STUDIES ON METHODS FOR SHORTENING THE COTTAGE CHEESE MAKING PROCESS

By

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INTRODUCTION

There has been little change in the methods used for making of cottage cheese in the last decade. While per capita consumption has greatly increased, the time required for the manufacture of cottage cheese has not been reduced. Therefore, the producer must increase size of equipment and plant space to increase production.

Methods have been developed for the continuous making of butter, ice cream, and processing of milk and more recently, for cheddar cheese. It would seem logical that a continuous method for the manufacture of cottage cheese could be developed. Before such a method can be developed, the various factors which influence the rate of coagulation of the milk must be established.

The work herein reported is a study of various factors affecting the rates of acid development in, and the period of time required for the coagulation of, milk in the manufacture of cottage cheese. A study was made of the various factors affecting the rates of acid production by lactic cultures. These included: (1) addition of stimulatory materials, (2) rates of inoculation, (3) depth of milk layer, and (4) the solids content of the milk. Also studied were modified methods for making cottage cheese.

REVIEW OF LITERATURE

Certain peptides or crude extracts containing peptides have a stimulatory effect on the growth of various species of bacteria in the family Lactobacteriaceae (7, 11, 12, 13, 14, 15, 17, 21, 24, 25, 26, 27, 28, 29, 35, 37). Interest in this general subject was intensified by the work of Sprince and Woolley (28), who coined the term "strepogenin" to describe these stimulatory peptides. They found that tryptic digests of many proteins were much richer sources of the growth factor than was the liver extract previously used as a starting point for analysis.

Sprince and Woolley (28), also noted that highly purified proteins isolated from pancreas were a far richer source of stimulation than was pancreas itself. In assaying autolysates of pancreas, it was found that the major portion of the strepogenin of this organ could be accounted for in the insulin and proteolytic enzymes normally found therein. This pointed to the conclusion that strepogenin was a part of the protein molecule. Furthermore, slow liberation of the factor during tryptic digestion suggested that this compound was an integral part of the protein rather than an impurity. It was also possible for a protein to contain all the amino acids and yet not possess strepogenin activity, for the amino acids might not be linked together in the

proper combination.

Peeler et al. (21), obtained evidence that acid-hydrolyzed casein contained at least two unidentified growth factors which were also present in liver extracts. Rickes, Koch, and Wood (23), showed that a pronounced increase in growth rate of Lactobacillus casei was observed when the levels of asparagine, serine, and glutamic acid were increased in the base medium. It was believed that these amino acids served directly or indirectly as precursors of strepogenin activity. Stokes et al. (29) presented evidence that trypsinized casein was about four times as potent as Wilson's liver fraction "L" when used in low concentrations for Lactobacillus casei and that high concentrations of asparagine inhibited the growth.

Evidence was presented to show that strepogenin is not a single amino acid or even a combination of only several amino acids (14, 17, 21, 29, 35, 36, 37,). It was also believed that some specificity of structure was involved. Strepogenin activity had been found to be present in many different protein substances such as extracts from liver, yeasts, insulin, hemoglobin, casein, crystalline mosaic virus, and pancreas. Woolley and Merrifield (37), showed that the substance with the highest strepogenin activity was synthetic insulin which had undergone partial hydrolysis.

Garvie and Mabbitt (7), showed that the rate of acid production by a slow variant of Streptococcus cremoris in milk was raised to that of the fast parent strain when peptone or acid hydrolysed peptone was added. Other sources

of available nitrogen gave similar results. This led to the conclusion that the factor which changed a fast culture to a slow culture was due to the loss of ability to utilize the nitrogen compounds of milk.

Speck and co-workers (17, 24, 25, 27), attempted to identify the constituent amino acids in pancreas extract which were the stimulators for the lactic acid bacteria. They were able to identify fourteen amino acids. It was noted that all of the amino acids that had been reported to be present in peptides possessing strepogenin activity were present in the two active peptides isolated from pancreas tissue. This gave further confirmation to the fact that strepogenin was not confined to a single compound.

MacLeod and Gordon (15), reported that Streptococcus lactis and Streptococcus cremoris possess peptidases capable of hydrolyzing a number of commercially available dipeptides and tripeptides into the constituent amino acids. It was also shown that if the C-terminal amino acid of the peptide was of D-configuration, no hydrolysis occurred. Dipeptides in which one of the constituent amino acids was L-leucine or L-valine, when substituted for these amino acids in chemically defined media, were shown to fulfill the nutritional requirements of each of the cultures for these essential acids.

Kihara and Snell (12, 13) and Kihara, Ikawa, and Snell (11), using the organism Lactobacillus casei, gave evidence which strongly supports the views that to serve as a sole

source of an essential amino acid for growth, a peptide must be hydrolyzed by the cell and that the growth-promoting activity of hydrolyzable peptides may be greater than , equal to, or less than that of the free limiting amino acid they supply depending upon whether the two independent processes of absorption and hydrolysis supply the limiting amino acid to the cell at a rate greater than, equal to or less than that which the free amino acid can be absorbed from the medium. These results lead one to believe that requirements for complex peptides (strepogenin) are apparent only and result from the fact that a single peptide must supply several, rather than only one, of the limiting amino acids in a form that can be absorbed and utilized by the cell more rapidly than the free amino acids of the medium. There is thus no evidence that peptides having strepogenin activity play any special role in metabolism of the organism other than as readily available sources of limiting amino acids.

Speck and Ledford (26), showed that by adding aqueous extract of pancreatic tissue to cottage cheese milk at the time of setting, they could speed up the time for development of the proper amount of lactic acid by as much as 40% and decrease the overall time for making of cottage cheese as much as 42%. They also stated that the finished product had no difference in flavor or appearance from the control product which was made by the standard "short-set" method.

The influence of rate of inoculation on propagation of mother cultures has been investigated by several workers.

Hales (8) recommended the use of 1.0% inoculation or greater so as to get desirable development of all the organisms present and to keep the blend of organisms as intact as possible.

Olson, Beachboard and Cohenour (20), recorded the activity on lactic cultures regularly inoculated at the rate of 0.1%, 0.4%, and 1.6% for a period of 6 weeks and found that best activity was maintained with the 1.6% inoculation. The activity decreased progressively with lower percentages of inoculation.

Babel (2, 3), stated that the first inoculation should be incubated until the milk is curdled and then cooled until the next propagation. Succeeding inoculations should be at the rate of 1.0% with incubation at 70° to 72°F. for 14 to 16 hours. He stated (2), that if an incubation period of 14 to 16 hours resulted in a culture having too great an acidity, the incubation time of the culture should be decreased rather than the amount of inoculum.

The influence of depth of milk layer on the rate of lactic acid production of cultures was reported by Harper and Huber (10), using the organisms Lactobacillus bulgaricus, Lactobacillus lactis, and Streptococcus thermophilus. They were able to show that differences in the ratio of the surface area to the depth of the milk was related to problems involving slow acid development during the manufacture of experimental Provalone cheese. It was also noted that the ratio of the surface area to the depth of the milk must be

similar to that which was used in carrying the starter culture to maintain the same rapid acid development.

Thurston and Barnhart (31), investigating the effects of heating skimmilk on the pH at coagulation by lactic cultures, showed that as the heat treatments of the skimmilk were raised from 145° to 198°F. for 30 minutes, the pH at coagulation increased from 4.60 to 5.03. They believed that this was caused by the effect of heat on the casein. They also found that the amount of acid produced at the time of coagulation was less as the temperature of heat treatment was increased. The time for coagulation under uniform setting conditions was about the same for lots of milk pasteurized at temperatures up to 155°F., but was reduced materially at 165°F., beyond which there was little further change in coagulation time.

Emmons, Price, and Swanson (5, 6) reported the same effect on the pH at time of coagulation. Their work covered both skimmilk and reconstituted non-fat dry milk solids.

The amount of starter culture which should be used in making cottage cheese seems to be one of preference by the individual cheese makers. Van Slyke and Price (33), suggested that when the short set method is used, the amount of starter to add to the skimmilk should be 4 to 5%. They believed that this was the maximum amount of starter that could be used without decreasing the firmness of the cottage cheese curd. They also stated that the starter should be strained before it is added to the skim milk for cottage cheese to

remove particles of curd and aid in thorough distribution.

