DESTRUCTION OF *E. COLI*, STRAIN W, DURING THE MANUFACTURE OF COTTAGE CHEESE

by

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Dedicated to my wife,

Morella

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

The percapita consumption of cottage cheese in the U.S. has increased from 1.2 pounds per person in 1930 to 4.8 pounds in 1976. This is due largely to its pleasant eating quality and excellent nutritional value.

From the public health point of view, it is important that cottage cheese be produced so that it is free from harmful organisms. The manufacturing procedures by which cottage cheese is produced make it one of the most perishable of all dairy products. The presence of coliform organisms in it for example is not necessarily excluded during processing. Spoilage of cottage cheese by coliform bacteria is one of the most important and common defects and occurs even at refrigeration temperature. Since cottage cheese is usually stored under refrigeration, growth of microorganisms is restricted to those able to grow at low temperatures. E. coli is not normally considered a psychrophilic, cold-loving, organism, but some genera are able to grow and multiply at low temperatures.

Inadequate cooking temperatures are considered to be responsible for the presence of coliform organisms in finished cottage cheese.

The objective of this study was to determine a minimum cooking temperature during the manufacture of cottage cheese necessary to completely destroy one of the most heat resistant coliform organisms, Strain W, E. coli.

REVIEW OF LITERATURE

Characteristics of Coliform Organisms

a. Source. Coliform organisms are generally lactose (+), non-spore-forming, Gram (-) rods, accepted to be either from the intestinal tract of man and warm-blooded animals (15, 43, 45) or from non-fecal origin (15). Their presence in milk products is regarded as an indication of unsanitary methods of manufacture (5, 11, 25, 36, 45).

Crossley (6) emphasized that coliform bacteria found during the manufacture of cheese do not come from excretal pollution. They occur as a result of adaption to peculiar conditions in each product which make it possible for them to survive and grow. Nielsen (27) observed that even in the best operated cheese plant, coliforms are obiquitous. Their presence in pasteurized milk at the time of setting is due to post-pasteurization contamination, improper handling of milk after pasteurization allowing multiplication of those organisms normally present, survival of pasteurization as a result of large concentration in the raw milk, inadequate pasteurization and heat resistant strains (5, 9).

b. Disease potential. Cheese as supplied to the consumer is ready to eat and thus the presence of coliform bacteria in cheeses is of concern to the industry as a potential health hazard (46). Some strains of E. coli are, in fact, pathogenic, causing diarrhea and food poisoning in infants (2, 11, 18, 19,

20, 28, 41). Although cheese has been shown to cause food-borne illness, epidemiological experiences place it among the relatively infrequent vehicles. However, when such outbreaks do occur, they usually involve a large number of people (13, 14, 40). Nielsen (30), Tuckey (47), and Crossley (6) however, reported that these organisms in cottage cheese have no public health significance other than indicating poor sanitation and process control. Nielsen (30) and Tuckey (47) also states that outbreaks of gastroenteritis caused by coliform bacteria are absent or extremely rare.

c. Heat resistance. The time-temperature relationship necessary for the destruction of coliform bacteria in milk has been reviewed in detail by Craige (5), Read et al. (38), and Yang and Jones (49). Thermal death studies showed that one or more coliform strains might survive a commercial high-temperature process if the number of cells present in raw milk were unusually large (5, 32, 49). Of the various heat resistant strains of coliforms, the one that has received most attention is E. coli, Strain E. (4).

Collins (4) found an average D value of 11.66 min at 51.5 C (125F), and 5.04 min at 55 C (131F) for Strain E, E. coli. Another strain of E. coli, Strain W (ATCC 9637) when heat-treated at 55, 60, and 62.5 C in commercially sterilized whole milk had a survival time of more than 140 min, 10 min, and 2 min, respectively, when recovered on VRB agar (7). This same strain was tested in raw milk by Read et al. (38) and shown to have mean D-values of 28.2, 5.1, 1.3, 0.00195,

0.00100, 0.00055, 0.00029 and 0.00016 min at 51.5, 54.0, 57.2, 76.0, 77.0, 78.0, 79.0, and 80.0 C, respectively.

Effect of Media on Coliform Organisms

a. Milk. There have been few publications reporting the growth patterns of coliform organisms in milk but it is accepted that they usually are present in raw milk. They may grow profusely on equipment and in milk if good sanitary control is not exercised (35, 43).

Yang and Jones (49) reported that viable counts of nonpathogenic E. coli Strain E suspended in skim milk at 7 C decreased with time; counts of a Strain A (Enterobacter aerogenes) and strain PE (enteropathogenic E. coli) declined during the first 2 to 4 days of storage, but thereafter increased rapidly.

b. Cottage cheese. Cottage cheese is a nutritious food not only for humans but also for numerous microorganisms. Consequently it is a highly perishable product and its market possibilities are often restricted by its short shelf-life (23, 25). Coliform organisms gain access to cottage cheese (or any cheese) from the milk and equipment, and occasionally from the starter. They may multiply to large numbers (11) in cheese and cause holes and objectionable flavors. In addition they may pose a public health treat (10).

Overcast and Britton (33) reported little change in coliform counts during storage of cottage cheese at 5 C but Skelton and Harmon (42) found considerable increase when

stored at 13 C. Goel et al. (17) found decreases in coliform counts in cottage cheese stored at 7 C, although in some instances they increased.

Adequate acid development in the curd in the cheese vat is one of the most effective means of limiting coliform numbers and their effect on cottage cheese (29). Goel et al. (17) also found that the pH of most cottage cheese samples declined during storage, however, this change often was not related to either survival or demise of coliform organisms.

c. Cottage cheese whey. Little is known on the effect of cottage cheese whey on coliform organisms.

Numbers of Coliform Organisms in Cottage Cheese

a. Standards. The U.S. Public Health Service and the Canada Department of National Health and Welfare require that cottage cheese contain less than 10 coliform organisms/g (12, 15, 29). Surveys in Canada, Kansas, Tennessee have shown that more than half of the cottage cheese samples examined contained more than 10 coliform organisms/g (39). Overcast et al. (34) observed a wide variation in coliform content in cottage cheese, with counts ranging from <1 to 751,000/g with a median of 422/g. Another survey in Connecticut, Iowa, Illinois, and Michigan indicated that greater care and stricter sanitary precautions are needed in the production and handling of cottage cheese (26).

The production of coliform-free cottage cheese is important since small number of these organisms in the finished

product are able to multiply during storage. Such increases result in the sale of cottage cheese that exceeds the legal maximum number of coliform organisms (39).

b. Keeping quality or shelf-life of cottage cheese. Keeping quality is commonly regarded as one of the chief problems, if not the most important problem, in the manufacture and merchandizing of cottage cheese (12). It depends largely on how many and what kinds of bacteria and other microorganisms gain access during and after its manufacture (33).

Surveys as enumerated by Emmons (12) in his review, as well as studies by Harmon et al. (22), and Morgan et al. (27), showed that the organisms most often causing spoilage of cottage cheese were molds, yeast, coliforms, psychrophilic bacteria, and acid-forming bacteria such as the starter bacteria. Bitter, musty, putrid, yeasty, acid and other off-flavors have been reported in many samples of cottage cheese (22, 26).

From the commercial point of view cheese manufacturers concern about coliform bacteria is related primarily to the defects these microorganisms can cause, such as gassiness and unclean flavors (29). Gas formation in cottage cheese indicates a coliform count in excess of 1×10^7/g.

In recent years there have been many changes in the manufacture of cottage cheese; from using raw skim milk, uncontrolled manufacturing methods, that yielded a poor quality and short shelf-life; to using pasteurized skim milk, improved stainless steel equipment, scientifically controlled

manufacturing methods, improved lactic cultures, new frozen concentrated cultures, direct acid method of manufacturing, resulting in a more uniform quality and long shelf-life cottage cheese (1). There has been a trend toward fewer and larger plants with more sophisticated equipment and skilled personnel. Under carefully controlled plant conditions, cottage cheese can be manufactured with a shelf-life of at least 15 days, with development of a little or no microbial off-flavor or visual defect (33).

Methods of Analysis of Coliform Organisms

a. Characterization. The coliform bacteria are members of the family Enterobacteriaceae. Included in this family are facultatively anaerobic, Gram-negative, non-spore-forming rods; also included are pathogenic groups such as Salmonella, Shigella and Yersenia. The coliform organisms can be differentiated from most other members of the family by their ability to ferment lactose with the production of acid and gas within 48 hrs. Typically coliforms are classified in the genera Escherichia and Enterobacter (formerly Aerobacter). In addition however, a few lactose-fermenting species of other genera are included in this group (8, 43).

