A BACTERIOLOGICAL STUDY OF COTTAGE CHEESE WITH PARTICULAR REFERENCE TO PUBLIC HEALTH HAZARD

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This is to certify that the

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A BACTERIOLOGICAL STUDY OF COTTAGE CHEESE WITH PARTICULAR REFERENCE TO PUBLIC HEALTH HAZARD

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A THESIS

Submitted to the School of Graduate Studies of Michigan State College of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

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THESIS

DEDICATION

This work is dedicated to my mother, my wife, and my four children, Connie Lou, Bobby, Janie, and Joie.

Without their patience and forebearance this paper would not have been started, nor could it have been finished.

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INTRODUCTION

Few city milk ordinances take into account the sanitary aspects involved in the production and handling of cottage cheese. In most instances, reference to cottage cheese is one of definition only.

In accordance with the various ordinances, careful sanitary inspections are made of milk plants, and, although the inspection covers all phases of milk processing, emphasis is always placed on fluid milk production.

In milk processing plants where cottage cheese is also manufactured and packaged, the sanitary methods used in manufacturing and packaging are in contrast to the methods used in the care and handling of milk. The use of improperly sanitized equipment, hand methods of conveying and packaging, and a general air of laxity are all common errors in the production of cottage cheese. Needless to say, such methods and equipment have not been used for the handling of market milk for many years.

It is interesting to note that at one time milk was sold in bulk to retailers for resale to their customers. The retailer would then ladle the milk into the customer's own container. However, as early as 1876, glass containers were used for the delivery of milk

to the consumer (2). Since that time recognition of the public health hazards involved in bulk milk sales, as well as other factors, has made it mandatory for milk to be handled in only legally approved containers. Yet, today, thirty-seven years after bulk milk sales were outlawed in Michigan, the sale of bulk cottage cheese is still permitted (3).

It is apparent that this product was not merely forgotten by the manufacturer and the public health officials, for there must have been some logical reason why it was ignored for so many years. This complacency seems to be founded on the premise that cottage cheese is too acid a product to require more than just casual care in its manufacture. It is true that in the early history of modern dairy production cottage cheese was a more acid product. Incomplete cooking of the curd, resulting in larger amount of lactose being incorporated within the curd, resulted in a consequent fermentation and an increase in acidity in the finished product. More recent practice, undoubtedly motivated by consumer demand, is for completely cooked curd with thorough rinsing and draining. This results in most of the lactose being expelled with the whey in the draining operation, and checks the rapid production of acid in the finished product. This bland cheese is quite different from the

cottage cheese of twenty or thirty years ago, and yet the manufacturing methods used are essentially the same. Without the protection of high acidity in the finished product, greater care and stricter sanitary precautions are necessary to produce a safe product.

In view of the above facts, this study was undertaken to determine if the commonly used methods of cottage cheese production and packaging actually constitute a public health hazard, or if, on the other hand, the product still remains sufficiently acid to warrant its casual handling by the manufacturer and disinterest on the part of the public health official.

STUDY I

COLIFORM INDEX OF MARKET COTTAGE CHEESE

Introduction

This study was made using samples obtained from eight dairies, six of which were under public health inspections as required by city ordinances, and two creameries which were without such inspections.

Packages of cheese were obtained at plant storage points and retail outlets. The samples were collected at intervals over a twelve-month period, and tests were made within twenty-four hours of the time collected.

According to Standard Methods for the Examination of Dairy Products (1), the coliform test constitutes by far the most delicate method available for detecting recontamination of dairy products. Inasmuch as the problem is entirely one of recontamination by handlers and improperly sanitized equipment, the coliform test was chosen as the most exact instrument to obtain the necessary data.

Dairy products were tested for the presence of coliforms through the use of 2 per cent brilliant green bile broth tubes or

desoxycholate agar in accordance with the standards set up by the A.P.H.A. (1). Both media were used so that comparative counts could be obtained and, if deemed advisable, isolation from the solid media could be accomplished by picking off individual colonies and subjecting them to further study.

Every effort was made to obtain samples representative of the production of each plant. Nothing was said or done to persuade the cheese processors to alter in any way their usual methods of handling. Samples were selected at random from the total day's production. Of course, the fact that samplings were being made would tend to make the handlers a little more careful in their methods, but, as will be shown, this does not seem to have made any significant difference in the final results.