Thurston and Gould (32), made cottage cheese with starter amounts ranging from 1.0 to 10.0%. The period required to develop a titratable acidity of 0.6% was 6 hours for the 1% inoculation, 4 hours and 30 minutes for 3%, 3 hours and 55 minutes for 5%, and 3 hours and 5 minutes for 10%.

Hales (9), stated that when the short set method was used, it was desirable to avoid the use of excessively high percentages of starter because the starter was a coagulated material and might interfere with good knitting of cottage cheese curd if too high a percentage was present.

Morgan, Jarman, and Willingham (19), using inoculation rates of 5, 10, 15, and 20%, showed that the average time from setting to cutting was 5 hours and 20 minutes, 3 hours and 47 minutes, 3 hours and 21 minutes, and 3 hours and 5 minutes, respectively. This was a savings of 1 hour and 13 minutes, 1 hour and 59 minutes, and 2 hours and 15 minutes for the 10, 15, and 20% inoculations, respectively. They stated that there was no significant difference between the yield obtained at the various rates of inoculation and that the whey losses from the various lots were approximately the same.

Stone, Lange, and Graf (30), showed that they were able to use 7 pounds of starter per 25 pounds of milk (28% starter) when making pilot vats of cottage cheese with reconstituted non-fat dry milk solids and rennet added at the rate of 0.25

ml per 100 pounds of milk plus starter. With good low heat dry milk powder, excellent batches of cheese were made, while some were of very poor quality depending on the quality of the specific low heat dry milk powder used.

There are numerous articles written which state that a specific per cent total solids should be used in the milk for the manufacture of cottage cheese (1, 6, 9, 22, 30, 33, 34). Angevine (1), stated that for small curd cottage cheese, 8.5 to 9.0% solids was most suitable with fresh skimmilk and 9.5% to not more than 10.5% solids if reconstituted non-fat dry milk solids were used. He also stated that for large curd cottage cheese, fresh skimmilk with 9% solids or fortified to 12% solids with low heat powder may be used. When making with all reconstituted non-fat dry milk solids, a solids content of 11% to 12% should be used.

Whitaker (34), recommended using from 9 to 12% solids when making cottage cheese with non-fat dry milk solids as the sole source of solids. He stated that if 9% solids were used, the acidity of the whey at the time of cutting should range from 0.50% to 0.52% and should be increased 0.02% for every 1% increase in solids content.

Hales (9), recommended the addition of 1% non-fat dry milk solids and/or condensed skim of low heat treatment to skimmilk for cottage cheese manufacture.

Attempts to find methods of making cottage cheese without the use of lactic starters so that more speed and uniformity of product could be obtained were presented by Deane

and Hammond (4), using meso-lactide at an amount equal to 8.8% of the solids-not-fat of the milk. They were able to coagulate the milk and reach a pH of about 4.6 in 2 hours at a temperature of 25°C. or 47 minutes at a temperature of 37.5°C. It was found necessary to use about 0.02% calcium chloride with the lactide to obtain normal expression of the whey during cooking. They also noted that the addition of rennet made it possible to cut the curd at higher pH values than normal and thus reduced the setting time. It was stated that a coagulation temperature above 37°C. caused excessive matting of the curd but if the temperature was below that, a cottage cheese made by this method had a bland flavor and was very similar in appearance to that made by starter cultures.

Mabbitt, Chapman, and Berridge (16), reported that glucono-delta-lactone, when dissolved in milk, hydrolyzed slowly to produce gluconic acid, and formed a suitable curd for making cheddar cheese. Deane and Hammond (4), using glucono-delta-lactone, found that it was unsatisfactory for cottage cheese making because of the long period required for coagulation of the milk.

EXPERIMENTAL METHODS

A. ROUTINE PROPAGATION OF CULTURES

The cultures used in these experiments were commercial lactic cultures taken from stock cultures maintained at Oklahoma State University. The lactic cultures consisted of strains of Streptococcus lactis and Streptococcus cremoris in combination with strains of Leuconostoc.

The method for propagation of these cultures was as follows: Approximately 9 ml quantities of reconstituted non-fat dry milk solids (10 gm added to 100 ml distilled water) were dispensed in screw capped test tubes containing calcium carbonate ($CaCO_3$) and autoclaved for 15 minutes at 15 psi. The milk was tempered to 72°F., inoculated with 0.1 ml (1%) of the culture, and incubated for 14 to 16 hours at 70° to 72°F. The cultures were then placed in a cold room at 45°F. until needed.

Before being used, the cultures were propagated twice in milk (without added calcium carbonate) in order to restore their vigor. Activity tests were frequently run in order to insure the selection of cultures which would be satisfactory.

B. GENERAL CHEESE MAKING PROCEDURE

The following is a brief description of the process used as the general or routine procedure for making of cottage cheese: Cultures were propagated as previously described. Milk was either pasteurized skimmilk (143°F. for 30 minutes) with a solids content varying from 8.5% to 9.75% or reconstituted non-fat dry milk solids (10 parts to 100 parts of water) with no further heat treatment applied.

The milk was dispensed at the rate of 9.5 pounds per batch with 0.5 pounds (5%) starter added. The inoculated milk was then tempered to 90°F. and allowed to stand for 1 hour at which time rennet was added at the rate of 0.01 ml per 10 pounds of milk. The rennet was diluted at the rate of 1:1,000 with sterilized distilled water and a 10 ml portion was added to the milk.

Titratable acidity readings were taken at hourly or half hour intervals until the titratable acidity reading of the whey reached a value of 5.2 (approximately 0.51%). At this point, the curd was cut with 3/8 inch knives and allowed to stand for 15 minutes prior to the start of cooking. In the cooking process, the temperature was brought up at the rate of 2°F. every 5 minutes until reaching 120°F. The curd was held at this temperature for 20 minutes or until cooked out.

Upon completion of cooking, the whey was partially drained and tap water was added at the rate of 4,000 ml per 10 pound lot. This was allowed to stand for 15 minutes,

drained and repeated. After partially draining the last tap water rinse, ice water was added at the rate of 4,000 ml per 10 pound lot and allowed to stand for 15 minutes. The ice water was drained and the cheese curd was allowed to drain for 1½ hours. The finished curd was stored in containers at 45°F. for at least 12 hours before further tests were performed.

C. FOUR-HOUR ACTIVITY TEST

The Four-hour activity test was used in the determination of the rate of lactic acid production by lactic cultures. This method was used because it more closely represents the making of cottage cheese than any of the other types of activity tests. Briefly the method used was as follows: Measured 9 ml quantities of reconstituted non-fat dry milk solids (10 parts added to 100 parts distilled water) were dispensed in rubber stoppered test tubes and heated in flowing steam for 30 minutes. The milk was then tempered to 90°F., inoculated with 0.5 ml (approximately 5%) of the culture, and incubated for 4 hours at 90°F. At the end of the incubation period, the milk was cooled immediately in an ice water bath. The entire contents of each tube was then titrated with N/10 NaOH, using phenolphthalein as the indicator. The ml of N/10 NaOH required was used as an indication of the activity of the culture.

D. RECONSTITUTED NON-FAT DRY MILK SOLIDS

The use of reconstituted non-fat dry milk solids for the routine propagation of cultures and making of cheese was done as follows: Non-fat dry milk solids were added to water at the rate of 10 parts of the solids to 100 parts of water which gave a final total solids content of the milk of approximately 9.1%. Distilled water was used in preparing the milk for activity tests. Unless otherwise stated, this procedure was used whenever reconstituted non-fat dry milk solids were used.

EXPERIMENTAL RESULTS AND DISCUSSION

A. INFLUENCE OF VARIOUS MATERIALS ON STIMULATION OF ACID PRODUCTION BY LACTIC CULTURES

Several authors have reported on the stimulatory effect of different materials on lactic acid producing organisms. Much of the work was done with organisms other than those used for the making of cottage cheese. Based on these facts, work was undertaken to determine if some of these materials had stimulatory effects on cottage cheese cultures and to what extent they would affect the rate of lactic acid production.

1. Pancreas extract. Pancreas extract, as reported by Speck and co-workers (17, 25), is an aqueous extract of pancreas tissue which has a stimulatory effect on lactic acid producing organisms. Trials were inititated to determine its stimulatory effect on mother culture and on acid production during cottage cheese making.

a. Stimulation of lactic cultures by the use of pancreas extract. In order to determine the stimulatory effect of pancreas extract on mother cultures with various abilities to produce lactic acid, 16 cultures with titration values ranging from 2.3 to 7.1 with the Four-hour activity test were propagated by the routine procedure previously

described. The pancreas extract* was made up as follows: The dried product was added to sterile distilled water at the rate of 1 gm per 25 ml of water and shaken until dissolved. To the tubes of milk prepared for the Four-hour activity test, 0.5 ml of aqueous pancreas extract (approximately 0.2%) was added and the Four-hour activity test was run. Controls containing no pancreas extract were also run. Titrations were run on all samples at 0 hours and 4 hours and the increase in acidity noted. The results are shown in Table I.