Coliform organisms have been reported to grow in a variety of culture media and under rather diverse physical and chemical conditions. Growth has been reported to occur over the temperature range -2 to 50 C and at pH values from 4.4 to 9.0. They grow well on nutrient agar and generally produce

visible colonies within 24 hrs at 37 C in the presence of bile salts, which inhibit the growth of most Gram-positive bacteria (8).

b. Methods of enumeration. Among the techniques that may be employed to isolate and enumerate coliform organisms are direct plating methods, the most probable number (MPN), and the membrane filter techniques. In direct plating, one may employ violet red bile (VRB) agar, deoxycholate-lactose agar or other suitable media. When VRB agar is used coliforms appear as typical red colonies (normally measuring at least 0.5 mm in diameter) within 24 $\pm$ 2 hrs after incubation at 32 C. The MPN technique is of more value in detecting small numbers of coliform organisms than the direct plating methods. By this technique populations are estimated on the basis of numbers of tubes of broth-media exibiting gas following a 48 $\pm$ 3 hrs incubation at 32 C. Generally three or five tubes are inoculated with a given quantity of sample. Hall (21) reported that lauryl sulfate tryptose (LST) broth was more effective than brilliant green lactose bile (BGLB) for numerating coliforms by the MPN procedure. Results from the primary inoculation of VRB agar and LST broth tubes are presumptive and should be confirmed by transferring typical colonies on solid media or aliquots from gas positive LST broth tubes to brilliant green lactose bile (BGLB) broth.

The presence of gas in the latter media indicates a positive confirmed-test. If the confirmed test is positive, the completed test is carried out by transferring aliquots

from BGLB broth onto appropriate media such as eosin methylene blue (EMB) agar or endo agar (EA), with the production of typical or atypical colonies. The pure coliform culture so isolated will produce gas in lactose broth fermentation tubes at 32 C within 48 hrs, and will consist only of gram-negative, non-spore-forming rod shape bacteria. The membrane filter technique is recommended as an alternative method for the examination of water and clear beverages. Standard Methods for the Examination of Dairy Products should be consulted for more specific directions (8, 43, 45).

Distinguishing fecal coliform. In order to distinguish between E. coli (generally considered to be fecal in origin) and E. aerogenes (nonfecal) the IMViC pattern of tests of the coliform isolates should be determined. These two organisms are identified as follow:

	I	M	V	C
E. coli	+	+	-	-
E. aerogenes	-	-	+	+

where I= indole production; M=methyl red reaction; V= Vogues-Proskauer reaction (acetoin production); and C= citrate utilization. It should be noted that other coliform types exist and are apt to provide a variety of similar patterns by the IMViC tests, intermediate between the two extremes.

Fecal coliforms are determined by the use of elevated incubation temperatures and the following two techniques: a.) The MPN method employing EC broth and a carefully controlled temperature of 45.5 $\pm$ 0.2 C; and b.) The membrane filter

technique using M-FC (M-Fecal Coliform) broth-soaked pads (8, 21, 45). Other techniques to enumerate or detect fecal coliform organisms are described by Defigueiredo and Splittstoesser (8) and Thatcher and Clark (45).

c. Diluent used in plating. Peptone added to distilled water was tested as a diluent by Straka and Stokes (44) and was proved to have advantages over distilled water or phosphate diluent for recovering bacteria. Oblinger and Kennedy (31) reported that Butterfield's diluent and perhaps 0.5% or 0.1% peptone may offer more consistent recoveries of bacteria from a variety of food samples than distilled water and 0.85% NaCl. King and Hurst (24) in 1963 demonstrated that the best diluent for four bacterial species, including E. coli, was 0.1% (w/v) peptone solution.

d. Surface and pour plate methods. Although both the surface and pour plate methods are used extensively for determination of total counts of microorganisms, few comparisons of the relative recoveries efficiencies of the two techniques have been published. Clark (3) in 1967 compared the two techniques and found that the surface plate method was 70% and 80% higher than the pour plate method at incubation temperatures of 0 C and 22-25 C. Punch and Olson (37) also compared the two techniques for estimating psychrophilic bacteria in milk. They found that both counts were equivalents and that colonies on the surface plate were larger, more uniform in size, and consequently easier to count than those on pour plates. Also enhanced pigmentation and more characteristic colony morphology was observed with the surface plate method.

EXPERIMENTAL MATERIAL AND METHODS

Experiment 1: Changes in Coliform Counts During Various Cottage Cheese Manufacturing Stages

Introduction. Fresh pasteurized skim milk (71.6 C/16 sec.) for the manufacture of cottage cheese was obtained from the KSU Dairy Processing Plant. The milk was inoculated with coliform organisms (E. coli) at the time of making cheese so as to have counts of 2.5×10^4 and 4.0×10^5 coliforms/ml. The low and high levels were used to compare the number of surviving coliform organisms in milk, whey and curd during the manufacture of cottage cheese. In Experiment 1, 4 vats of cottage cheese were made with the low concentration of organisms (2.5×10^4) and 4 with the high concentration (4.0×10^5).

Selection and preparation of E. coli cultures. A heat resistant E. coli, Strain W, reported by Read et al. (1961) (38) was obtained from the American Type Culture Collection (ATCC no 9637) and used in this experiment.

The freeze-dried stock culture first was cultured on nutrient agar slants at 4 C and transferred at 2-week intervals. A portion of the culture on the agar slant was transferred with a loop into 100 ml of 10%, autoclaved, nonfat dry milk (NFDM). One milliliter was transferred every day to maintain the organisms in the log phase of growth; incubated at 32 C for 12 hrs, and then held in a refrigerator until used in the experiments. Cell counts of inoculums were kept relatively constant between 10^6 and 10^7 coliforms/ml. A measured

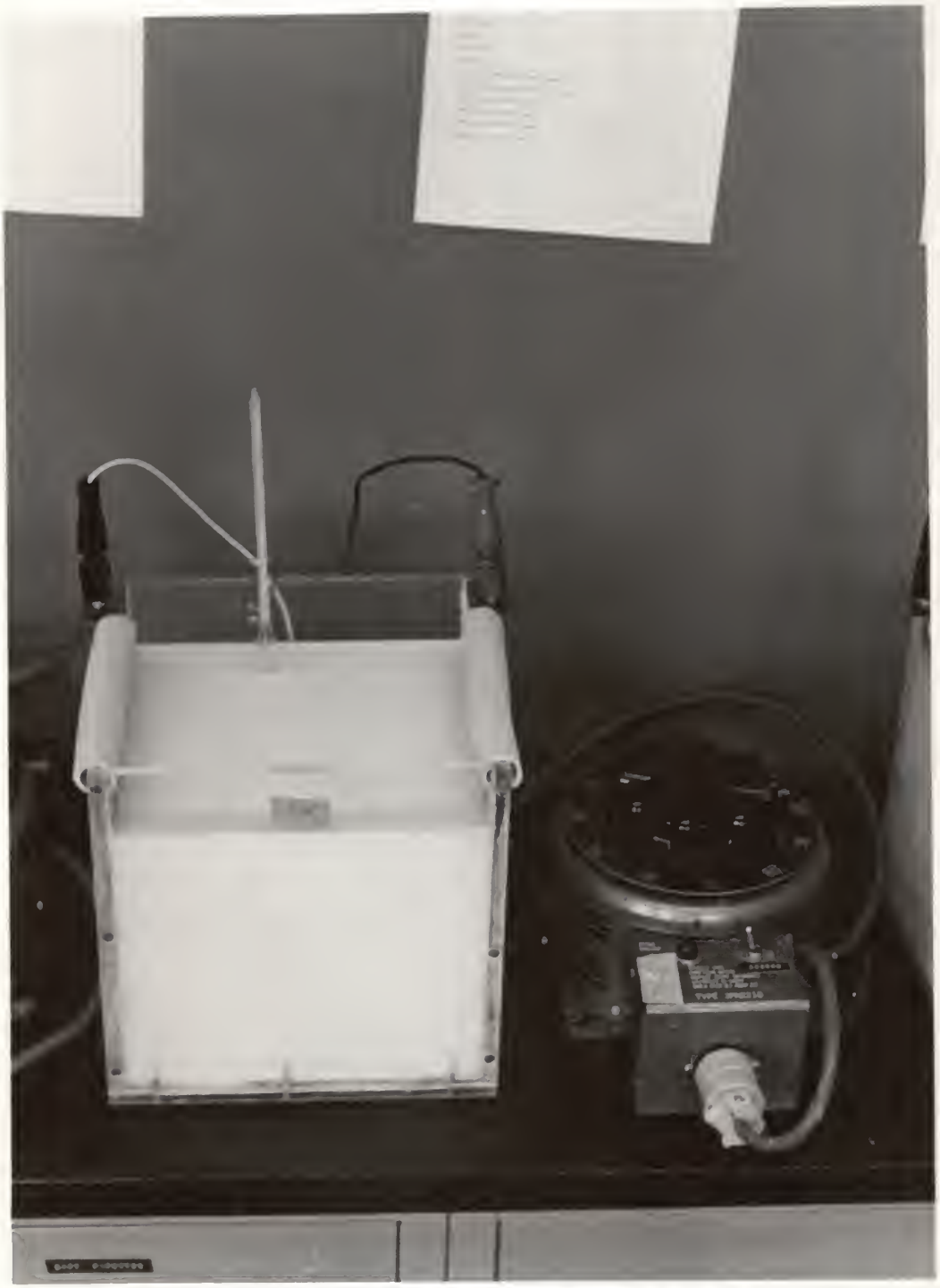
amount of inoculum was added each time to milk at the time of setting the cheese milk, to have a final count of either 2.5×10^4 or 4.0×10^5 coliforms/ml.