Procedure

A Waring blendor was sterilized by washing thoroughly with a detergent wetting agent, and soaking for thirty minutes in a 500 ppm sodium hypochlorite solution. After soaking, it was rinsed in running water for about ten minutes. Checks for free chlorine and possible carry-over of alkali from the chlorine solution were made by thiosulphate titration and by checking with the Beckman pH meter.

There was no indication that the chlorine solution was carried over after the blendor had been rinsed for ten minutes in running water.

One hundred ml of sterile distilled water was put into the sterile Waring blendor. The machine was run for a few seconds and then one-ml amounts of the water were placed in each of three brilliant green bile broth tubes. Dilutions of 1-10 and 1-100 were made on desoxycholate agar by putting 0.1 ml of the water into a Petri dish and by putting one ml of the water into a 99 ml dilution blank and transferring one ml of this dilution to a Petri dish. Desoxycholate agar was then added to these Petri dishes and mixed with the water being tested. These two tests constituted a sterility control on the Waring blendor.

Using aseptic precautions, 10 gm of cottage cheese to be sampled was weighed out and added to the 90 ml of sterile water in the Waring blendor. After rendering the mixture homogeneous, 1.0 ml amounts were transferred to each of five brilliant green bile fermentation tubes, and dilutions of 1-10 and 1-100 were made into desoxycholate agar by the same methods described above in plating the water used as a sterility control. These samples, as well as all samples in this study, unless specifically stated otherwise, were incubated at 37 C.

A sufficient quantity of the mixture was also removed at this point to determine the pH of the cheese with a Beckman pH meter.

Results and Discussion

The pH determination on over 150 samples of market cottage cheese revealed that the pH was between 4.7 and 5.5, with over 80 per cent of the samples falling between 5.0 and 5.5. The pH of such cheese consequently was within the range tolerated by the coliform organisms.

Counts on the brilliant green bile tubes were taken at twenty-four and forty-eight hours. It was found that about 67 per cent of the samples collected contained coliform in the amount of 220 organisms or more per hundred gm of cheese. The percentage of positive samples collected from each dairy varied from 33 to 100 per cent (Table I).

There seemed to be no correlation between the individual dairy plant's ability to produce coliform-free bottle products and its ability to produce coliform-free cottage cheese. Plants with excellent records in these respects had rather high percentages of coliform-contaminated samples at times, and this was variable from plant to plant.

TABLE I

COTTAGE CHEESE PACKAGED AT PLANT

Dairy No.	No. of Samples	No. of Samples Showing Coli Colonies on Desoxycholate Agar		Total No. Positive Samples	Per Cent Positive Samples
		1-10	1-100		
1	16	1	6	7	44
2	18	4	1	5	28
3	25	10	4	14	56
4	12	3	4	7	60
5	14	6	2	8	57
6	12	4	6	10	83
7	16	6	8	14	87
8	18	8	10	18	100
Totals	131	42	41	83	63

84% of all samples had a pH of 5.0 to 5.6.

TABLE I (Continued)

No. of Positive Samples in 5 Tubes of Brilliant Green Bile Broth					Total No. Positive Samples	Per Cent Positive Samples
1/5	2/5	3/5	4/5	5/5		
-	-	7	-	2	9	56
1	1	2	-	2	6	33
4	1	2	-	8	15	60
3	-	-	2	2	7	60
4	2	1	1	1	9	64
2	1	1	2	4	10	83
-	1	1	2	10	14	87
-	1	1	2	14	18	100
14	7	15	9	43	84	64

TABLE II

COTTAGE CHEESE IN BULK AT RETAIL OUTLETS

Dairy No.	No. of Samples	Number of Samples Showing Coli Colonies on Desoxycholate Agar		Per Cent Positive Samples	Number of Positive Samples in 5 Tubes of Brilliant Green Bile Broth					Total No. Positive Samples	Per Cent Positive Samples
		1-10	1-100		1/5	2/5	3/5	4/5	5/5		
1	10	9	9	90	-	3	1	1	4	9	90
2	11	11	11	100	-	2	2	3	4	11	100

Ninety-five per cent of all samples of cheese that were obtained from bulk displays in meat markets, groceries, and delicatessens were found to be contaminated with coliforms (Table III). These samples showed heavier contamination than those obtained from plant-filled cartons.