Since one is concerned with the amount of acid produced during the four hour incubation period, the results are expressed in terms of the titration values at the end of the four hours minus the initial (0 hours) titration values. The initial titration values for the cultures without the pancreas extract ranged from 1.7 to 2.1 ml and averaged 1.95 ml, while those for the cultures with the pancreas extract added ranged from 1.0 to 2.3 ml and averaged 2.1 ml. With the Four-hour activity test, the titration values should be 6.0 ml or higher for satisfactory cultures; therefore with the initial values of about 2.0 ml, the increase during the four hours of incubation should be 4.0 ml or more.

It will be noted that the pancreas extract had a stimulatory effect on all cultures tested. The average increase

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*Dried pancreas extract was obtained from Dr. Marvin Speck, Department of Animal Industry, North Carolina State College, Raleigh, North Carolina.

TABLE I

INFLUENCE OF PANCREAS EXTRACT ON RATE OF ACID PRODUCTION BY LACTIC CULTURES

CULTURE NO.	NO. TRIALS	INCREASE IN ACID DURING FOUR-HOUR TEST		INCREASE DUE TO PANCREAS EXTRACT
		PANCREAS EXTRACT ADDED		
		None	0.2%	
		ml	ml	ml
2	2	0.55	2.50	1.95
8	2	1.00	2.90	1.90
9	2	4.60	5.60	1.00
26	3	0.60	2.85	2.25
27	3	3.45	4.25	0.80
28	3	3.80	5.15	1.25
29	3	3.85	4.95	1.10
30	3	3.05	4.50	1.45
37	4	2.55	4.50	1.95
41	8	5.00	5.80	0.80
44	8	5.00	6.00	1.00
50	4	5.05	5.90	0.85
51	4	5.20	6.15	0.95
63	3	4.55	5.40	0.85
76	5	3.70	4.90	1.20
78	20	4.75	5.10	0.35
Average Value		3.56	4.78	1.22
No. Satisfactory Cultures		7	13	

in acid during the four hour test was 3.56 ml for the control cultures and 4.78 ml for the cultures with the pancreas extract added, a difference of 1.22 ml which was attributable to the added stimulant. Of the 16 lactic cultures tested, 9 failed to produce an increase in titration value of 4.0 ml or more which is considered adequate for making of cottage cheese. Upon the addition of the pancreas extract, only 3 cultures had titration values of less than 4.0 ml. It will be noted that the three slowest cultures, Nos. 2, 8, and 26, were greatly stimulated by the pancreas extract but were still too weak to use for cheese making. Without pancreas extract, they produced 0.55, 1.00. and 0.60 ml titration values, respectively, while with the extract, they had titration values of 2.50, 2.90, and 2.85 ml, respectively.

In order to determine if pancreas extract had a stimulatory effect on stored cultures, further studies were initiated. Four lactic cultures were prepared by the standard procedure in quantities of 100 ml per culture. These cultures were then stored at 45°F. and the Four-hour activity test was run on 0 days, 1, 3, 5, 7, and 9 days. Pancreas extract at the rate of 0.2% was added to one set of activity tests and none was added to the control set. The results are shown in Table II.

When pancreas extract was added to the culture, titration values held up well until the fifth day when there was a rapid drop in titration values. Cultures with pancreas extract added still produced enough lactic acid to be

TABLE II

EFFECT OF PANCREAS EXTRACT ON STORED MOTHER CULTURES

DAYS STORED AT 45°F	INCREASE IN ACID DURING FOUR-HOUR TEST (Averages of Four Trials)	
	CONTROL	0.2% PANCREAS EXTRACT ADDED
	ml	ml
0	4.80	5.70
1	4.70	5.70
3	4.35	5.60
5	3.55	5.05
7	2.20	3.75
9	0.80	1.25

considered active enough for use in cheese making after the fifth day while the titration values of the control cultures failed to show sufficiently high titration values after three days of storage.

Based upon the results obtained from the above trials, tests were initiated to determine if the pancreas extract had a lasting effect. Tubes for the Four-hour activity test were prepared. Triplicate tubes for the control and with pancreas extract added at the rate of 0.2% were inoculated. One tube for each was titrated at 0 hours and another at 4 hours while the third tube was chilled immediately after the the four hours of incubation. This tube was held at 45°F. for 14 hours and the Four-hour activity tests were run in milk without the pancreas extract added. This was also repeated the following day. The results are shown in Table III.

The results indicate that pancreas extract does not have a lasting stimulatory effect on the lactic culture and that it must be present to cause stimulation. This would lead one to believe that pancreas extract contains a nutritional factor which is lacking in milk or not present in large enough amounts for maximum production of lactic acid.

b. Stimulatory effect of pancreas extract in cheese making. In order to determine the stimulatory effect of pancreas extract on the acid production during the cottage cheese making process, two 10 pound lots of milk were made into cottage cheese with the conventional procedure except that 0.2% pancreas extract was added to one vat and none to

TABLE III

INFLUENCE OF PANCREAS EXTRACT ON SUBSEQUENT TRANSFERS OF LACTIC CULTURES

CULTURE NO.	CONTROL			PANCREAS EXTRACT		
	TRANSFER			PANCREAS EXTRACT (0.2%)	1st TRANS. FROM PANCREAS EXTRACT	2nd TRANS. FROM PANCREAS EXTRACT
	FIRST	SECOND	THIRD			
	ml	ml	ml	ml	ml	ml
2	0.6	0.3	0.4	2.5	0.6	0.5
8	0.6	0.6	0.6	2.7	0.7	0.7
9	4.7	4.6	4.6	5.6	4.6	4.5
26	0.6	0.6	0.6	2.7	0.7	0.7
27	3.7	3.3	3.5	4.7	3.5	3.3
28	3.8	3.7	3.6	5.5	4.0	4.1
29	3.6	3.3	3.5	5.4	3.4	3.3
30	3.0	3.0	2.9	4.9	3.4	3.2

the other. The time allowed to bring the temperature from 90° to 120°F. was shortened from 75 minutes to 45 minutes for the vat containing pancreas extract. This was done because of the information obtained from work by Speck and Ledford (26). Five trials were run using reconstituted non-fat dry milk solids at the rate of 1 pound solids to 10 pounds of water which gave a final solids content of approximately 9.1%. The results are shown in Table IV.

The results show that the lots of cottage cheese made with the addition of pancreas extract, on the average, were cut approximately 1 to 1½ hours sooner than the controls and required only about one-half the time to cook out. The savings in time for making the cottage cheese from start to finish ranged from 2 hours and 05 minutes to 2 hours and 40 minutes.

The lots of cottage cheese which were made with pancreas extract had no detectable off flavors or odors and the bodies and textures were similar to those of the control lots except for slightly rubbery body defects.

It would seem that cottage cheese made with the use of pancreas extract would be feasible in a commercial operation. By the use of pancreas extract, it would be possible to make a complete batch of cottage cheese and also clean up in an 8 hour working day.

2. Amino acids and related compounds. Research has been reported with the organism Lactobacillus casei which indicated that what had previously been reported as

TABLE IV

INFLUENCE OF PANCREAS EXTRACT ON TIME REQUIRED TO MAKE COTTAGE CHEESE

TRIAL NO.	CULTURE NO.	PROCESS	ml N/10 NaOH TO NEUTRALIZE 9 ml OF MILK			PERIOD OF TIME FROM:			
			INITIAL	CUTTING	DRAINING	SETTING TO CUTTING	CUTTING TO DRAINING	TOTAL TIME	REDUCTION IN TIME ATTRIBUTABLE TO PANCREAS EXTRACT
			ml	ml	ml	hr:min	hr:min	hr:min	hr:min
1	41	Control	2.2	5.2	5.7	4:35	2:00	6:35	
	41	P.E.*	2.4	5.4	5.5	3:20	1:05	4:25	2:10
2	44	Control	2.2	5.3	5.9	4:40	2:00	6:40	
	44	P.E.	2.4	5.2	5.4	3:00	1:00	4:00	2:40
3	51	Control	2.3	5.3	5.8	4:05	1:55	6:00	
	51	P.E.	2.5	5.2	5.4	2:50	1:05	3:55	2:05
4	63	Control	2.2	5.1	5.6	4:25	2:05	6:30	
	63	P.E.	2.4	5.2	5.4	3:00	1:05	4:05	2:25
5	63	Control	2.2	5.2	5.7	4:35	2:00	6:35	
	63	P.E.	2.4	5.2	5.4	3:05	1:05	4:10	2:25

*Pancreas extract added at the rate of 0.2%.

strepogenin activity was not due to complex peptides but rather that it was due to the lack of certain limiting amino acids which were needed to meet specific requirements. Kihara and Snell (12), recommended that certain compounds should be added to the basal medium for testing strepogenin activity and if this was done, no strepogenin activity could be shown with complex peptides. Based on these facts, work was undertaken to determine if any of the compounds which were recommended by Kihara and Snell would have a stimulatory effect on lactic cultures.