Manufacture of cottage cheese by the direct-acid-set method. The experimental cottage cheese was manufactured in a 10-liter vat made of 1 cm thick plexiglass with base dimensions of 21.5 x 21.5 cm and a height of 23.75 cm. The vat was fitted with two stainless steel-plate electrodes at opposite sides of the vat and connected to a 110 volt alternating current (Fig. 1). The voltage and the rate of heating was controlled by an in-line rheostat.

The direct-acid-set method developed by Vitex Laboratories (the so-called in-line acidification method (16)), was modified to facilitate the manufacture of cottage cheese in the 10-l experimental vat.

Refrigerated skimmilk was acidified to pH 4.95 to 5.00 with a special food grade acidulant (Vitex 750) which was diluted with an equal volume of distilled water. Acidified milk then was heated to 26.5 ± 0.5 C, pH was measured and a specified amount of D-glucono-delta lactone (GDL) weighed according to the pH value of the heated-acidified milk. The temperature was increased to 32 C and the GDL dissolved in water (1 Kg GDL/1.386 l of water) and mixed into the milk. After this, Vitex coagulator (51.6 m./378 l of milk) was diluted with at least 5 times its volume of room temperature distilled water and added to the milk. The milk in the vat was allowed to set and coagulate for 60 ± 5 min. The curd

Figure 1. Experiment cheese vat with electrical heating apparatus.



was cut at a pH of 4.6 - 4.7 using horizontal and vertical 1-cm knives, and acidified with Vitex 750 (88.5 ml/378 l of milk) to prevent matting. The curd was cooked and stirred constantly up to 54 C where it was held 15 min.

Sampling stages for microbiological analysis. Samples of milk, whey and curd were taken and analyzed for coliform counts at nine (9) different stages during the manufacture of cottage cheese. These sampling stages were:

1. Milk inoculated with E. coli (4 C)
2. Milk after acidification (pH 4.95 to 5.00)
3. Milk at 32 C
4. Whey + curd mixture (after cutting 32 C)
5. 38 C
    a. Whey + curd mixture
    b. Whey
6. 43 C
    a. Whey
    b. Curd not washed
    c. Curd acid washed
    d. Curd acid and chlorine washed
7. 49 C
    a. Whey
    b. Curd not washed
    c. Curd acid washed
    d. Curd acid and chlorine washed
8. 54 C
    a. Whey

b. Curd not washed

c. Curd acid washed

d. Curd acid and chlorine washed

9. 54 C + 15 min holding

a. Whey

b. Curd not washed

c. Curd acid and chlorine washed

References will be made to these stages in designating samples throughout the result and discussion sections of the thesis.

Samples preparation. Milk and whey samples were drawn from the full, agitated vat by using 5 ml sterile pipettes and dispensed into sterile screw cap test tubes. Curd samples were obtained from the vat with a sanitized metal spoon, drained for 20 sec on a sanitized sieve, and 11 g weighed aseptically into a sterile, tared blender jar. For the washed curd samples about 50 g of curd particles was collected from the vat and placed in a beaker. Water acidified to pH 5.00 at 27 C was added and the mixture allowed to set undisturbed for 15 min. After that, the water was drained and a sample of 11 g weighed. Acidified water at pH 5.00 containing 10 ppm chlorine at 5 C was added to the remaining curd particles and held for 15 min, the water then was drained and 11 g weighed. Whey plus curd samples were drawn also with a sanitized metal spoon and 11 g weighed (approximately half whey and half curd particles). All curd and whey—plus-curd samples were blended in sterile, 200 ml, Mason jars with 99 ml 0.1% peptone water

for 30 sec. Two 5-sec interspaced stops were incorporated to allow the curd particles to contact the blades for more effective cutting.

Enumeration of E. coli organisms. All routine coliform counts were made using Difco dehydrated violet red bile (VRB) agar, prepared according to Difco's directions 24-48 hrs prior to use.

Plates for the pour plate method were prepared according to the standard procedure recommended in Standard Methods (43). After solidification these plates were dried for an hour at 32 C; the plates were opened and 1/3 of the surface exposed in order to evaporate excess moisture and avoid the formation of spreaders. The surface plate method was followed as described by Punch and Olson (37), excepts that VRB agar was used and that the rods were maintained in a beaker containing alcohol. Prior to spreading the inoculum, the rods were flamed.

A special procedure (48) was used in order to get dilutions of 10^0 from the curd samples. It consisted of preparing tubes with 10 ml of double concentrated VRB agar, cooled to 44-46 C and adding 10 ml of the blended curd sample. The mixture was well mixed and poured in a petri dish. The plate was then dried as indicated in the pour plate technique. All plates were inverted and incubated for 24 $\pm$ 2 hrs at 32 C. Colonies were counted as indicated in the Standard Methods (43) and recorded.

One tenth percent (w/v) peptone solution was used

throughout this experiment for all dilutions.

Experiment 2: Changes in Coliform Counts of Cottage Cheese Whey and Curd Held for up to 60 Min at 38 - 54 C

Introduction. In this experiment samples of cottage cheese whey and curd were held in water baths at constant temperatures (38, 43, 49, and 54 C) for up to 60 min and samples analyzed for coliform organisms every 10 min. Six vats of cottage cheese were made by the direct-acid-set method with only one level of inoculation with Strain W, *E. coli* (4.0×10^5). Three vats were used to prepare whey samples and three for curd samples.

Sample preparation. Samples of whey were collected from experimental cottage cheese vats by using a sanitized metal soup spoon while the whey and curd were agitated at the following temperatures: 38, 43, 49, or 54 C. The whey was poured through a sanitized sieve into previously warmed 800 ml, sterile, wide mouth Mason jars. Approximately 300 ml of whey were collected at each temperature in this manner. At 0 min, samples were removed from the jars with sterile 5-ml pipettes, poured into a sterile screw cap test tubes and using appropriate dilutions, plated immediately as in Experiment 1. The jars containing the remaining whey were placed in corresponding previously set-up water baths at the respective temperature (38, 43, 49, or 54 $\pm$ 0.5 C).

Samples of cottage cheese curd were removed in sanitized metal spoons and excess whey drained for 10 sec in sanitized

sieves. The curd particles were transferred to Mason jars. At 0 min, 11-g samples were weighed, blended, diluted and plated as in Experiment 1. The jars, each containing approximately 200 g of curd, were placed in the appropriate water baths. Samples of curd were removed every 10 min for up to 60 min and plated immediately.

Statistical analysis. A 2-factor split-plot factorial design was used to analyze the data in this experiment. The main units tested were difference between temperatures (38, 43, 49, and 54 C) and subunits tested were difference between times (0, 10, 20, 30, 40, 50, and 60 min).

The model used was:

$$Y_{cb} = u + \alpha_c + \beta_b + (\alpha\beta)_{cb} + \gamma_m + (\alpha\gamma)_{cm} + E_{cbm}$$

where:

Y_{cb} = response (log coliform count).

u = mean.

α_c = temperature effects.

β_b = block effects.

$(\alpha\beta)_{cb}$ = Error (a) random experimental error, as a deviation from the mean, for the c^{th} treatment of the b^{th} block.

γ_m = time effects.

$(\alpha\gamma)_{cm}$ = interaction between temperature and time.

E_{cbm} = Error (b) random experimental error, as a deviation from the mean, for the cm^{th} treatment combination of the b^{th} block.

RESULTS AND DISCUSSIONS

Experiment 1: Changes in Coliform Counts During Various Cottage Cheese Manufacturing Stages

High level of inoculation (4.0×10^5 E. coli/ml). As can be seen in Fig. 2 there was little variation in the log counts of coliform organisms during the first five-stages of manufacture of cottage cheese. These sampling stages included: 1. The inoculated milk, 2. Milk after acidification to pH 5.00, 3. After heating to 32 C, 4. Mixtures of whey and curd after cutting, and 5. After heating to 38C.

A slight increase in coliform organisms was observed in the following transition stages: from 2 to 3; from 4 to 5; and from 5 to 6. This indicated that when heat was applied at these stages, the coliform organisms were reaching an optimum growth temperature. The stages 5 through 9 comprised the cooking stages. It was observed that a lower concentration of coliform organisms was found in the cottage cheese whey once it was separated from the cottage cheese curd particles (stage 6). It appears that a larger concentration of coliform organisms was entrapped in the curd particles.

At 43 C (stage 6), it was observed that the log coliform count of the curd was almost 2 log cycles greater than the whey. At stage 6, treating curd with water acidified to pH 5.00 and water acidified to pH 5.00 containing 10 ppm chlorine had little effect in destroying coliform organisms.

