at least 227 persons in 96 separate outbreaks in approximately 8 states of the U. S. became ill with acute gastroenteritis about 24 h after consuming imported French Camembert or Brie cheese *(2, 17)*. Symptoms of the disease included vomiting, diarrhea, fever, and myalgia. Some victims observed blood in their stools. *E. coli* serogroup 0124:B17 was isolated from stools of several patients and from samples of cheese believed to have caused the illness. Although the presence of coliform bacteria in cheese was recognized as early as 1895 *(15)*, it was not until 76 years later that the first well-documented cheese-related U. S. outbreak of foodborne illness caused by *E. coli* was recorded.

When the outbreak of foodborne illness just described occurred, information was lacking on the fate of enteropathogenic strains of *E. coli* during the manufacture and curing of Camembert cheese. Consequently experiments were done to obtain these data. Results of the tests are in this paper. A preliminary report of the data has been given *(13)*.

Materials and Methods

Cultures

The following enteropathogenic strains of *E. coli* were supplied by the Food and Drug Administration: 0128:B12 (NY), 0125:B15 (8H1B), 0125:B15 (3H1C), 0124:B17 (5H4B), 0124:B17 (4H4A), and 0124:B17 (7H4A). Most of these strains were originally isolated from the French Camembert cheese that caused illness. Each culture was transferred to nutrient broth daily for 3 days before it was used for an experiment. Incubation was at 37 C for 24 h. Sufficient of a 24-h old nutrient broth culture (37 C incubation) was added to pasteurized milk to provide approximately 100 *E. coli* cells/ml of milk.

Starter cultures used to make cheese included a commercial mixed strain lactic culture and *Streptococcus cremoris* C_1. The starter cultures were transferred to sterile skimmilk daily for 3 days before they were used to make cheese. Incubation was at 22 C for approximately 16 h. Milk to be made into cheese was inoculated with 2% of a 16 to 24-h old lactic culture prepared in skimmilk.

Penicillium camemberti, obtained from K. B. Raper, Department of Bacteriology, University of Wisconsin, Madison, was grown on Czapek agar slants at 22 C for 1 week. The mold on one agar slant was blended (Waring blendor) with 50 ml sterile buffered distilled water and the mixture was added to 40 lb. milk at the time of cheesemaking.

Manufacture of cheese and sampling procedure

[1]Research supported by the College of Agricultural and Life Sciences, University of Wisconsin-Madison and by contributions from the food industry to the Food Research Institute.
[2]Present address: Cheese Division, Borden Foods, Plymouth, Wisconsin 53073.

TABLE 1. TYPICAL MANUFACTURING SCHEDULE FOR CAMEMBERT CHEESE MADE FROM 40-LB. LOTS OF PASTEURIZED MILK INOCULATED WITH ENTEROPATHOGENIC STRAINS OF *E. coli*

Step	Time (h:min)	Temperature	pH
Add starter (363 ml/40 lb. milk)			
Add mold (1 agar slant/40 lb. milk)			
Add rennet (4.8 ml/40 lb. milk)	0:0	33.3 C (92 F)	
Cut curd	0:45	33.3 C (92 F)	6.22
Dip curd into hoops	1:05	33.3 C (92 F)	
Hoops turned first time	3:05	24-29 C (75-84 F)[1]	
Hoops turned second time	5:05	24-29 C (75-84 F)[1]	5.29
Add salt (69 g to cheese from 40 lb. milk)	23:50	(75-84 F)[1]	4.65

[1]Room temperature

Sixteen vats of Camembert cheese were made as outlined in Table 1. The cheese was ripened for 1 week in a humid chamber at 15.6 C (60 F) and then for up to 9 additional weeks at 10 C (50 F). Each strain of *E. coli* was used to inoculate two vats of cheese made with the commercial starter culture. Only *E. coli* 0128:B12 was used in tests with antibiotic-contaminated milk or when S. *cremoris* served as the starter culture. Again, each experiment was done in duplicate. Antibiotic-contaminated milk was prepared by adding sufficient penicillin so milk contained 0.3 unit antibiotic per milliliter. This quantity of penicillin is sufficient to markedly but not completely inhibit acid production by the starter culture *(9, 11)*.