L-ascorbic acid, L-cysteine, L-glutamine, L-serine, guanylic acid, uracil, and spermine were tested for their stimulatory effect on lactic cultures. Each of the above compounds was made up by diluting the dried or crystalline compound with a desired amount of double, glass distilled, sterilized water. Concentrations of 1, 2, 4, 8, 10, 20, 40, 80, 100, 200, 400, 500, and 600 ppm of the various compounds were added to reconstituted non-fat dry milk solids prepared for the Four-hour activity test. The tests were then run in the usual manner. Three trials were run with four cultures. Similar results were obtained for all cultures.

Spermine showed only slight stimulation at concentrations of 1 to 10 ppm added but no stimulation with concentrations of 20 ppm or higher. Based on the Four-hour activity test, spermine at concentrations of less than 10 ppm showed titration values of 0.4 ml greater than the controls which contained no spermine.

The results presented in Table V show the effect of guanylic acid and L-cysteine on the rate of production of lactic acid by lactic cultures. Guanylic acid in concentrations of 40 ppm or greater had a stimulatory effect on lactic cultures with a maximum of stimulation at approximately 400 ppm. Concentrations over the 400 ppm had slightly less stimulatory effect than that which was obtained with 400 ppm of guanylic acid. The maximum value of the increase in lactic acid production due to guanylic acid was 1.20 ml based on the Four-hour activity test.

The data in Table V shows that L-cysteine reduces the rate of production of lactic acid by lactic cultures. At concentrations of less than 20 ppm, L-cysteine had a slight inhibitory effect on the culture. At concentrations of 20 ppm there was marked inhibition and as the concentration of L-cysteine was increased, greater inhibition occurred.

No stimulation or inhibition was noted for L-ascorbic acid, L-glutamine, L-serine, or uracil at concentrations of 100 ppm or less. L-serine showed inhibition with concentrations greater than 100 ppm and L-glutamine showed inhibition with concentrations greater than 300 ppm. L-ascorbic acid and uracil showed no effect even with concentrations of 600 ppm.

Guanylic acid was then added to a Four-hour activity test in combination with pancreas extract. Guanylic acid was added at the rate of 400 ppm and pancreas extract was added at the rate of 2,000 ppm (0.2%). The results are

TABLE V

INFLUENCE OF CYSTEINE AND GUANYLIC ACID ON RATE OF ACID PRODUCTION BY LACTIC CULTURES

ppm CYSTEINE OR GUANYLIC ACID	INCREASE IN ACID DURING FOUR-HOUR TEST		CHANGE DUE TO:	
	GUANYLIC ACID	CYSTEINE	GUANYLIC ACID	CYSTEINE
	ml	ml	ml	ml
0	5.65	5.65	0.00	0.00
1	5.65	5.60	0.00	-0.05
2	5.65	5.60	0.00	-0.05
4	5.65	5.45	0.00	-0.20
8	5.70	5.45	≠0.05	-0.20
10	5.70	5.45	≠0.05	-0.20
20	5.75	4.85	≠0.10	-0.80
40	5.80	4.30	≠0.15	-1.35
80	5.90	3.80	≠0.25	-1.85
100	5.95	3.30	≠0.30	-2.35
200	6.40	2.65	≠0.75	-3.00
400	6.85	1.65	≠1.20	-4.00
500	6.75	1.50	≠1.10	-4.15
600	6.70	1.45	≠1.05	-4.20

shown in Table VI. These results indicate that guanylic acid had, at the most, only a slight stimulatory effect on the lactic culture when added with pancreas extract. This would lead one to believe that pancreas extract either contains guanylic acid or a compound which can replace it. It was also observed that with each of the four cultures used, the pancreas extract was more stimulatory than the guanylic acid with the concentrations used.

It would seem from these results, that the compounds which were used by Kihara and Snell (12), except for cysteine and guanylic acid, had little or no effect on the growth of lactic cultures. It could also be concluded that guanylic acid plays a part in stimulation of the lactic culture to produce lactic acid but that pancreas extract can replace it and has a greater stimulatory effect. Cysteine in any amount seemed to have a detrimental effect on the production of lactic acid although the effect was only slight until greater than 10 ppm of the cysteine was added.

B. INFLUENCE OF VARIOUS FACTORS ON THE RATE OF ACID PRODUCTION BY LACTIC CULTURES

Several factors other than adding of stimulants were considered which might have an effect on lactic acid production by lactic cultures. Considerable work has been done which shows that temperature plays a vital part in the rate of lactic acid production. Work has also been done which indicates that rate of inoculation, depth of milk layer, and total solids in the milk also play a vital role in rate of

TABLE VI

INFLUENCE OF GUANYLIC ACID AND PANCREAS EXTRACT IN COMBINATION ON THE RATE OF ACID PRODUCTION BY LACTIC CULTURES

CULTURE NO.	NO. TRIALS	INCREASE IN ACID DURING FOUR-HOUR TEST			
		CONTROL	GUANYLIC ACID	PANCREAS EXTRACT	COMBINATION
		ml	ml	ml	ml
41	3	3.95	4.45	5.10	5.10
44	3	4.15	4.60	5.20	5.30
63	4	4.60	5.20	5.60	5.65
78	4	4.85	5.00	5.20	5.35

lactic acid produced. Accordingly, experiments were initiated to determine how much the amount of inoculation used in the propagation of mother cultures, amount of inoculation used in making of cottage cheese, depth of milk layer, and total solids in the milk effected the production of lactic acid by lactic cultures.

1. Effect of amount of inoculum used in propagating mother cultures. In order to determine the influence of the rate of inoculation for propagating mother cultures on the rate of production of lactic acid, cultures were carried through successive transfers using different rates of inoculation. Four active cultures were selected and carried through 10 successive transfers with inoculation rates of 0.1, 0.3, 1.0, 3.0, and 10.0% for each culture. The Four-hour activity test was run after each transfer and subsequent ripening. The results are shown in Table VII and summarized in Table VIII.

The results in Table VII indicate that when transferring mother culture one should not use a heavier inoculation than 1.0%. The results also show that there is little difference in activity of the cultures with 1.0% inoculation or less. One factor which is not revealed in the results of the activity tests is that of flavor. Cultures which were transferred with 0.1% inoculations had a green flavor after the 10 days of transferring while those which had been transferred using 1.0% inoculations maintained a good flavor. This would seem to be due to the fact that the Leuconostoc

TABLE VII

INFLUENCE OF AMOUNT OF CULTURE USED IN TRANSFERRING MOTHER CULTURES ON RATE OF ACID PRODUCTION

TRANSFER	CULTURE NO.	INCREASE IN ACID DURING FOUR-HOUR TEST				
		PER CENT CULTURE ADDED				
		0.1 ml	0.3 ml	1.0 ml	3.0 ml	10.0 ml
1		4.70	4.60	4.50	4.30	3.60
2		5.10	4.70	4.80	4.50	4.60
3		4.35	4.10	4.15	4.05	3.90
4		4.75	4.50	4.55	4.30	4.10
5	41	4.60	4.55	4.50	4.50	4.25
6		5.10	4.90	4.60	4.65	4.50
7		5.20	5.00	4.60	4.70	4.30
8		4.85	5.15	4.65	4.30	4.30
9		4.45	4.60	4.40	4.20	4.10
10		5.05	5.35	5.10	4.80	4.50
1		4.60	4.60	5.00	4.60	4.20
2		5.10	5.30	5.50	5.40	4.90
3		4.65	5.05	4.90	4.75	4.50
4		5.05	5.25	5.10	5.05	4.45
5	44	5.00	5.00	5.20	5.00	4.75
6		5.20	5.30	5.30	5.10	4.80
7		5.15	5.40	5.30	4.95	4.85
8		5.05	5.15	5.40	5.00	4.75
9		4.35	4.80	4.85	4.50	4.10
10		4.70	4.80	5.30	5.20	4.75