At 49 C (stage 7), the highest number of coliform

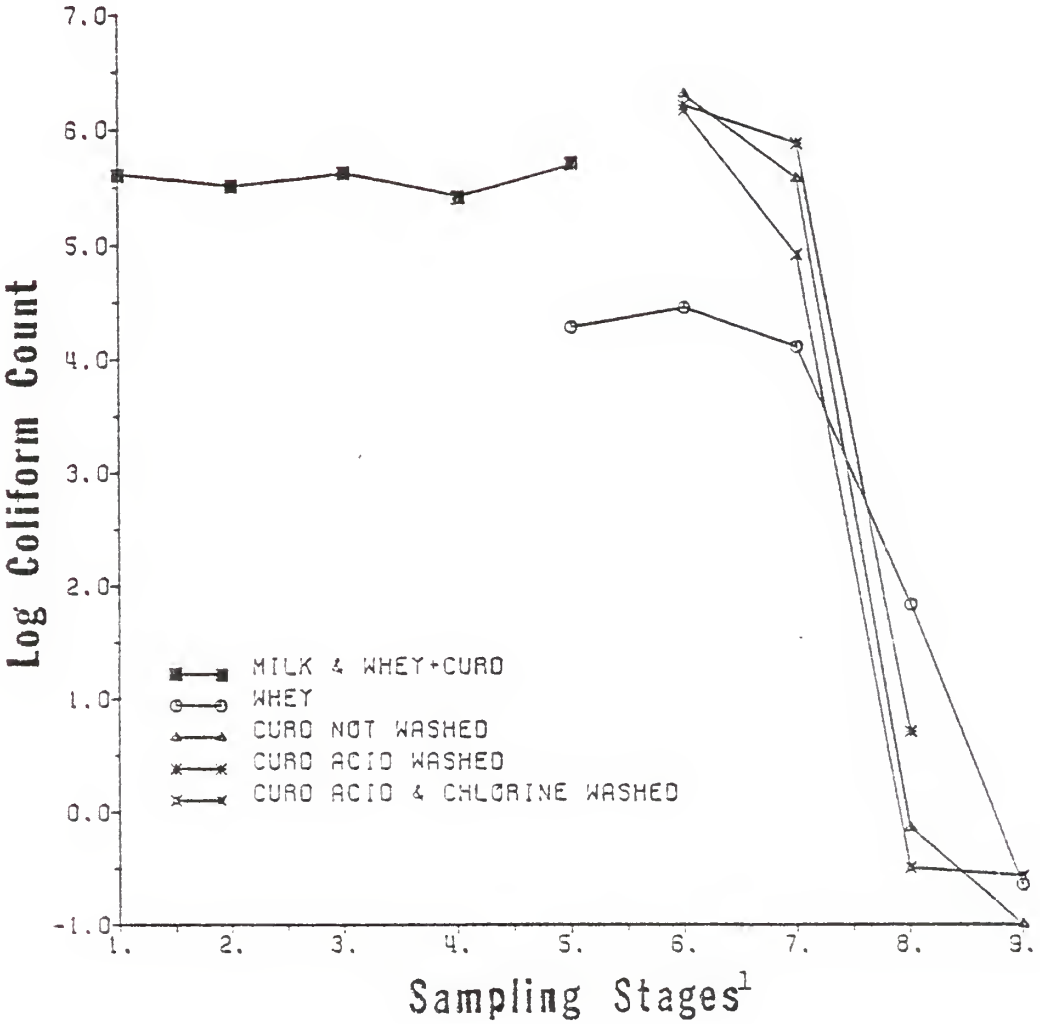


Figure 2. Log counts of coliform organisms at various stages during the manufacture of cottage cheese. Skim-cheese milk was inoculated with approximately 4.0 x 10^5 *E. coli*/ml.

[1]Stages at which samples were taken during the manufacture of cottage cheese: 1. Inoculated milk; 2. Milk at pH 4.95 - 5.00; 3. Milk at 32 C; 4. After cutting the curd; 5-8. Cooking temperatures (38, 43, 49, and 54 C); 9. 54 C + 15 min holding.

organisms was observed in the curd that was acid washed. The reason might be that when the curd particles at 49 C were put in contact with the wash water for 15 min that had a temperature of 27 C, the resultant temperature was approximately 30-32 C. This is the ideal growth temperature for the coliform organisms. This of course is speculative and more research is needed to support this theory. The acid and chlorine washing of the curd was not very effective in reducing the number of coliform organisms as observed by the slopes of these lines in Fig. 2. Chlorine water acidified to pH 5.00 accomplished two things: it increased the effectiveness and bactericidal activity of chlorine, and it reduced the tendency of the wash water to raise the pH of the curd during washing.

It is important to note that at 54 C (stage 8), the number of coliform organisms remaining in the whey was higher than in the curd particles. Since the whey was usually at the top of the vat, it might be that the heating action was less at that particular location of the vat due to evaporative cooling. However, at 54 C complete destruction of coliform organisms was observed in both the curd and the whey held for 15 min.

Fig. 3 presents the same data shown in Fig. 2, with data presented as percent of the original number of coliform organisms surviving. In this graph it can be seen that the number of coliform organisms increased about 45% in the mixture of whey and curd when heating the mixture from 32 to 38 C (stages 4 to 5). This was not very apparent when comparing log

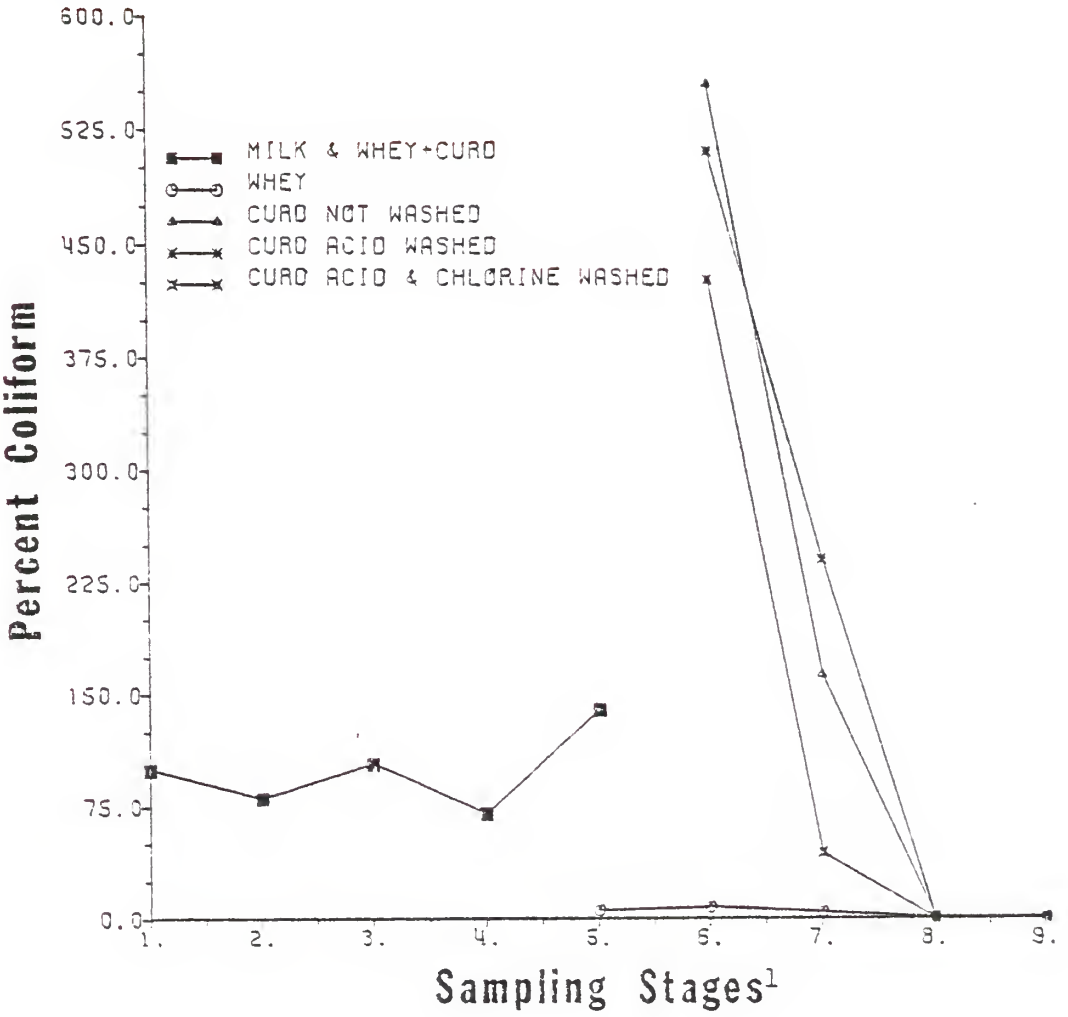


Figure 3. Percent surviving of coliform organisms at various stages during the manufacture of cottage cheese. Skim-cheese milk was inoculated with approximately 4.0×10^5 E. coli/ml.

[1]Stages at which samples were taken during the manufacture of cottage cheese: 1. Inoculated milk; 2. Milk at pH 4.95 - 5.00; 3. Milk at 32 C; 4. After cutting the curd; 5-8. Cooking temperatures (38, 43, 49, and 54 C); 9. 54 C + 15 min holding.

counts (Fig. 2).