All Camembert cheese (average fat in dry matter, 53.7%; average moisture content, 59.2%) made in these trials complied with the Federal standard (minimum of 50% fat in dry matter, no requirement for moisture) for this product. The schedule for sampling the cheese is indicated in Fig. 1 and 2.

Enumeration of enteropathogenic E. coli

A Most Probable Number (MPN) technique was used to enumerate enteropathogenic *E. coli* in samples of milk, curd, or cheese. Each value reported in Fig. 1 and 2 represents the average MPN of two trials.

Twenty grams of curd or cheese were blended (Waring blendor) for 3 min with 180 ml of sterile 2% sodium citrate solution. All subsequent dilutions also were made in the sodium citrate solution. One-milliliter quantities of the appropriately diluted samples were added to tubes with 9 ml of nutrient broth. After incubation for 24 h at 37 C, some material from each tube was streaked onto EMB agar (Difco) and plates were then incubated at 37 C for 24 h. Typical *E. coli* colonies were picked and streaked onto a blood base agar (Difco) slant in preparation for a serological test to confirm that the isolate recovered from the sample was the same strain of *E. coli* that was used to inoculate the milk. Growing the *E. coli* on blood base agar served to minimize problems with false-positive and cross reactions. The slide agglutination test and OB antisera (Difco) were used to confirm that the isolates were of the same *E. coli* strain that was added to milk when cheese was made.

Measurement of moisture, pH, and fat

Approximately 3 g of cheese in an aluminum foil moisture dish (Sargent-Welch no. S-25725) were dried for 16 h in a forced-draft air oven at 110 C. The percent of weight lost was considered to be the percent moisture. The pH of milk was measured with a Beckman pH meter equipped with glass electrodes, whereas the pH of cheese was determined with a saturated calomel half-cell, gold electrode, and a Leeds and Northrup portable potentiometer. Fat in cheese was determined by the Babcock procedure.

RESULTS AND DISCUSSION

Behavior of E. coli during cheese manufacture

Twelve vats of Camembert cheese were made using a commercial lactic starter culture and six strains of *E. coli* (Fig. 1). Two vats of cheese were made using each strain of *E. coli*. Average values from each set of two vats appear as Cheese No. 1-6 in Fig. 1. Two additional vats of cheese were made using the commercial lactic starter culture, *E. coli* 0128:B12, and milk contaminated with penicillin (average values from the two vats of cheese appear as Cheese No. 7 in Fig. 2). Another two vats of cheese were made using S. *cremoris* C_1, *E. coli* 0128:B12, and normal

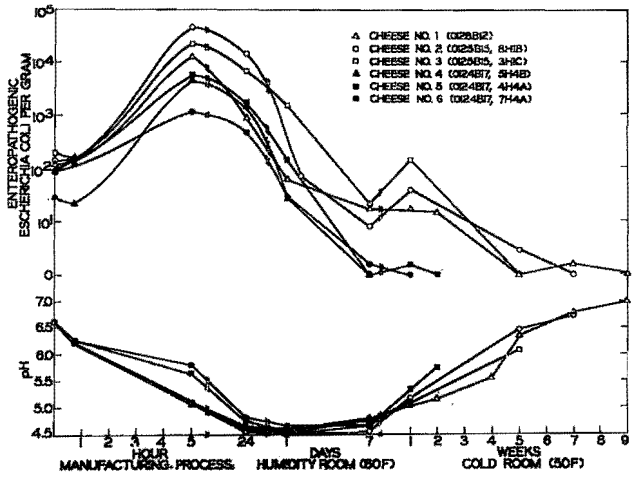

Figure 1. Behavior of enteropathogenic strains of *E. coli* during the manufacture and ripening of Camembert cheese made with a commercial starter culture.