The mechanization of all steps in the processing and packaging of cottage cheese can do much toward eliminating this contamination if the product is finally packaged into a single-service container at the plant. This was demonstrated by Dairy 5 (Table III). This dairy converted its cheese processing from an almost completely hand process to a completely mechanized one. Prior to this mechanization there was no evidence that Dairy 5 was able to produce cheese samples with fewer than 64 per cent being contaminated with coliforms. After complete mechanization, the percentage dropped to 33.

It is the author's opinion that the degree of contamination can easily be reduced to nearly zero by closer application of the same sound cleaning and sanitizing practices that are commonly used on other milk-handling equipment used in this plant.

TABLE III

COTTAGE CHEESE PACKAGED AT PLANT

Dairy No.	No. of Samples	No. of Samples Showing Coli Colonies on Desoxycholate Agar		Total No. Positive Samples	Per Cent Positive Samples
		1-10	1-100		
		Hand Processed and Packaged			
5	14	6	2	8	57
		Mechanically Processed and Packaged			
5	12	3	1	4	33

TABLE III (Continued)

No. of Positive Samples in 5 Tubes of Brilliant Green Bile Broth					Total No. Positive Samples	Per Cent Positive Samples
1/5	2/5	3/5	4/5	5/5		
			Hand Processed and Packaged			
4	2	1	1	1	9	64
			Mechanically Processed and Packaged			
3	1	-	-	-	4	33

Summary

Consumer preference has brought into practice the production of a bland cottage cheese. The pH of such cheese lies within the range tolerated by the coliform organisms. With coliforms as the indicator, the foregoing data show that cottage cheese may well be considered a potential public health hazard.

Large percentages of all cottage cheese samples collected contained coliform in amounts of 220 organisms per 100 gm of cheese or more.

Nearly all samples of bulk packaged cottage cheese were heavily contaminated with coliform organisms.

The mechanization of cottage cheese manufacturing and packaging is undoubtedly part of the solution to the production of a more sanitary cottage cheese; however, it must be conceded that elaborate precautions with hand methods may result in fairy satisfactor cheese production. Such production may compare favorably with average production by machine methods. This is pointed out by the results obtained by Dairy 2 (Table I), using hand methods, and Dairy 5 (Table III), using machine methods.

In general, however, these data show an unsatisfactory condition existing in the field of cottage cheese manufacturing and packaging.

STUDY II

LINE RUNS IN COTTAGE CHEESE PROCESSING PLANTS

Introduction

Inasmuch as the data in Study I revealed that a large percent of market cottage cheese samples was contaminated with coliform, it was decided to determine the source of this contamination. This would seem desirable to know, so that control measures could then be more intelligently applied directly to the points that can be shown to need improvement.

If, for example, the coliform organisms were getting into the cheese during processing, a study could be made of methods of cheese processing that might eliminate this contamination. If, on the other hand, the source of contamination was found at the packaging table, then efforts should be directed toward more sanitary packaging procedures.

Line samplings were made in six of the eight dairies from which cottage cheese samples had been obtained in Study I. The samplings followed the line of production through the dairy plant.

Procedure

The line sampling was as follows: (1) raw skim milk in vat before pasteurization; (2) the same milk after pasteurization; (3) milk in cheese vats after passing through piping and pumps, but before starter had been added; (4) the same milk after starter had been added; (5) the milk after processing into curd and with the curd draining in the vat; (6) cream to be used in creaming the finished curd; (7) the finished package.

These samples of milk and cheese were brought into the laboratory and tested within twenty-four hours after collection. All samples were collected in sterile tubes or water bottles using sterile pipettes or wooden tongue depressors to convey the sample into the tube or bottle. Brilliant green bile broth was used and incubation of all samples took place at 37 C for twenty-four and forty-eight hours, reading being taken at the end of both periods. One ml amounts of milk were put into 10 ml of brilliant green bile broth in fermentation tubes. The cheese was tested in the same manner as outlined in Study I under Procedure, with the exception that desoxycholate agar was not used.