At 43 C the percent of coliform organisms remaining in the whey was less than 25% of the original count while in the curd not washed, this increased to 550%; 500% in the curd acid washed; and 425% in the curd acid and chlorine washed. This indicates a reduction of 75% when the curd was washed with acidified water at pH 5.00 containing 10 ppm chlorine.

Fig. 4 presents the raw coliform counts. This graph followed exactly the same pattern as the percent of surviving coliforms. An initial count at the time of inoculation of approximately 4.0×10^5 coliform/ml was observed; while in the curd not washed at stage 6 (43 C) the count observed was about 2.4×10^6 coliform/g. This represented an increase of 2.0×10^6 coliform organisms.

Low level of inoculation (2.5×10^4 E. coli/ml). Fig. 5 which represents the log coliform counts following inoculation of the cheese milk with the low concentration of coliform organisms (2.5×10^4) at the time of and during the cheese making followed essentially the same pattern as the high concentration (4.0×10^5), shown in Fig. 2.

The rate of destruction of coliform organisms during the cooking stages was greater in the low level (Fig. 6) than in the high level (Fig. 3). Comparing the percentages of coliforms remaining in the curd particles at 43 C (stage 6), we can see the following differences between low (Fig. 6) and high level (Fig. 3) of inoculation:

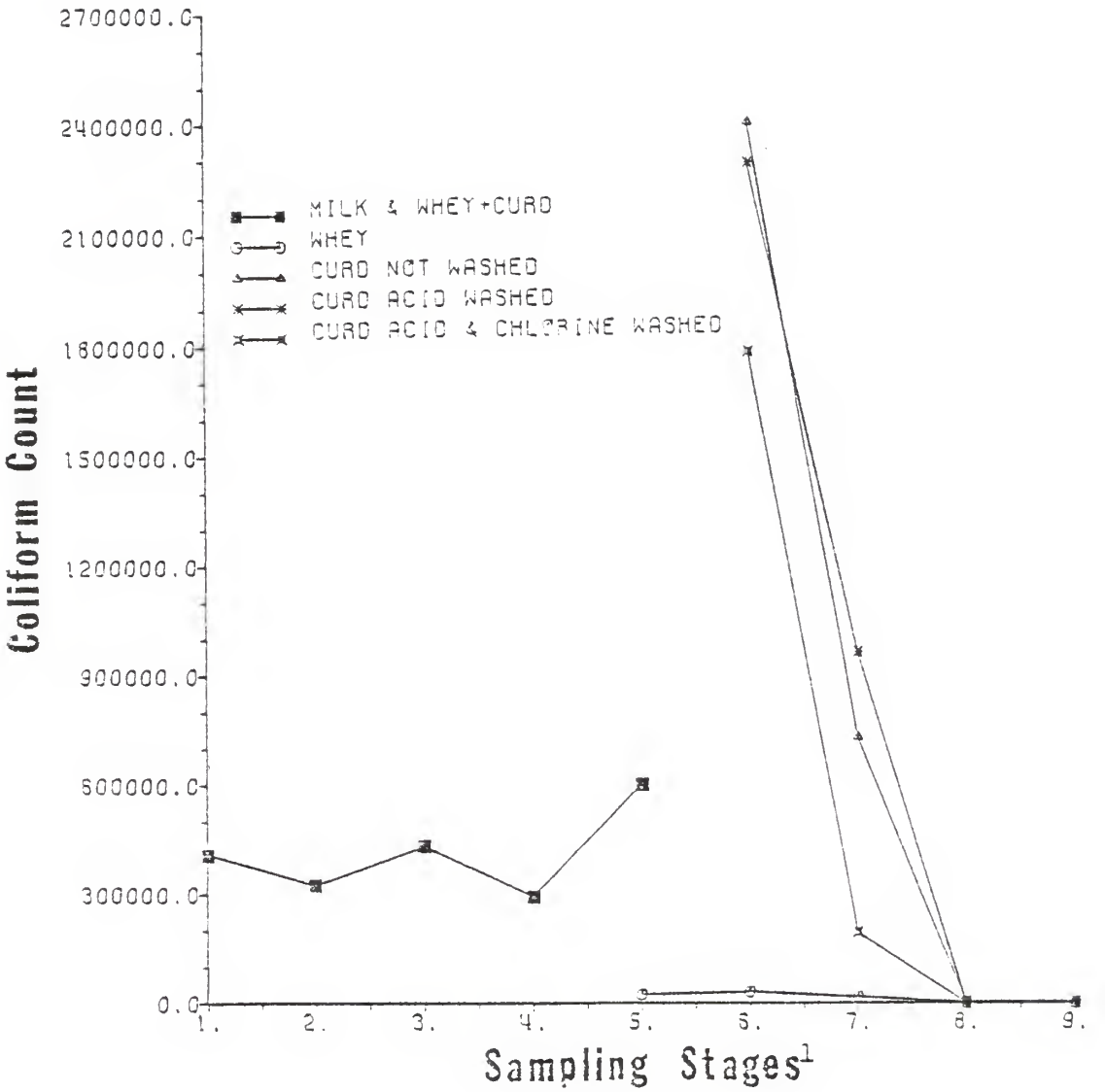


Figure 4. Coliform count at various stages during the manufacture of cottage cheese. Skim-cheese milk was inoculated with approximately 4.0 x 10^5 *E. coli*/ml.

[1]Stages at which samples were taken during the manufacture of cottage cheese: 1. Inoculated milk; 2. Milk at pH 4.95 - 5.00; 3. Milk at 32 C; 4. After cutting the curd; 5-8. Cooking temperatures (38, 43, 49, and 54 C); 9. 54 C + 15 min holding.

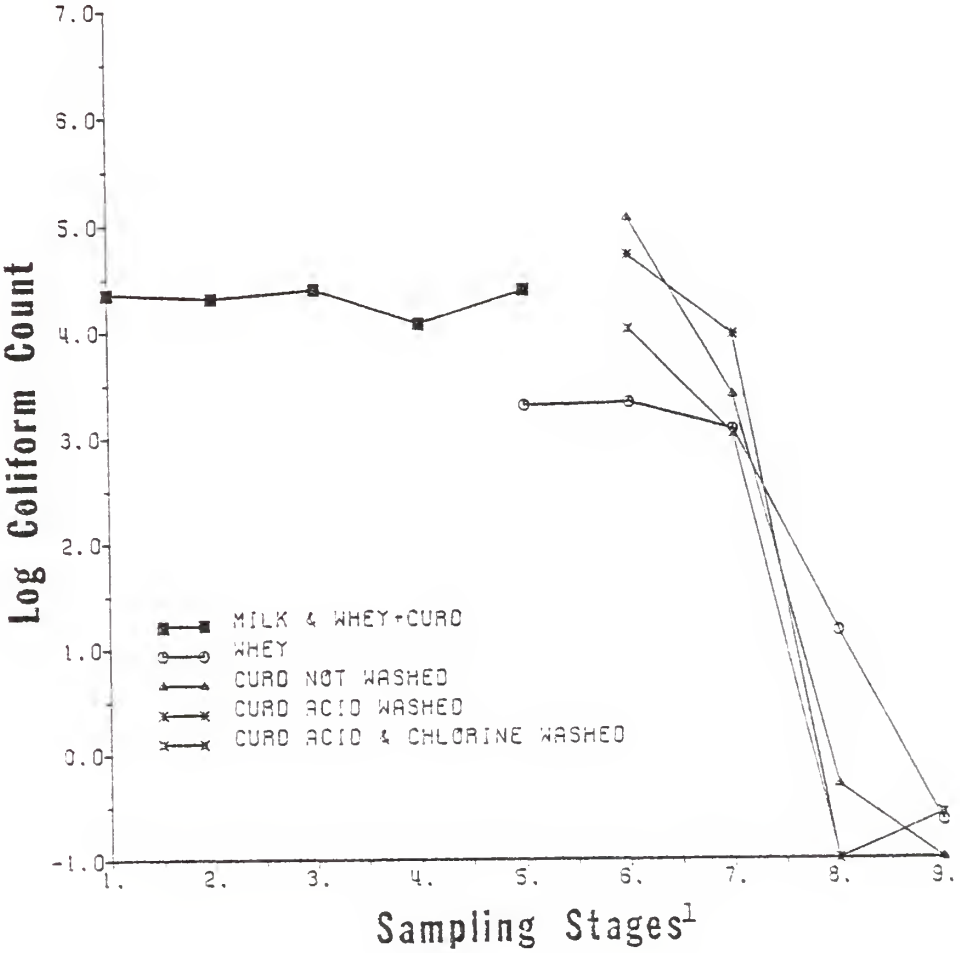


Figure 5. Log counts of coliform organisms at various stages during the manufacture of cottage cheese. Skim-cheese milk was inoculated with approximately 2.5 x 10^4 E. coli/ml.

[1]Stages at which samples were taken during the manufacture of cottage cheese: 1. Inoculated milk; 2. Milk at pH 4.95 - 5.00; 3. Milk at 32 C; 4. After cutting the curd; 5-8. Cooking temperatures (38, 43, 49, and 54 C); 9. 54 C + 15 min holding.