TABLE VII (Con't)

INFLUENCE OF AMOUNT OF CULTURE USED IN TRANSFERRING MOTHER CULTURES ON RATE OF ACID PRODUCTION

TRANSFER	CULTURE NO.	INCREASE IN ACID DURING FOUR-HOUR TEST				
		PER CENT CULTURE ADDED				
		0.1 ml	0.3 ml	1.0 ml	3.0 ml	10.0 ml
1		4.45	4.50	4.30	4.15	4.00
2		4.50	4.30	4.00	3.90	3.40
3		4.40	4.25	4.25	4.15	3.90
4		4.65	4.60	4.35	4.45	4.30
5	63	5.05	4.95	4.65	4.60	4.30
6		5.00	5.00	4.85	4.50	4.40
7		4.90	4.95	4.90	4.60	4.20
8		5.00	5.00	5.10	4.40	4.00
9		4.90	5.10	5.10	4.15	3.90
10		4.90	5.25	5.15	4.20	3.70
1		4.60	4.50	4.30	4.20	4.00
2		4.45	4.00	4.45	3.90	3.45
3		4.90	4.50	4.45	4.40	4.00
4		4.95	4.85	4.70	4.70	4.25
5	78	4.90	4.90	4.80	4.60	4.30
6		5.10	4.90	4.85	4.40	4.30
7		5.00	4.95	4.90	4.30	4.40
8		5.00	4.90	5.10	4.25	4.10
9		4.90	4.85	5.00	4.00	3.80
10		4.85	4.70	4.90	4.00	3.65

TABLE VIII

SUMMARY OF INFLUENCE OF AMOUNT OF CULTURE USED IN TRANSFERRING MOTHER CULTURES ON RATE OF ACID PRODUCTION

CULTURE NO.	TRANSFERS	AVERAGE INCREASE IN ACID DURING FOUR-HOUR TEST				
		PER CENT CULTURE ADDED				
		0.1 ml	0.3 ml	1.0 ml	3.0 ml	10.0 ml
41	1 - 5	4.70	4.49	4.50	4.33	4.09
	6 - 10	4.93	5.00	4.67	4.53	4.34
	1 - 10	4.82	4.75	4.59	4.43	4.22
44	1 - 5	4.88	5.04	5.14	4.96	4.56
	6 - 10	4.89	5.09	5.23	4.95	4.64
	1 - 10	4.89	5.07	5.19	4.96	4.60
63	1 - 5	4.61	4.52	4.31	4.25	3.98
	6 - 10	4.94	5.06	5.02	4.37	4.04
	1 - 10	4.77	4.79	4.66	4.31	4.01
78	1 - 5	4.76	4.55	4.54	4.36	4.00
	6 - 10	4.97	4.86	4.95	4.19	4.05
	1 - 10	4.87	4.71	4.85	4.28	4.03
average all cultures	1 - 5	4.74	4.65	4.62	4.48	4.16
	6 - 10	4.93	5.00	4.97	4.51	4.27
	1 - 10	4.83	4.83	4.80	4.49	4.21

grow more slowly than Streptococcus lactis or Streptococcus cremoris but will continue to grow when the pH is low enough to stop the S. lactis or S. cremoris. It would seem that with 1.0% inoculation, peaks of acid production would be reached sooner than with the 0.1% inoculation thus allowing the Leuconostoc a longer period of time to develop and to produce flavor compounds.

When transfers of inoculum were greater than 1.0%, the titration values for the Four-hour activity test were somewhat lower. This would be due to the fact that with the heavier inoculation, peak acid production would be reached sooner than with 14 to 16 hours of incubation before running the Four-hour activity test, the cultures would have passed their peak ability to produce lactic acid. If the incubation time were shortened, the titration values for the 3% and 10% inoculation rates would probably have been higher.

If the heavier inoculation rates (3% and 10%) caused a gradual weakening of the cultures, the activities on the last five propagations would be much lower than on the first five. The data in Table VIII show that this is not true. Except for one culture (No. 78) the average activity values for the last five determinations were equal to or higher than those for the first five. This suggests that the heavy inoculation rates do not weaken the cultures and that the relatively low activity values resulted from the culture having passed the peak for greatest activity.

From all indications, a 1.0% inoculation is desired

when transferring mother cultures and an incubation period ranging from 12 to 16 hours is desirable at incubation temperatures of 70° to 72°F.

2. Effect of depth of milk layer. In order to determine if the depth of the milk in the vat influences the rate of lactic acid production by lactic cultures, cultures were propagated in equal quantities on milk in different shaped containers. Reconstituted non-fat dry milk solids were made up in 200 ml portions and heated in flowing steam for 30 minutes and tempered to 90°F. The milk was then inoculated with 5% of the desired culture which had been propagated by the routine procedure. One-hundred ml of the inoculated milk was poured into a sterilized 10 X 1 inch test tube and the remaining 100 ml of milk was poured into a 500 ml erlenmeyer flask. The milk in the test tube had a depth of 7¼ inches and the depth of the milk in the erlenmeyer flask was three-fourths of one inch. Both containers were incubated at 90°F. for 4 hours. At the end of the 4 hour incubation, 9 ml samples from each container were titrated with N/10 NaOH to determine the amount of lactic acid produced in a 4 hour period. Three or four trials were run on each of five cultures. The results are shown in Table IX.

The results indicate that the depth of the milk has a slight influence on the amount of lactic acid produced. In all cases, more lactic acid was produced in the tubes than in the flasks. The difference in lactic acid production based on ml of N/10 NaOH ranged from 0.33 ml to 0.77 ml with

TABLE IX

EFFECT OF DEPTH OF MILK LAYER ON THE RATE OF PRODUCTION OF LACTIC ACID

CULTURE NO.	NO. TRIALS	AVERAGE ml N/10 NaOH REQUIRED TO NEUTRALIZE ACID AFTER 4 HOUR INCUBATION IN:		DIFFERENCE
		TUBE	FLASK	
		ml	ml	ml
41	3	4.70	4.37	0.33
44	4	5.43	4.90	0.53
63	4	5.78	5.40	0.38
68	3	6.50	5.73	0.77
78	4	6.03	5.53	0.50
Average		5.69	5.19	0.50

an average of 0.50 ml. This would lead one to believe that, because Streptococcus lactis and Streptococcus cremoris are facultative anaerobes, the oxidation-reduction potential is more favorable when the ratio of surface area to volume is small resulting in less absorption of oxygen.

3. Influence of rate of inoculation of cheese milk. The usual rate of inoculation for the making of cottage cheese with the "short set" is approximately 5% of culture. This portion of the study was undertaken to determine if larger quantities of starter could be used and to determine what effect the use of larger quantities would have on the time required to make cottage cheese and on the quality of the finished product. Work previously reported has indicated that 28% starter could be used and a good cottage cheese obtained.

a. Preliminary trials. Preliminary trials were run with 9 ml portions of milk to determine the influence of the rate of inoculation on the rate of acid production and time required for coagulation. By the use of small portions in closed test tubes, there was less chance of contamination and other factors entering into the results.

Reconstituted non-fat dry milk solids were placed in half-pint bottles. One bottle containing a 120 gm portion and 3 bottles containing 100 gm portions were covered with double parchment and heated in flowing steam for 30 minutes and then cooled to 90°F. To the 120 gm portion of milk, 80 ml of freshly ripened culture was added and shaken well.

This was equivalent to a 40% inoculation. One-hundred grams of this 40% inoculation was weighed into a 100 gm portion of milk which was equivalent to a 20% inoculation. This was well shaken and a 100 gm portion of this milk was weighed into another 100 gm portion of milk which was equivalent to a 10% inoculation. This was well shaken and a 100 gm portion of this milk was weighed into another 100 gm portion of milk which was equivalent to a 5% inocualtion. Ten 9 ml portions of the above lots of inoculated milk were pipetted into sterile screw capped test tubes using sterilized pipettes and placed in a 90°F. incubator and held at this temperature. One tube of each lot of inoculated milk was removed at 0, 1, 1½, 2, 2½, 3, 3½, 4, 4½, and 5 hours and placed in an ice water bath. Coagulation time was noted for each lot and pH and titratable acidity were also run. The results are shown in Table X.