Results and Discussion

Coliform organisms were fairly plentiful at all sampling points in the line of production, with the exception of those samples taken out of the pasteurizing vat at the end of the pasteurizing period. All test runs made followed the pattern indicated in Table IV. Raw skim milk, as would be expected, was found to contain coliforms in all samples taken. After pasteurization, this coliform count dropped to zero. After the milk had reached the cheese vats, having passed through pumps and piping, 80 per cent of all samples collected yielded coliform. The addition of a starter did not change this figure. It is apparent that the piping, pumps, and vats concerned contributed heavily to the increase in coliform-positive samples.

After the skim milk had been processed into curd and the curd was piled at the side of the vat to drain, only 18 per cent of the samples taken at this point yielded coliforms. This drop in coliform-positive samples may be attributed to two factors. One would be the cooking of the curd, in which moderate heat (120 to 130 F) gently shrinks the curd and expels the whey and occluded bacteria, and, secondly, the washing of the curd with clear water to free the curd of whey, which follows the cooking.

TABLE IV

LINE RUNS AT PROCESSING PLANTS

Per Cent of Samples Positive in Brilliant Green Bile						
No. of Runs	Raw Skim Milk	Skim Milk in Vat After Pasteur-ization	Skim Milk With Starter Added	Washed Curd	Cream to be Added	Finished Package
11	100	0	80	18	45	60

The cream that is intended to be used to cream the curd just prior to packaging showed a significant coliform content in 45 per cent of all samples collected. This cream was taken from the same vat as that which is bottled for market cream sales. It is important to note that the cream, when it was ready to be used for cottage cheese creaming, had a high coliform content, although the cream taken from the same vat for bottle use did not. This was due primarily to the fact that it was stored and handled in ten-gallon milk cans of questionable cleanliness.

The finished package showed an additional increase in coliform content due to the bad packaging methods employed in all plants. Sixty per cent of all such samples collected contained coliform organisms.

Summary

The line-run samplings in this study indicate a need for more complete sanitizing of all equipment used in cheese making. With properly sanitized piping and pumps, milk from the pasteurizing vats should reach the cheese vats with only a slight increase, if any, in coliform content. The processing of cheese, with comparatively mild heat and constant hand manipulation by the cheese maker

would be expected to contribute coliforms to the product. This, however, is more than overcome by the thorough washing which the curd receives before being piled at the side of the vat to drain. The careful sanitization of all milk cans that are to be used for the storage of cream will aid in obtaining a more sanitary product. Hand packaging is admittedly the weakest link in the processing-packaging procedure. Here again, elaborate precaution such as a separate clean, light room, stainless steel equipment and packaging machines, and the careful and repeated sanitization of equipment and hands of the operator during the packaging procedure will possibly result in a much higher-quality product. There is, of course, no substitute for complete mechanization throughout from pasteurization to the finished package.

STUDY III

SURVIVAL OF SELECTED ORGANISMS IN COTTAGE CHEESE

Introduction

The usual processing and packaging methods used in cottage cheese production leave several avenues through which various organisms may be introduced into the finished product.

Some of the organisms which may be introduced in this way include those of the enteric group, as well as various streptococci and staphylococci.

It was decided, therefore, to introduce some of these organisms into cottage cheese in the laboratory and determine the length of time that they would survive or possibly multiply.

The organisms that were used were Escherichia coli, Streptococcus faecalis, Salmonella typhosa, and Micrococcus pyogenes, var. aureus.

A number of studies have been made on the survival of various organisms in various types of media. Several of these studies reveal the importance of pH in the survival or growth of organisms. Fabian and Winslow (4), in 1939, did certain work to determine the influence of anions on bacterial viability. They found

that a bacterial population of over a hundred and sixty million on media at a pH of 4.9 dropped to a fifth of this number when the pH of the media was lowered to 4.6. A further drop of the pH to 4.4 lowered the number of surviving bacteria to only about 7 per cent of the original number. This same critical sensitivity to pH was exhibited in another part of their work when a bacterial population was carried at a pH of 8.6. When the pH was raised to 9.0, only about 1 per cent of the original number of bacteria survived.

Darby and Mallman (5), in 1939, in their studies on media for coliform organisms, demonstrated that, at a pH of 6.8, an initial planting of thirty-eight coliform organisms in a selected medium resulted in the growth of these organisms in forty-eight hours to 880,000,000. This same experiment repeated at pH 7.8, the culture tubes being seeded with thirty-five organisms, resulted in the growth of the organisms to over 2.5 billion. This again demonstrated that, although bacteria may survive at extremes of pH, the optimum pH for their growth or for their destruction lies at a critical point or within very narrow ranges of pH.