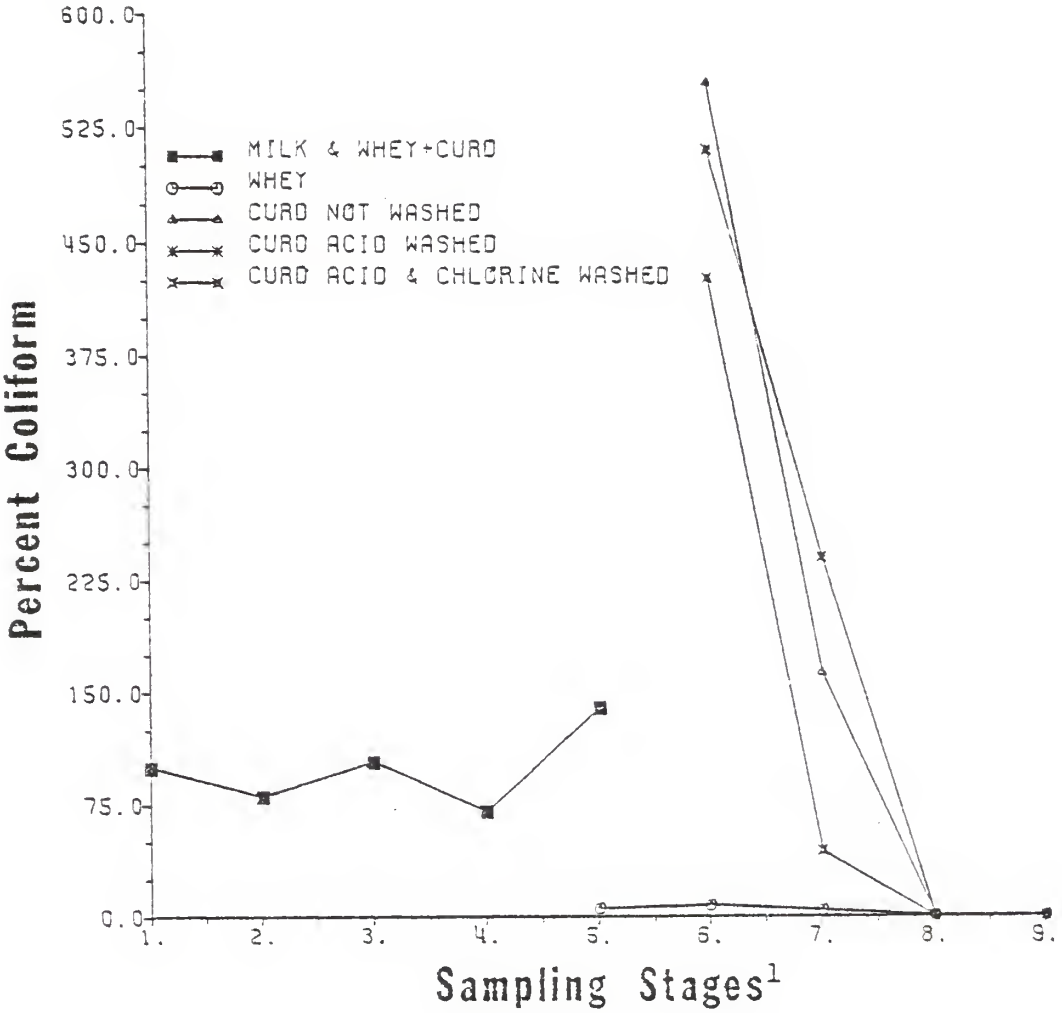


Figure 6. Percent surviving of coliform organisms at various stages during the manufacture of cottage cheese. Skim-cheese milk was inoculated with approximately 2.5×10^4 *E. coli*/ml.

[1]Stages at which samples were taken during the manufacture of cottage cheese: 1. Inoculated milk; 2. Milk at pH 4.95 - 5.00; 3. Milk at 32 C; 4. After cutting the curd; 5-8. Cooking temperatures (38, 43, 49, and 54 C); 9. 54 C + 15 min holding.

	High level	Low level
Curd not washed	550% survival	560% survival
Curd acid washed	500% "	400% "
Curd acid & chlorine washed	425% "	80% "

This comparison demonstrates not only that low coliform levels are more easily removed, but that the acid and chlorine wash water had the greatest effect in destroying this lower level of coliform organisms. At 49 C, the percentage of coliform organisms remaining in the acid and chlorine washed curd was about 8% while in the high level (Fig. 3) about 48% remained. In compliance with these results, several investigators (5, 34, 49) have reported that the initial concentration of the heated suspension influences the thermal death time of a particular organism. It means that the greater the number of organisms present in the cheese milk, the more difficult it is to destroy them and higher temperatures are required.

Fig. 7 presents the raw coliform counts for the lower level of inoculation and shows that the counts followed the same pattern as with the higher level of inoculation (Fig. 4). It was observed that from the initial count at the time of inoculation of approximately 2.5×10^4 coliform/ml, the count in the curd not washed at 43 C (stage 6) was about 1.2×10^5 coliform/g. This was an increase of 9.3×10^4 coliform organisms.

A cooking temperature below 54 C during the manufacture of cottage cheese was inadequate for completely destroying

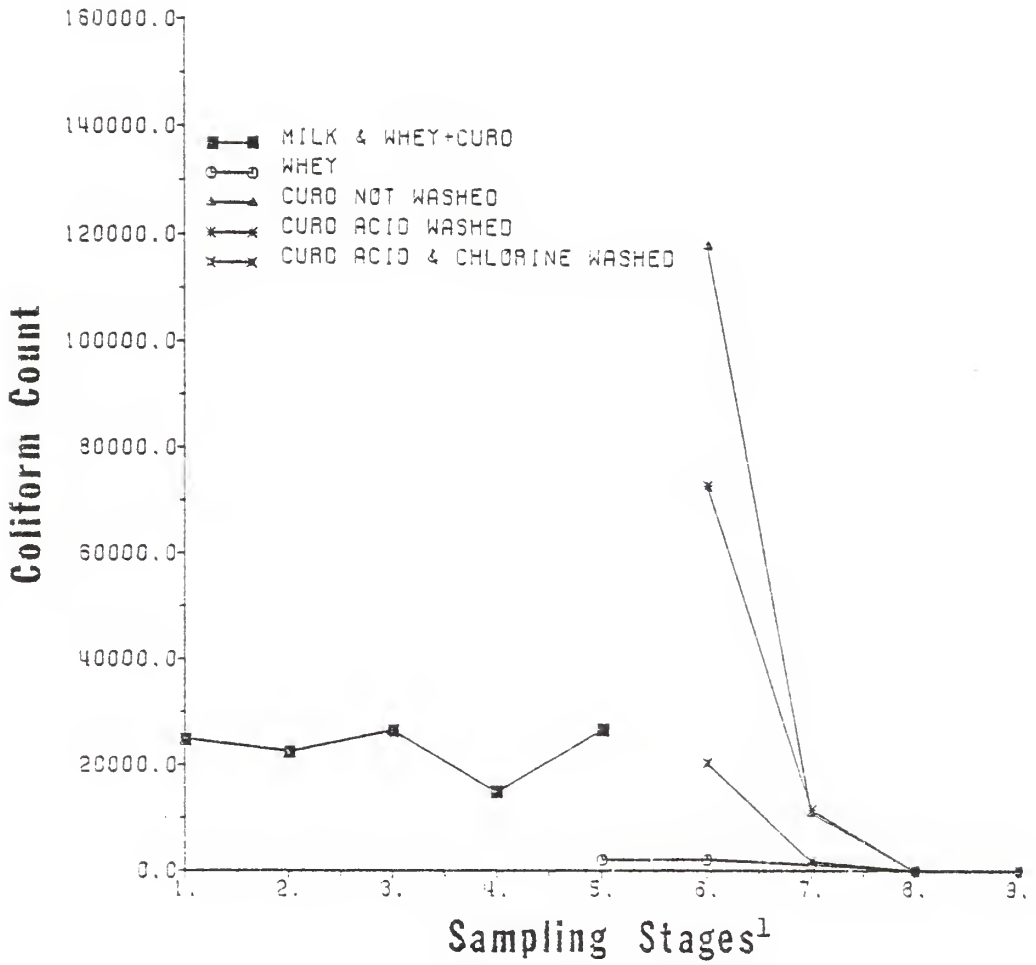


Figure 7. Coliform count at various stages during the manufacture of cottage cheese. Skim-cheese milk was inoculated with approximately 2.5×10^4 *E. coli*/ml.

[1]Stages at which samples were taken during the manufacture of cottage cheese: 1. Inoculated milk; 2. Milk at pH 4.95 - 5.00; 3. Milk at 32 C; 4. After cutting the curd; 5-8. Cooking temperatures (38, 43, 49, and 54 C); 9. 54 C + 15 min holding.

coliform organisms.