It will be noted that with the increase in amount of inoculation, the initial acidity increased from 1.80 ml for the 5% inoculation to 4.10 ml for the 40% inoculation. It will also be noted that time for first sign of coagulation was shortened and occured at pH of approximately 5.0 and a titratable acidity value of approximately 5.4 ml. This coagulation point was reached at 3½ hours with 5% inoculation, 3 hours with 10% inoculation, 2 hours with 20% inoculation, and 1 hour with 40% inoculation. This was not a solid coagulation but only denoting the time when coagulation had begun. Solid coagulation did not occur for

TABLE X

INFLUENCE OF RATE OF INOCULATION ON COAGULATION TIME, pH, AND TITRATABLE ACIDITY
(Average of Four Trials)

TIME	RATE OF INOCULATION							
	5%	10%	20%	40%	5%	10%	20%	40%
	pH VALUES				N/10 NaOH REQUIRED TO TITRATE 9 ml OF MILK			
hr					ml	ml	ml	ml
0	6.45	6.27	6.08	5.41	1.80	2.10	2.90	4.10
1	6.23	6.02	5.72	4.98*	2.10	2.65	3.80	5.35*
1½	6.16	5.82	5.39	4.71	2.35	3.15	4.55	6.25
2	6.00	5.53	5.07*	4.61	2.80	3.95	5.50*	6.60
2½	5.68	5.28	4.72	4.55	3.45	4.85	6.25	6.75
3	5.30	4.94*	4.69	4.49	4.30	5.75*	6.55	6.95
3½	4.99*	4.72	4.65	4.45	5.35*	6.30	6.80	7.05
4	4.71	4.68	4.61	4.42	6.20	6.55	6.95	7.15
4½	4.65	4.64	4.56	4.40	6.35	6.80	7.05	7.20
5	4.57	4.50	4.48	4.40	6.70	6.95	7.15	7.20

*First Evidence of Coagulation

approximately another one-half hour.

Although there was quite a difference in the titratable acidity values for the initial readings, there was somewhat of a narrower range of values after 5 hours of incubation. At 5 hours, the titratable acidity value for the 5% inoculation was 6.7 and for the 40% inoculation 7.20. The peak acid production per given time was reached when the pH was approximately 4.7 and the titratable acidity was approximately 6.30 ml. This would indicate that there is a marked decrease in acid development once solid coagulation has taken place.

Another point of interest is the amount that the lag period is decreased when the amount of starter is increased. With the use of 40% starter, there was little or no lag period noted while with 5% starter, the maximum production of lactic acid did not start until after approximately 2 hours.

As previously mentioned, the time for coagulation was reduced as the amount of starter added was increased. It will be noted that for every 5% of starter added over the original 5% of starter, there is a decrease in coagulation time of approximately one-half hour up to a total of 20% starter added. After this point, time which can be saved is less for the amount of starter added due to the lag phase which the organisms go through before rapid growth is evident.

b. Cheese making. From the results obtained in the preliminary trials, it appeared the larger amounts of culture could be used to shorten the cheese making process.

Accordingly, 10 pound lots of reconstituted milk were made into cottage cheese. The routine procedure for making cottage cheese was used except that the amount of starter was varied from 5% to 40%. The results are shown in Table XI.

The results show that the time from setting to cutting was reduced as the amount of starter added was increased. When 10% starter was used, setting time was reduced approximately 1 hour. When 20% starter was used, setting time was reduced approximately 1 hour and 15 minutes. When 40% starter was used, setting time was reduced approximately 2 hours and 15 minutes. The time to cook the curd was not affected by the amount of starter added, although there were slight differences in time required for the different lots. It will be noted that by the use of 10% starter rather than the conventional 5%, the time for making cheese was reduced approximately 1 hour. The quality of the cheese made with 10% starter was comparable to that made with 5% starter except for a slightly shattered curd with the 10% starter. Cottage cheese made with 20 and 40% starter was deemed unsatisfactory because of the large amount of shattered curd, mealy texture, and poor color.

From the results obtained, the author believes that the use of 10% starter for making of cottage cheese would result in a product of high quality with a reduction in time of approximately 1 hour as compared to the use of 5% starter.

4. Influence of solids content of milk. A factor which is known to influence the time required to make cottage

TABLE XI

INFLUENCE OF RATE OF INOCULATION ON THE MAKING OF COTTAGE CHEESE

STARTER ADDED	ml N/10 NaOH TO NEUTRIALIZE 9 ml OF MILK			TIME			FLAVOR	BODY & TEXTURE	APPEARANCE
	INITIAL	CUTTING	FINAL	SETTING TO CUTTING	COOKING	TOTAL			
%	ml	ml	ml	hr:min	hr:min	hr:min			
5	2.05	5.20	5.63	4:38	2:10	6:48	Good	Good	Good
10	2.43	5.03	5.55	3:41	2:06	5:47	Good	Slightly Shattered	Good
20	3.10	5.18	5.58	3:23	2:13	5:36	Good	Shattered & Mealy	Chalky
40	4.45	4.90	5.33	2:20	2:15	4:35	Good	Badly Shattered & Very Mealy	Dull to Yellow

cheese and also the quality of the finished cheese is the amount of solids used in the milk. Too low a solids content will give a poor bodied cheese which is weak, while too high a solids content will cause the cheese to be coarse and have a chalky appearance. Tests were undertaken to determine what the optimum amount of solids in milk for cottage cheese made with fortified skimmilk and with reconstituted non-fat dry milk solids would be.

a. Preliminary trials. It was desirable to know at what pH and at what titratable acidity coagulation was first taking place. Reconstituted non-fat dry milk solids were made up at rates so that the solids content ranged from 7 to 16%. Eight 9 ml portions of each lot of milk were pipetted into screw capped test tubes and autoclaved for 15 minutes at 15 psi. The tubes were then cooled to 90°F. and inoculated with 0.5 ml of a freshly prepared culture and placed in an incubator at 90°F. One tube of each lot of inoculated milk was removed at 0, 1, 2, 3, 3½, 4, 4½, and 5 hours and placed in an ice water bath. The period required for the first appearance of coagulation was noted for each lot and pH and titratable acidity were also run. The results are shown in Table XII.

The results show the period required for the lots of milk to start to coagulate were 3½ hours for the lots with 7 to 10%, inclusive, of solids present. The coagulation period lengthened as the solids contents increased above 10%, being 4 hours for the lots with 11% and with 12% solids, 4½

TABLE XII

EFFECT OF SOLIDS CONTENT OF MILK ON RATE OF COAGULATION, pH, AND TITRATABLE ACIDITY (Average of 3 trials)

TIME	PER CENT SOLIDS							
	7		8		9		10	
	pH	TA	pH	TA	pH	TA	pH	TA
hr		ml		ml		ml		ml
0	6.15	1.50	6.25	2.03	6.19	2.17	6.09	2.37
1	6.05	1.93	5.90	2.50	5.93	2.53	5.97	2.77
2	5.85	2.50	5.64	3.33	5.69	3.50	5.70	3.80
3	5.14	3.67	5.07	5.00	5.16	5.20	5.21	5.68
3½	4.90	5.03*	4.91	5.50*	5.00	6.00*	5.06	6.63*
4	4.83	5.30	4.72	6.18	4.81	6.80	4.87	7.03
4½	4.55	5.53	5.66	6.91	4.73	7.35	4.80	7.63
5	4.49	6.20	4.57	7.05	4.66	7.50	4.71	7.90

TIME	PER CENT SOLIDS							
	11		12		14		16	
	pH	TA	pH	TA	pH	TA	pH	TA
hr		ml		ml		ml		ml
0	6.13	2.63	6.18	3.00	6.16	3.37	6.12	3.60
1	5.99	3.03	5.98	3.33	6.04	3.47	6.01	3.83
2	5.74	3.98	5.78	4.20	5.81	4.60	5.81	5.25
3	5.38	5.70	5.45	5.83	5.49	6.18	5.55	6.60
3½	5.14	6.85	5.26	7.60	5.31	8.05	5.38	8.12
4	5.02	7.10*	5.01	8.00*	5.18	8.23	5.21	8.65
4½	4.90	8.62	4.96	8.70	5.09	9.65*	5.13	9.80
5	4.77	8.65	4.87	9.10	5.00	9.85	5.06	9.99*

*First Evidence of Coagulation

hours for the lots with 14% solids, and 5 hours for the lots with 16% solids. It may be noted that the titratable acidity at coagulation increased as the solids content increased, while the pH values were rather constant at about 5.0.

b. Cheese making. From the results obtained in the preliminary trials, it was decided that 4 vats of cheese would be made at one time using 9, 10, 11, and 12% solids. The cottage cheese was made by the standard procedure except that the solids content was varied in each lot.