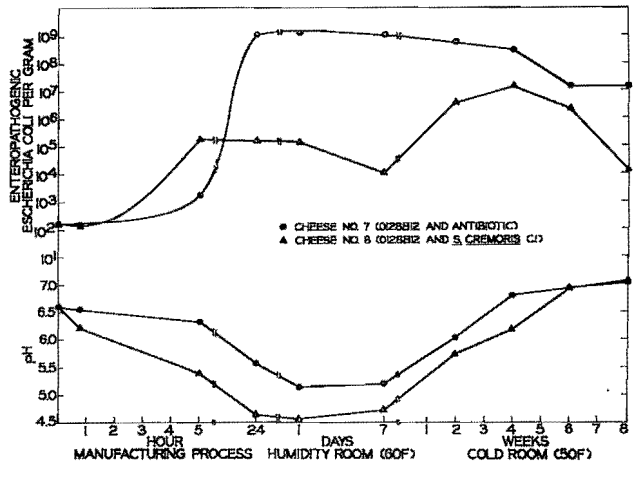

Figure 2. Behavior of an enteropathogenic strain of *E. coli* during the manufacture and ripening of Camembert cheese made from milk contaminated with penicillin (Cheese No. 7) or with S. *cremoris* C_1 (Cheese No. 8).

milk (average values from the two vats of cheese appear as Cheese No. 8 in Fig. 2).

Relatively little growth of *E. coli* occurred during the first 45 min of the cheesemaking operation during which the rennet acted to coagulate milk held at 33 C (Fig. 1, Table 1). A marked increase in numbers of *E. coli* appeared during the interval between cutting the curd and turning hooped cheese for the second time. The increase during this time can be attributed both to growth and concentration of cells through entrapment in the curd. Such entrapment accounts for a 10-fold increase in numbers and the balance of the increase resulted from growth of *E. coli.*

After taking into account the increase through entrapment of cells in curd, an average generation time of 38 min was calculated for growth of *E. coli* in experiments reported in Fig. 1. This compares favorably with an average of 32 min reported as the generation time for coliform bacteria during the interval between adding rennet to milk and milling the curd in the manufacture of Cheddar cheese *(21).* It also agrees well with the values of 35 and 36 min reported as the generation time for *Salmonella typhimurium* during the same segment of the procedure used to make Cheddar cheese *(6, 14).* Generation times during the 260-min segment of the Camembert cheesemaking operation mentioned before were different when S. *cremoris* C_1 (23 min) or antibiotic-contaminated milk (69 min) were used.

A marked decline in numbers of *E. coli* occurred during overnight storage of all cheeses made with normal milk and the commercial lactic starter culture. The pH of the cheese averaged 4.65 at this point and the acid probably inactivated a portion of the *E. coli* population. Other investigators *(5, 8, 20, 21)* also have noted that coliform bacteria are either inhibited or inactivated by pH values of 5.0 or below when they occur during production of Cheddar or cottage cheese or in other cultured products.

The marked decline in number of *E. coli* during overnight storage of hooped cheese failed to occur when cheese was made with S. *cremoris* C_1 or from milk contaminated with penicillin (Fig. 2). In fact, *E. coli* grew most rapidly during this time in the latter cheese and approached a population of 10^9/g. The pH values of cheese made in these trials did not drop as rapidly or as low as when normal milk and a commercial lactic starter culture were used (Fig. 1). This may account for growth of *E. coli* in one and no inactivation in the other instance.

Behavior of E. coli during ripening of cheese

After overnight storage, cheese was removed from hoops, salt was rubbed on the outside, and cheese was moved to storage at 15.6 C (60 F) and high humidity. During the first day at 15.6 C (60 F) there was a further decline in number of *E. coli* in cheeses made with the commercial lactic starter culture (Fig. 1). This decline was not evident in cheese made with S. *cremoris* C_1 or milk that contained penicillin (Fig. 2).