Procedure

Pure stock cultures of each of the four bacteria used were transferred to agar slants, and after forty-eight hours' incubation at 37 C, each slant was rinsed with 5.0 ml of normal saline. One ml of this saline-bacteria mixture was transferred to 99-ml sterile water blanks, and from these blanks 1.0 ml quantities were transferred to agar plates to obtain a gross count of the numbers of bacteria present in 1.0 ml quantities.

M. aureus was plated on blood agar; the other three cultures were plated on nutrient agar.

Cartons of cottage cheese were tested, and only cartons free from coliforms and S. faecalis were selected for these experiments.

Twelve lots of 100 gm of cheese were mixed in the Waring blendor with 100 ml of sterile water. To each of these lots of cheese, 1.0 ml quantities of the bacterial suspensions were added. The four bacteria-cheese mixtures were divided into three groups so that each mixture would have a representative sample incubated at each of the three temperatures: 37 C; 10 C, the approximate temperature found in a mechanical refrigerator; and room temperature, 24 C.

After incubation at these temperatures, transfers were made from the cheese-bacteria mixtures in the amounts of 1.0, 0.1, and 0.01 ml into suitable media for the recovery of the bacteria remaining viable.

The dilutions of the coli-inoculated cheese were planted into brilliant green bile broth fermentation tubes.

The dilutions of the S. faecalis-inoculated cheese were planted into dextrose azide broth (7), and after incubation for twenty-four hours all positive tubes were transferred into ethyl violet azide broth (6).

The dilutions of the M. aureus-cheese mixture were also divided into three lots which were incubated at the three temperatures mentioned above. Survival was determined by smearing a loop from each sample on blood agar, incubating for forty-eight hours at 37 C and identifying by hemolysis and microscopic examination.

The dilutions of the S. typhosa-cheese mixture were incubated in the same manner as described above. After incubation, 1.0 ml amounts were transferred into tetrathionate broth, and, after twenty-four hours' incubation in this medium at 37 C, transplants were made into Kligler iron agar slants. Survival of the S. typhosa was determined by characteristic appearance of the Kligler slant.

Results and Discussion

The survival of bacteria in cottage cheese seemed to be closely linked to pH. When samples were incubated at 37 C, the pH dropped rapidly, and in ninety-six hours coliform organisms were destroyed at a pH of approximately 4.0. Cottage cheese, however, is stored and handled generally at temperatures ranging from 40 to 50 F. At these temperatures coliforms survived for about 182 hours, with the pH dropping to a minimum of 4.6.

This same picture seemed to hold true in the case of each of the other bacteria used. S. faecalis survived until a pH of 4.0 had been reached, and required 192 hours to reach this pH, when incubated at 37 C. When incubated at 10 C, S. faecalis required 240 hours to reach a pH of 4.6, at which point it was apparently destroyed.

The M. aureus survived until a pH of 4.2 had been reached. This pH was reached at an incubation temperature of 37 C in ninety-six hours. At 192 hours, samples incubated at 10 C reached a pH of 4.6, and when the pH dropped to 4.5, M. aureus was apparently destroyed.

S. typhosa survived for the shortest time of all the bacteria used. In forty-eight hours the samples incubated at 37 C and 24 C

had dropped to a pH of 3.8, and the bacteria were apparently destroyed. At incubation temperature of 10 C S. typhosa survived until a pH of 4.8 had been reached. This required ninety-six hours of incubation.

Summary

It is apparent that growth of the bacteria used as test organisms is not a problem in cottage cheese handling. Survival was of sufficient duration to be considered a potential public health hazard. Cottage cheese is usually manufactured and consumed within about a seven-day period. After that time, unless special storage precautions have been taken, the cheese may develop off-flavors and become generally unmarketable. Inasmuch as all the bacteria used as test organisms in this work survived for a sufficient length of time to be conveyed from packager to consumer, under the conditions in which cottage cheese is usually handled, it must be concluded that insanitary packaging of cottage cheese is a potential public health hazard.