(1). Reconstituted non-fat dry milk solids. Four trials of 4 vats each containing 10 pounds of milk per vat were made into cottage cheese by the routine procedure. The milk was made by adding non-fat dry milk solids to water so that the final solids content of the 4 vats were 9, 10, 11, and 12%, respectively. No further heat treatment was given the milk. Starter was propagated by the routine method except that the solids content of the starter culture was the same as the solids content of the vat that in which it was to be used. The Results are shown in Table XIII.

The results show the lots of milk with 11% solids resulted in the best quality cheese. With either higher or lower solids, defects in body and texture were evident. As the solids of the milk was increased, the time required for making the cheese increased. Although the lots with 11% solids in the milk required more time for making than the lots with lower solids contents, the better quality may justify the longer time.

TABLE XIII

INFLUENCE OF SOLIDS CONTENT OF MILK IN CHEESE MAKING USING RECONSTITUTED NON-FAT DRY MILK SOLIDS (Average of 4 trials)

SOLIDS CONTENT OF MILK	ml N/10 NaOH TO NEUTRALIZE 9 ml OF MILK			TIME			FLAVOR	BODY & TEXTURE	APPEARANCE
	INITIAL	CUTTING	FINAL	SETTING TO CUTTING	COOKING	TOTAL			
%	ml	ml	ml	hr:min	hr:min	hr:min			
9	2.23	5.13	5.33	4:33	2:10	6:43	Green	Pasty	Mushy
10	2.40	5.58	5.80	4:50	2:05	6:55	Good	Slightly Pasty	Slightly Mushy
11	2.60	6.20	6.45	5:15	1:50	7:05	Good	Good	Good
12	2.85	6.45	6.70	5:35	2:00	7:35	Acid	Mealy & Shattered	Good

The data show that as the solids content increased, the titratable acidity at cutting also increased. With 9% solids, the acidity at cutting averaged 5.13 ml while that for the lots with 12% solids averaged 6.45 ml.

One fact which is not noted in the table is that 2 of 4 vats of 10% solids and all vats of 11% and 12% solids had floating curd and as the solids were increased the amount of floating curd increased. There was no reasonable explanation for this fact.

It would seem that, with the use of reconstituted non-fat dry milk solids as the total solids content of the milk, 11% solids is the most favorable amount of solids for the best yield and finished product.

(2). Fortified skimmilk. In a further study of the influence of solids content of the milk, four trials were conducted using fortified skimmilk. With the first trial, skimmilk was obtained from the Oklahoma State University Creamery. The milk had been pasteurized at 143°F. for 30 minutes and held in the cold box for one day. A Mojonnier test (18), was run to determine the solids-not-fat content of the skimmilk. The milk was then divided into four 10 pound lots and non-fat dry milk solids were added at the rates of 0, 1, 2, and 3% to each lot. The milk was then made into cheese by the routine procedure. The results are shown in Table XIV.

The effect of fortifying skimmilk with non-fat dry milk solids were essentially the same as those obtained by using

TABLE XIV

INFLUENCE OF FORTIFYING MILK WITH NON-FAT DRY MILK SOLIDS

SOLIDS CONTENT OF MILK	ml N/10 NaOH TO NEUTRALIZE 9 ml OF MILK			TIME			FLAVOR	BODY & TEXTURE	APPEARANCE
	INITIAL	CUTTING	FINAL	SETTING TO CUTTING	COOKING	TOTAL			
%	ml	ml	ml	hr:min	hr:min	hr:min			
9.0	2.20	5.20	5.60	4:45	2:25	7:10	Good	Weak	Mushy
10.0	2.40	5.50	5.90	4:55	1:55	6:50	Good	Weak	Slightly Mushy
11.0	2.60	6.10	6.40	5:10	1:50	7:00	Good	Slightly Weak	Good
12.0	2.90	6.40	6.80	5:25	1:45	7:10	High Acid	Good	Good

reconstituted milk in the previous experiment (Table XIII) except that, the milk which contained 12% solids resulted in the best finished product. There was no criticism of the cheese for body and texture or appearance. The time required for making the cheese was slightly longer than that required for the 10% or 11% milk but was not considered sufficently longer to affect the time required to make cheese.

Milk was then obtained from the Oklahoma State University herd from cows known to produce milk with low solids content. The milk was separated and the skim milk pasteurized in a 10 gallon can at 143°F. for 30 minutes. The Mojonnier test (18), was run to determine the solids-not-fat content of the skimmilk. The milk was then divided into four 10 pound lots and non-fat dry milk solids were added at the rates of 0, 1, 2, and 3% to each lot. The milk was then made into cheese by the routine procedure. The results are shown in Table XV.

The effects of fortifying low solids milk with non-fat dry milk solids are essentially the same as those obtained from the regular fortified skimmilk. The results in Table XV are the average of 3 trials. The total solids content of the milk before fortifying ranged from 8.15% to 8.50% which gave an average of 8.32% solids. The lots of milk which had an average of 11.32% solids resulted in the best finished product. The only criticism was that of a very slight weakness in the body of the cheese.

TABLE XV

INFLUENCE OF FORTIFYING MILK WITH NON-FAT DRY MILK SOLIDS
(Average of 3 trials)

SOLIDS CONTENT OF MILK	ml N/10 NaOH TO NEUTRALIZE 9 ml OF MILK			TIME			FLAVOR	BODY & TEXTURE	APPEARANCE
	INITIAL	CUTTING	FINAL	SETTING TO CUTTING	COOKING	TOTAL			
%	ml	ml	ml	hr:min	hr:min	hr:min			
8.32	2.11	5.05	5.41	4:35	2:40	7:15	Green	Weak	Mushy
9.32	2.35	5.21	5.57	4:50	2:15	7:05	Good	Weak	Slightly Mushy
10.32	2.53	5.54	5.88	5:10	2:00	7:10	Good	Slightly Weak	Good
11.32	2.81	5.89	6.32	5:20	1:50	7:10	Good	Slightly Weak	Good

The total time for making the cheese was approximately the same for all four vats. It will be noted that it took longer for the 11.32% solids milk to reach cutting time but the cooking time was somewhat reduced compared to the other vats of cheese. The 8.32% solids milk reached cutting time the fastest but the cooking time was increased greatly. For the 8.32% solids milk, the cooking temperature was raised to 130°F. in an attempt to complete the cooking and firm the body but the finished cheese was still of poor quality. It would seem from these results that, the best cheese made with fortified skimmilk should have a total solids content of approximately 12.0%.

It would seem that the solids content of the milk has a considerable effect on the finished cottage cheese as well as on the time required to cook the curd. It seems that although it requires slightly longer to make a vat of cheese of high solids milk, the gain in quality of the finished product is worth the little extra time required in processing. The author believes that 11.0% solids should be used when making cottage cheese with reconstituted non-fat dry milk solids and 12.0% solids when fortified skimmilk is used.

C. MODIFICATION OF THE CHEESE MAKING PROCESS

While undertaking the above experiments, the author was at all times looking for methods which would shorten the time required for making cottage cheese. It has been shown that some time can be saved by the standard procedures for

making of cottage cheese even if all factors involved are used to their maximum efficiency. A factor which had not been explored was that of preliminary ripening such as that used in the cheddar cheese operation. It was the purpose of this part of the experiment to determine if by some means, it would be possible to add rennet in greater quantities than normal thus allowing the milk to set faster but to still have the same desirable quality of cottage cheese made by the standard procedures.

1. Preliminary trials. Before attempts to make cottage cheese by the use of an excess amount of rennet, preliminary experiments were undertaken to determine at what titratable acidity the most favorable rennet action occurred.