The number of viable *E. coli* continued to decline during storage at 15.6 C (60 F) of cheese made with the commercial starter culture. In fact, cheese made with *E. coli* 0124:B17 (5H4B) was free of viable cells of this bacterium at the end of the 1-week storage at 15.6 C (60 F). Numbers of *E. coli* 0124:B17 (4H4A) and 0124:B17 (7H4A) in cheese were very low at this point. In contrast to these observations, 7 days at 15.6 C (60 F) had essentially no effect on *E. coli* in cheese made from milk that contained penicillin. There was a 10-fold decline during this time in the population of *E. coli* in cheese made with S. *cremoris* C_1. The number of *E. coli* approximated 10^4/g at this point which is the lowest population achieved in such cheese during the entire ripening process.

Storage at 10 C (50 F) of cheese made with the commercial starter culture was marked by a further decrease in numbers of viable *E. coli* (Fig. 1). Cheese made with *E. coli* 0124:B17 (4H4A) was free of viable cells of the bacterium, as measured by our tests, after 1 week at 10 C (50 F). Another week was required to do the same when cheese contained *E. coli* 0124:B17 (7H4A). Five and 7 weeks at 10 C (50 F) were required to free cheese of *E. coli* 0125:B15 (3H1C) and 0125:B15 (8H1B), respectively, whereas *E. coli* 0128:B12 persisted in the cheese for > 7 but < 9 weeks. Two strains of *E. coli* appeared to grow slightly during the first week at 10 C (50 F). This growth coincided with an increase in the pH of the cheese.

Storage at 10 C (50 F) failed to eliminate viable cells of *E. coli* from cheese made with S. *cremoris* C_1 or milk that contained penicillin (Fig. 2). Growth of *E. coli* in cheese made with S. *cremoris* C_1 was clearly evident during the first one-half of the holding period. This was followed by a decline in numbers during the second one-half but the population at the conclusion of ripening still exceeded 10^4/g. The population of *E. coli* in cheese made from milk with penicillin declined only slightly during storage at 10 C (50 F) and such cheese still contained $> 10^7$/g when ripening was completed.

Limited growth of *E. coli* in Camembert cheese during storage at 10 C (50 F) when the pH of the cheese had increased caused us to further explore the possibility that surviving cells might initiate growth at this time and cause the cheese to become

highly contaminated with *E. coli* at the end of the ripening period. Two-month old Camembert cheese, pH 6.4, was contaminated with *E. coli* 0128:B12 to provide 10^2-10^3 cells/g. The cheese and bacteria were thoroughly mixed, and portions were stored at 10 C (50 F) and 15.6 C (60 F). Viable *E. coli* could not be recovered from the cheese two weeks later. Although these limited observations do not provide additional information about the apparent limited growth of some *E. coli* strains in cheese at (50 F), they do suggest that ripened Camembert cheese is unfavorable for growth of this bacterium. The reason for lack of growth by *E. coli* in the ripened cheese remains to be determined.

It is evident from the data obtained in these experiments that the behavior of *E. coli* was variable during manufacture and ripening of Camembert cheese. This variation was directly related to *(a)* the kind and activity of the starter culture and *(b)* the strain of *E. coli.*

Production of Camembert cheese with few if any *E. coli* cells can be achieved by *(a)* use of adequately pasteurized antibiotic-free milk, *(b)* preventing contamination of the milk after it is pasteurized, and *(c)* use of an active lactic starter culture that will promptly produce sufficient acid so that a pH value of approximately 4.7 will be attained in cheese within 24 h.

ACKNOWLEDGMENT

The authors thank Dr. J. C. Olson, Jr., Food and Drug Administration, for arranging to supply the cultures of *E. coli* used in this study. Thanks also go to Mrs. E. C. Christenson, Wisconsin State Laboratory of Hygiene, for her advice and assistance with the serological tests used to identify *E. coli* isolates.

REFERENCES

1. Agenjo, C. C. 1945. Study of food-poisoning caused by cheese. Trab. Inst. Biol. Anim., Madrid 8:265-290.

2. Barnard, R., and W. Callahan. 1971. Follow-up on gastroenteritis attributed to French cheese. Morbidity Mortality 25:445.