Experiment 2: Changes in Coliform Counts of Cottage Cheese Whey and Curd Held for up to 60 Min at 38 - 54 C

Results of this experiment are presented in Table 1, and Figs. 8 and 9. When log coliform counts (in the curd), were compared at different temperatures, at any one time (0 through 60 min), there was no significant difference ($p < .05$) between 38 and 43 C (see Table 1). Coliform counts for the curd decreased significantly when temperatures increased to 49 C for all holding times except 0 min. At 54 C the coliform counts decreased significantly even at 0 min. For the whey, again there was no difference between 38 and 43 C, and differences between these temperatures and 49 C showed up to be statistically significant only after holding the wheys at 49 C for 40 min. When the whey was held at 54 C there was a significant decrease in coliform organisms at every holding time.

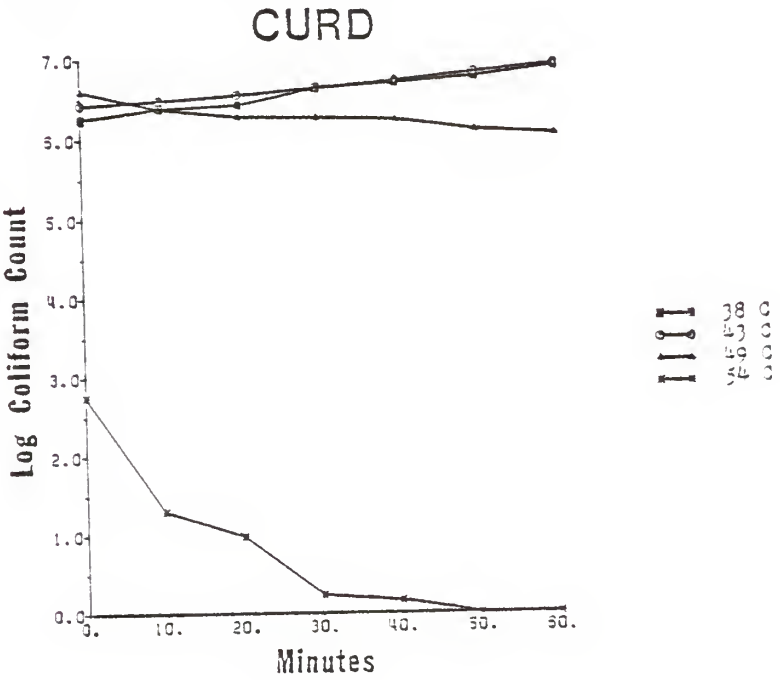
When comparing times for destruction of coliform organisms, changes were not as obvious as when temperatures were compared. The results indicated that at 38 C, and at 43 C, there was a general increase in coliform organisms with time in both the curd and the whey, however, when the temperatures were increased to 49 C the trends reversed, and the longer the samples were held, the lower the coliform counts. These rates of decrease in coliform organisms with time, increased substantially when the temperatures were increased to 54 C. These trends are shown graphically in Figs. 8 and 9.

Table 1. Changes in coliform counts of cottage cheese whey and curd held for up to 60 min at 38 - 54 C.

Comparison between temperatures				Comparison between times			
Temp. (C)	Time (min)	Curd (mean log coliform)	Whey coli-count)[1]	Temp. (C)	Time (min)	Curd (mean log coliform)	Whey coli-count)[1]
38	0	6.26 a	2.55 a	38	0	6.26 a	2.55 a
43		6.43 a	2.69 a		10	6.39 ab	2.61 ab
49		5.90 a	2.62 a		20	6.42 ab	2.73 ab
54		2.69	0.38		30	6.62 ab	2.80 abc
					40	6.68 ab	2.87 bc
					50	6.76 ab	3.07 cd
38	10	6.39 a	2.61 a		60	6.86 b	3.20 d
43		6.48 a	2.73 a				
49		5.56	2.51 a				
54		1.29	0.00	43	0	6.43 a	2.69 a
					10	6.48 a	2.73 ab
					20	6.54 a	2.78 ab
38	20	6.42 a	2.73 a		30	6.63 a	2.85 ab
43		6.54 a	2.78 a		40	6.71 a	2.88 ab
49		5.41	2.40 a		50	6.80 a	2.92 ab
54		0.91	0.00		60	6.90 a	3.03 b
38	30	6.62 a	2.80 a	49	0	5.90 a	2.62 a
43		6.63 a	2.85 a		10	5.56 ab	2.51 a
49		5.38	2.33 a		20	5.41 abc	2.40 ab
54		0.18	0.00		30	5.38 bc	2.33 ab
					40	5.34 bc	2.13 b
					50	5.14 bc	1.72 c
38	40	6.68 a	2.87 a		60	5.04 c	1.62 c
43		6.71 a	2.88 a				
49		5.34	2.12				
54		0.13	0.00	54	0	2.69	0.38
					10	1.29 b	0.00 a
					20	0.91 b	0.00 a
38	50	6.76 a	3.07 a		30	0.18 a	0.00 a
43		6.80 a	2.92 a		40	0.14 a	0.00 a
49		5.14	1.71		50	0.00 a	0.00 a
54		0.00	0.00		60	0.00 a	0.00 a
38	60	6.87 a	3.20 a				
43		6.90 a	3.03 a				
49		5.04	1.62				
54		0.00	0.00				

[1]Log coliform count below zero where reported as 0.00. Means in each column for each holding temperature and time not significantly different ($p < .05$) are indicated with a common letter. Each mean represents the average of 3 replications.

Figure 8. Changes in coliform counts of cottage cheese curd held for up to 60 min at 38-54 C. Skim-cheese milk was inoculated with approximately 4.0 x 10^5 *E. coli*/ml.



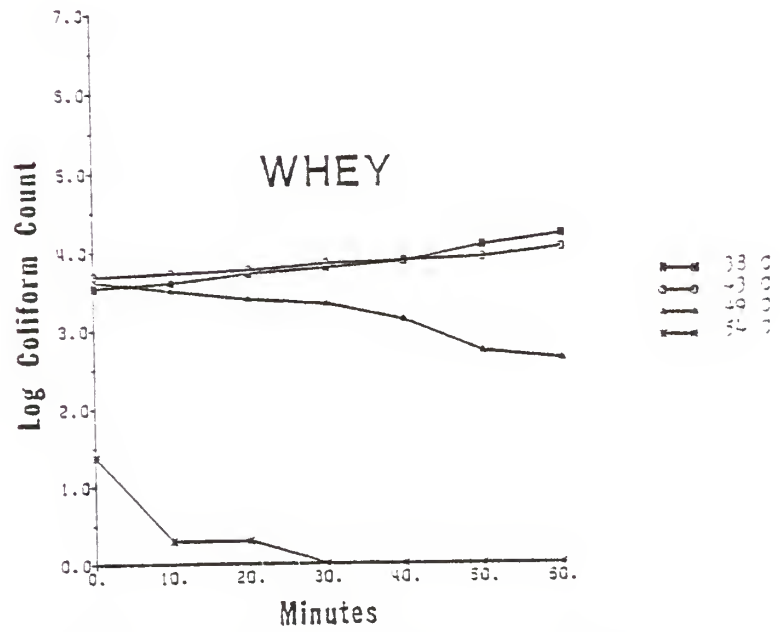


Figure 9. Changes in coliform counts of cottage cheese whey held for up to 60 min at 38-54 C. Skim-cheese milk was inoculated with approximately 4.0 x 10^5 *E. coli*/ml.

CONCLUSIONS

Based on the results obtained in this study, the following conclusions may be drawn:

1. Acidification of milk to pH 4.95 - 5.00 had little effect in destroying coliform organisms.

2. Wash-water acidified to pH 5.00 had no effect on coliform organisms in cottage cheese curd when high concentration of these organisms were present.

3. Wash-water acidified to pH 5.00 containing 10 ppm chlorine was very effective in destroying coliform organisms in curd when present in low numbers.

4. Cooking temperatures of 54 C and wash water acidified to pH 5.00 containing 10 ppm chlorine were required to obtain complete destruction of E. coli, Strain W, during the manufacture of cottage cheese.

5. The counts of coliform organisms followed similar survival or destruction patterns when present in cottage cheese milk at either low (2.5×10^4) or high levels (4.0×10^5).

6. When samples of cottage cheese whey and curd were held at constant temperatures, a significant decreased in coliform count with time occurred only at 54 C.

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LITERATURE CITED

(1) Angevine, N.C. (1976) Cures for some cottage cheese problems. J. Cultured Dairy Products 11:14.

(2) Charter, R.E. (1965) Escherichia coli type 0.114 isolated from infantile diarrhea and calf scours. J. Pathol. Bacteriol. 72:33.

(3) Clark, D.S. (1967) Comparison of pour and surface plate methods for determination of bacterial counts. Canadian J. of Microbiol. 13:1409.

(4) Collins, E.B. (1961) Resistance of certain bacteria to cottage cheese cooking procedures. J. Dairy Sci. 44: 1989.

(5) Craige, J.E. (1946) Significance of concentration of coliform organisms in raw milk upon survival of pasteurization. J. Milk and Food Technol. 9:191.

(6) Crossley, E.L. (1946) The coliform flora of milk and dairy products. J. Dairy Res. 14:233.