Seeing that the great majority of dairies use hand methods in producing cottage cheese, extraordinary precautions must be taken if a clean, sanitary product is to be obtained. The thorough

washing and sanitizing of equipment is, of course, of first importance. The careful scrubbing and sanitizing of milk cans for the storage of cream to be used in cheese production is essential. Beyond the use of clean sanitized equipment the most difficult part of the sanitary production of cottage cheese is the training of personnel to be used in this work. Because the sanitary quality of the work is so completely dependent upon the care of the operator, he should understand the necessity for clean hands, sanitized in a solution of a quaternary ammonium compound, clean clothing, and constant watchfulness for minor errors in handling which will contribute to the poor sanitary quality of the finished package of cottage cheese.

TABLE V

SURVIVAL OF *E. COLI* IN COTTAGE CHEESE

Hours Incubation	pH	Incubation Temp.	In BGB Tubes Pos. 1 ml Inocul.	In BGB Tubes Pos. 0.1 ml Inocul.	In BGB Tubes Pos. 0.01 ml Inocul.	MPN
48	4.7	37 C	5/5	2/5	3/5	1,000
	4.7	24	5/5	3/5	2/5	1,000
	5.0	10	5/5	5/5	4/5	6,000
	5.0	10	5/5	4/5	4/5	5,000
96	3.9	37	0/5	0/5	0/5	0
	4.0	24	0/5	0/5	0/5	0
	4.9	10	5/5	5/5	4/5	5,000
	4.9	10	5/5	5/5	5/5	6,000+
144	4.9	10	5/5	3/5	2/5	1,000
	4.9	10	5/5	2/5	1/5	700
168	4.7	10	5/5	3/5	2/5	1,000
	4.7	10	5/5	2/5	0/5	500
182	4.6	10	4/5	0/5	0/5	130
	4.6	10	4/5	1/5	0/5	130

TABLE VI

SURVIVAL OF S. FAECALIS IN COTTAGE CHEESE

Hours Incubation	Incubation Temp.	pH	Number of Tubes Positive in Ethyl Violet Azide Broth			
			1 ml Inoc.	0.1 ml Inoc.	0.01 ml Inoc.	MPN
48	37 C	4.7	5/5	5/5	5/5	6,000
	24	4.6	5/5	5/5	5/5	6,000
	10	5.4	5/5	5/5	5/5	6,000
	10	5.3	5/5	5/5	5/5	6,000
96	37	4.5	5/5	5/5	5/5	6,000
	24	4.5	5/5	5/5	5/5	6,000
	10	5.2	5/5	5/5	5/5	6,000
	10	5.2	5/5	5/5	5/5	6,000
144	37	4.4	5/5	4/5	2/5	2,000
	24	4.5	5/5	4/5	3/5	3,000
	10	5.1	5/5	5/5	4/5	5,000
	10	5.1	5/5	5/5	3/5	4,000
192	37	3.9	0/5	0/5	0/5	0
	24	4.1	0/5	0/5	0/5	0
	10	4.9	5/5	2/5	0/5	500
	10	4.9	5/5	3/5	0/5	900
240	37	-	-	-	-	-
	24	-	-	-	-	-
	10	4.6	3/5	0/5	0/5	75
	10	4.6	2/5	0/5	0/5	45

TABLE VII

SURVIVAL OF M. AUREUS IN COTTAGE CHEESE

Hours Incubation	Incubation Temp.	pH	Hemolysis in Blood Agar	Staph. Colonies Under Microscope
24	37 C	4.7	+	+
	24	4.6	+	+
	10	5.4	+	+
	10	5.3	+	+
48	37	4.4	+	+
	24	4.3	+	+
	10	4.9	+	+
	10	4.9	+	+
96	37	4.2	-	+
	24	4.2	-	+
	10	4.8	+	+
	10	4.8	+	+
120	10	4.8	+	+
	10	4.8	+	+
168	10	4.6	+	+
	10	4.6	+	+
192	10	4.5	-	-
	10	4.4	-	-

TABLE VIII

SURVIVAL OF S. TYPHOSA IN COTTAGE CHEESE

Hours Incubation	Incubation Temp.	pH	Growth in Kligler Slants
24	37 C	3.8	-
	24	3.8	-
	10	5.0	+
	10	5.0	+
48	37	3.6	-
	24	3.7	-
	10	4.9	+
	10	4.9	+
72	37	3.5	-
	24	3.6	-
	10	4.8	+
	10	4.8	+
96	37	3.4	-
	24	3.5	-
	10	4.6	-
	10	4.6	-

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