3. Brag, H., and A. Kämpe. 1969. Hygienic quality of Camembert cheese. Svensk. Vet. Tidn. 21:78-80.

4. Dobrier, I. B. 1961. The incidence of pathogenic serotypes of coliform bacteria in foods. Gigiena i Sanit. 26:104-106.

5. Goel, M. C., D. C. Kulshrestha, E. H. Marth, D. W. Francis, J. G. Bradshaw, and R. B. Read, Jr. 1971. Fate of coliforms in yogurt, buttermilk, sour cream, and cottage cheese during refrigerated storage. J. Milk Food Technol. 34: 54-58.

6. Goepfert, J. M., N. F. Olson, and E. H. Marth. 1968. Behavior of *Salmonella typhimurium* during manufacture and curing of Cheddar cheese. Appl. Microbiol. 16:862-866.

7. Hall, H. E., D. F. Brown, and K. H. Lewis. Examination of market foods for coliform organisms. Appl. Microbiol. 15:1062-1069.

8. Harmon, L. G., and C. K. Smith. 1956. Influence of environment and processing on spoilage organisms in cottage cheese. J. Milk Food Technol. 19:252-259.

9. Jezeski, J. J., H. A. Morris, E. A. Zottola, E. George, and F. F. Busta. 1961. The effect of penicillin in milk on the growth and survival of *Staphylococcus aureus* during the manufacture and curing of Cheddar and Colby cheese. J. Dairy Sci. 44:1160. (Abstr.)

10. Manzullo, A., and D. J. Estevez. 1965. Investigation for the presence of pathogenic *Escherichia coli* in raw milk and cream in a zone of Buenos Aires. Revta Asoc. Med. Argent. 79:36-41.

11. Marth, E. H., and B. E. Ellickson. 1959. Problems created by the presence of antibiotics in milk and milk products—A review. J. Milk Food Technol. 22:266-272.

12. Ogawa, H., A. Nakamura, and R. Sakazaki. 1968. Pathogenic properties of enteropathogenic *Escherichia coli* from diarrheal children and adults. Japan J. Med. Sci. Biol. 21:333-349.

13. Park, H. S., E. H. Marth, and N. F. Olson. 1972. Behavior of enteropathogenic strains of *Escherichia coli* during manufacture of Camembert cheese. J. Dairy Sci. 55:669. (Abstr.)

14. Park, H. S., E. H. Marth, J. M. Goepfert, and N. F. Olson. 1970. The fate of *Salmonella typhimurium* in the manufacture and ripening of low-acid Cheddar cheese. J. Milk Food Technol. 33:280-284.

15. Russell, H. L. 1895. Gas-producing bacteria and the relation of the same to cheese. Wis. Agr. Expt. Sta. 12th Annu. Rep., pp. 139-150.

16. Sakazaki, R., K. Tamura, and M. Saito. 1967. Enteropathogenic *Escherichia coli* associated with diarrhea in children and adults. Japan J. Med. Sci. Biol. 20:387-399.

17. Schnurrenberger, L. W., R. Beck, and J. Pate. 1971. Gastroenteritis attributed to imported French cheese. Morbidity Mortality 20:427-428.

18. Shifrin, I. A., and R. Y. Osatilovskaya. 1963. Outbreak of food poisoning caused by *Escherichia coli.* Gigiena i Sanit. 28:80-82.

19. Singh, R. S., B. Ranganathan, and H. Laxminarayna. 1970. Incidence of pathogenic *Escherichia coli* in milk and milk products. Proc. 18th Intern. Dairy Congr. 1E:148.

20. Skelton, W. R., and L. G. Harmon. 1964. Growth rate of coliform organisms in cottage cheese and reconstituted nonfat dry milk. J. Milk Food Technol. 27:197-201.

21. Yale, M. W., and J. C. Marquardt. 1943. Coliform bacteria in Cheddar cheese. New York Agr. Exp. Sta. Tech. Bull. No. 270.

22. Yang, H. Y., and G. A. Jones. 1969. Physiological characteristics of enteropathogenic and non-pathogenic coliform bacteria isolated from Canadian pasteurized dairy products. J. Milk Food Technol. 32:102-109.