(7) Dabbah, R., Moats, W.A., and Edwards, V.M. (1971) Heat survivor curves of food-borne bacteria suspended in commercially sterilized whole milk. II. Bacteria other than Salmonellae. J. Dairy Sci. 54:1772.

(8) Defigueiredo, M.P., and Splittstoesser, D.F. (1976) Food Microbiology: Public Health and Spoilage Aspects. The AVI Publishing Company, Inc. Westport, Connecticut.

(9) Dommet, T.W. (1970) Studies of coliform organisms in cheddar cheese. Aust. J. Dairy Technol. 25:54.

(10) Dommet, T.W. (1974) Variations in levels of coliforms and Escherichia coli type 1 in Queensland cheddar cheese. Aust. J. Dairy Technol. 29:198.

(11) Elliot, J.A., and Millard, G.E. (1976) A comparison of two methods for counting coliforms in cheese. J. Inst. Can. Sci. Technol. Aliment 9:95.

(12) Emmons, D.B. (1959) Recent research in the manufacture of cottage cheese. Part II. Dairy Sci. Abstr. 25:175.

(13) Fabian, F.W. (1946) Cheese as the cause of epidemics. J. Milk and Food Technol. 9:129.

(14) Fabian, F.W. (1947) Cheese and its relation to disease. Amer. J. Public Health 37:987.

(15) Frazier, W.C. (1967) Food Microbiology. McGraw-Hill, NY., NY.

(16) Gerson, M.F. (1970) New non-cultured curd process for producing cottage cheese by the batch method. Am. Dairy Rev. 32:52.

(17) Goel, M.C., Kulshrestha, D.C., Marth, E.H., Francis, D.W., Bradshaw, J.G., and Read, Jr. R.B. (1971) Fate of coliforms in Yogurt, Buttermilk, Sour Cream, and Cottage Cheese during regrigerated storage. J. Milk and Food Technol. 35:653.

(18) Gorbach, S.L. (1970) Acute diarrhea - a toxin disease? New England J. Med. 283:44.

(19) Gorbach, S.L., Kean, B.H., Evans, D.G., Evans, Jr. D.J., and Bessudo, O. (1975) Travelers' diarrhea and toxigenic Escherichia coli. New England J. Med. 292:933.

(20) Gorbach, S.L., and Khurama, C.M. (1972) Toxigenic Escherichia coli. New England J. Med. 287:791.

(21) Hall, H.E. (1964) Methods of Isolation and Enumeration of Coliform Organisms. U.S. Dept. of Health, Education and Welfare. Public Health Service no. 1142, Wash., D.C..

(22). Harmon, L.G., Trout, G.M., and Bonner, M.D. (1955) A market survey of cottage cheese. I. Some characteristics influencing consumer acceptance and shelf-life. Mich. Agr. Expt. Sta., Quart. Bull. 38:146.

(23) Huang, E., and Armstrong, J.G. (1970) The effect of microbial inhibitors on the storage life of cottage cheese exposed to ambient temperatures. J. Inst. Can. Sci. Technol. 3:147.

(24) King, W.L., and Hurst, A. (1963) A note on the survival of some bacteria in different diluents. J. Appl. Bact. 26:504.

(25) Marth, E.B. (1970) Spoilage of cottage cheese by psychrophilic microorganisms. J. Cultured Dairy Products 5:14.

(26) Martin, W.H., Foltz, V.D., and Rutz, W.D. (1960) A survey of cottage cheese quality. J. Milk and Food Technol. 23:306.

(27) Morgan, M.E., Anderson, E.O., Hankin, L., Dowd, L.R. and Foter, M.J. (1952) Chemical, bacteriological, and organoleptic characteristics of retail cottage cheese. Storrs Agr. Expt. Sta., Bull. 284.

(28) Neter, E., Webb, C.R., Shumway, C.N., and Murdock, M.R. (1951) Study on etiology, epidemiology and antibiotic therapy of infantile diarrhea, with a particular reference to certain serotypes of Escherichia coli. Am. J. Public Health 41:1490.

(29) Nielsen, V.H. (1976) Coliform bacteria in cheddar cheese present no public health problem. Amer. Dairy Rev. 38:50b.

(30) Nielsen, V.H. (1976) More research needed on contamination by coliform bacteria. Amer. Dairy Rev. 38:50.

(31) Oblinger, J.L., and Kennedy, Jr. J.E. (1976) Evaluation of diluents used for total counts. J. Milk and Food Technol. 39:114.

(32) Olson, J.C., Macy, Jr. J., and Halvorson, H.O. (1951) Thermal death time studies of coliform bacteria in milk. Tech. Bull. Minn. Agr. Expt. Sta., 202.

(33) Overcast, W.W., and Britton, J.V. (1959) A study of the microbial flora of cottage cheese during storage. J. Dairy Sci. 42:910.

(34) Overcast, W.W., Skean, J.D., and Britton, J.B. (1961) Coliform bacteria in cottage cheese. J. Dairy Sci. 44:970.

(35) Panes, J.J., and Thomas, S.B. (1959) The multiplication of coli-aerogenes bacteria in milk stored at 3-5°. J. Appl. Bact. 27:272.

(36) Potashnik, L., Sklan, D., Rosen, B., and Gordin, S. (1972) Coliforms and keeping quality of Streptococcus lactis based cultured milk products. J. Milk and Food Technol. 35:651.

(27) Punch, J.D., and Olson, Jr. J.C. (1964) Comparison between standard methods procedures and a surface plate method for estimating psychrophilic bacteria in milk. J. Milk and Food Technol. 27:43.

(38) Read, Jr. R.B., Schwartz, C., and Litsky, W. (1961) Studies of thermal destruction of Escherichia coli in milk and milk products. Appl. Microbiol. 9:415.

(39) Roth, L.A., Clegg, L.F.L., and Stiles, M.E. (1971) Coliforms and shelf-life of commercially produced cottage cheese. J. Inst. Can. Technol. Aliment. 4:107.

(40) Schurreberger, L.W., Beck, R., and Plate, J. (1971) Gastroenteritis attributed to imported french cheese. Morbidity and mortality. Weekly Report 20:427.

(41) Shore, E.G., Dean, A.G. Holik, K.J., and Davis, B.R. (1974) Enterotoxin-producing Escherichia coli and diarrheal disease in adult travelers. A prospective study. J. Infect. Dis. 129:577.

(42) Skelton, W.R., and Harmon, L.G. (1964) Growth rate of coliform organisms in cottage cheese and reconstituted non-fat dry milk. J. Milk and Food Technol. 27: 197.

(43) Standard Methods for the Examination of Dairy Products (1972) 13th ed. APHA, New York.

(44) Straka, R.A., and Stokes, J.L. (1957) Rapid destruction of bacteria in commonly used diluents and its elimination. J. Appl. Microbiol. 5:21.

(45) Thatcher, F.S., and Clark, D.S. ed. (1968) Microorganisms in Food: Their Significance and Methods of Enumeration. Univ. of Toronto Press, Toronto, Canada.

(46) Thatcher, F.S., and Clark, D.S. ed. (1974) Microorganisms in Foods. 2. Sampling for Microbiological Analysis: Principles and Specific Applications. Univ. of Toronto Press, Toronto, Canada.

(47) Tuckey, S.L. (1959) Problems in cottage cheese production in 1959. J. Dairy Sci. 42:1246.

(48) Urban, J. (1977) Personal communications. Department of Microbiology, Kansas State University, Manhattan, Kansas.

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

DESTRUCTION OF *E. COLI*, STRAIN W, DURING THE MANUFACTURE OF COTTAGE CHEESE

by

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B.S., Escuela de Quimica Industrial, Caracas, Venezuela, 1972

AN ABSTRACT OF A MASTER'S THESIS

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Cottage cheese was manufactured in 10-l experimental vats from skimmilk inoculated with two levels (2.5×10^4 and 4.0×10^5 organisms/ml) of a heat resistant strain of Escherichia coli, Strain W (ATCC 9637).

The effect of various processing conditions during its manufacture included the following stages: 1. Milk inoculated with E. coli; 2. Milk after acidification to pH 4.95 - 5.00; 3. Milk at 32 C; 4. After cutting the curd at 32 C; 5-8. Cooking temperatures (38, 43, 49, and 54 C); 9. 54 C + 15 min holding. A combination of surface and pour plate on violet red bile (VRB) agar, followed by an overlay of VRB was used to enumerate coliform organisms. A second study included holding samples of cottage cheese whey and curd at constant temperatures (38, 43, 49, and 54 C) in water baths for up to 60 min and samples analyzed every 10 min. Only the high level of inoculation was used.

Little variation in coliform counts occurred during the first five stages of manufacture of cottage cheese. Wash water acidified to pH 5.00 had no effect in destroying coliform in the curd when these organisms were present in high concentration. On the other hand wash water acidified to pH 5.00 containing 10 ppm chlorine was very effective in destroying them when present in low concentration in the curd. Cooking temperatures of 54 C were required to obtain complete destruction of E. coli, Strain W, during the manufacture of cottage cheese. From the second study it was observed that a significant decreased in coliform count occurred only at 54 C.