Reconstituted non-fat dry milk solids were divided into 280 ml portions and heated with flowing steam for 30 minutes. The milk was then cooled in an ice water bath and 20 ml of freshly prepared culture was added. Nine ml portions of the inoculated milk were then pipetted with sterile pipettes into sterile screw capped test tubes. The inoculated milk was then warmed to 90°F. and placed in a 90°F. incubator. At intervals of 0, 30, 60, 90, 100, 120, 130, 140, and 150 minutes, one tube was removed from the incubator and titrated with N/10 NaOH. At the same time, rennet was added to another tube at the rate of four times normal (8.8^{-6} ml rennet per ml of milk) and allowed to continue to incubate for a total incubation period of 180 minutes. Coagulation time, titratable acidity, and condition of the curd were noted for all samples.

The results are shown in Table XVI.

The results indicated that, if the rennet was added when the titratable acidity had reached a value somewhere between 4.2 ml and 4.8 ml (approximately 0.41 to 0.47% acidity), the best cottage cheese curd would probably be obtained.

With this information, four 10 pound lots of cottage cheese were made from non-fat dry milk solids by the routine procedure except that four times the normal amount of rennet was added to each vat. The rennet was added when the vats reached titratable acidity values of 4.2, 4.4, 4.6, and 4.8 ml. The curd was cut 30 minutes after adding rennet and routine procedures were then followed for cooking.

None of the vats of cheese were satisfactory becuause as soon as cooking was started, the cheese matted badly and the final product was badly matted and very rubbery. It appeared impossible to cook out cottage cheese with an excess of rennet added.

2. Preliminary ripening plus added acid. From the results obtained by adding four times the normal amount of rennet, it was deemed necessary to devise a method which would result in a very rapid increase in acid to counteract the rubbery texture resulting from the action of the rennet at a low acidity. It was thought that citric acid added at the time of cutting the curd would possibly give the counteracting results desired.

In a preliminary trial, 10 pound lots of milk were made

TABLE XVI

EFFECT OF ADDING RENNET TO COTTAGE CHEESE MILK AT VARIOUS TITRATABLE ACIDITIES
(Average of 4 trials)

TIME	TITRATABLE ACIDITY	COAGULATION TIME	EFFECT ON CURD
min	ml	min	
0	2.4	50	Firm, whey off
30	2.7	50	Firm, whey off
60	3.0	40	Firm, whey off
90	3.7	35	Firm, whey off
100	3.9	30	Firm, slight whey off
110	4.2	30	Firm, no whey off
120	4.5	30	Firm, no whey off, "best"
130	4.8	20	Firm, slight whey off
140	5.1	10	Firm, considerable whey off
150	5.4	10	Not firm, considerable whey off

into cottage cheese. Ten per cent starter was used and the titratable acidity was allowed to reach a value of 4.6 ml at which time four times normal amounts of rennet were added to each vat and allowed to stand for 30 minutes. The curd was then cut and a solution of 50% citric acid was added to each vat at the rate of 15, 20, 25, and 30 ml per vat, respectively. Cooking was begun 5 minutes after cutting and the temperature of the curd was raised from 90° to 120°F. in 30 minutes and allowed to stand at this temperature for 5 minutes. The cheese was then rinsed twice in tap water and once in ice water and allowed to drain.

The final titratable acidity values on the four vats were 5.8 ml for 15 ml of citric acid added, 6.2 ml for 20 ml of citric acid added, 6.6 ml for 25 ml of citric acid added, and 7.0 ml for 30 ml of citric acid added. The flavor of all vats of cheese was that of high acid. The body and texture for the first vat was rubbery, the second and third vat slightly rubbery, and the fourth vat was good. The appearance of the cheese was good except for the first vat which was slightly dull.

One serious problem was still noted. After one day, the cheese curd became more rubbery and continued to expel whey. This was believed due to continued rennet action. It was assumed that a temperature of 120°F. for 5 minutes failed to stop the action of the rennet.

An experiment was carried out to determine the temperature and period of exposure required to stop rennet action

when the large amount of rennet was used. Reconstituted non-fat dry milk solids were used for the milk and 9 ml portions were pipetted into 28 screw capped test tubes which were then autoclaved for 15 minutes at 15 psi and cooled to 90°F. The tubes were then divided into 4 groups of 7 tubes each and four times the normal amount of rennet was added to 6 of the 7 tubes in each group. The remaining tube had a thermometer placed in and was used as the temperature control for the remaining 6 tubes. Each group of tubes was placed in a separate water bath and temperatures were adjusted so that the temperature for group 1 was 119°F.; group 2, 124°F.; group 3, 129°F.; and group 4, 135°F. One tube from each group was removed from the water bath and placed in an ice water bath at 0, 5, 10, 15, 20, and 25 minutes.

Four-hundred and fifty ml of reconstituted milk was autoclaved for 15 minutes at 15 psi, cooled to 90°F. and the pH was then adjusted to 5.00 with citric acid. This milk was then pipetted into sterilized screw capped test tubes at the rate of 8 ml of acidified milk per test tube. To these tubes, 2 ml of the rennetted milk which had been heated at different temperatures and periods was added to duplicate tubes and 4 tubes were used with no rennet and 4 tubes with unheated rennet were used as controls. All tubes were placed in a 95°F. incubator and observed for two days for coagulation. The results are shown in Table XVII.

TABLE XVII

TIME AND TEMPERATURE REQUIRED TO DESTROY THE ACTION OF RENNET ENZYMES IN COTTAGE CHEESE
(Average of 3 trials)

PERIOD OF EXPOSURE	TEMPERATURE			
	119°F	124°F	129°F	135°F
	Rennet Coagulation			
min				
0	+	+	+	+
5	+	+	-	-
10	+	+	-	-
15	+	+	-	-
20	+	+	-	-
25	+	-	-	-

The results indicate that the rennet action was destroyed at a temperature of 124°F. for 25 minutes or a temperature of 129°F. for 5 minutes. These results thus indicated, for all practical purposes, that a temperature of 129°F. should be reached and maintained for a period of 5 minutes when cheese was made with four times the normal amount of rennet.

With these results, two lots of cottage cheese were made with a control vat and a vat of cheese made with four times normal amount of rennet. The control vats of cheese were made according to the general cheese making procedure using reconstituted non-fat dry milk solids. The vat with four times normal amount of rennet contained 10% starter and the titratable acidity was allowed to reach a value of 4.6 ml at which time four times normal amount of rennet was added and the milk allowed to stand for 30 minutes. The curd was then cut and a solution of 50% citric acid was added to the vat at a rate of 30 ml per 10 pounds of milk. Cooking was begun 5 minutes after cutting and the temperature of the curd was raised from 90° to 129°F. in 35 minutes. The curd was rinsed and drained according to general procedures. The results are shown in Table XVIII.

The results show that the time from setting to cutting was reduced by approximately 1 hour and 50 minutes and the time to cook was reduced approximately 1 hour and 25 minutes. This gave an approximate time reduction of 3 hours and 15 minutes. The quality of the acid-rennet cheese was similar

TABLE XVIII

EFFECT OF ACID-RENNET PROCESS ON QUALITY OF COTTAGE CHEESE

LOT NO.	PROCESS	ml N/10 NaOH TO NEUTRALIZE 9 ml OF MILK			TIME			REMARKS
		INITIAL	CUTTING	FINAL	SETTING TO CUTTING	COOKING	TOTAL	
		ml	ml	ml	hr:min	hr:min	hr:min	
1	Control	2.2	5.2	5.7	4:35	2:10	6:45	Good
	Acid-Rennet	2.5	4.6	7.0	2:45	0:40	3:25	High acid flavor, body and texture good
2	Control	2.3	5.3	5.8	4:35	2:05	6:40	Good
	Acid-Rennet	2.6	4.6	6.9	2:50	0:45	3:35	High acid flavor, slight rubbery body and texture

to that of the control vat except for a high acid flavor in both lots and a slight rubbery body and texture of lot No. 2. The high acid flavor was not undesirable after the curd was creamed and salted. Although the second lot had a slightly rubbery body and texture, it was not considered to be serious. There was no noticeable increase in rubbery body after 4 days of storage at 45°F.

SUMMARY AND CONCLUSIONS

In attempts to shorten the time required for making cottage cheese, a study was made of the various factors which influence the rates of acid production by lactic cultures used for cottage cheese making. Those factors which resulted in considerable stimulation of acid production were applied to cottage cheese making to determine their influence on the quality of the finished product.

Preliminary ripening of the milk followed by rennet coagulation and the addition of acid during the cooking were also used to reduce the time required to make cottage cheese.

Of various materials added to milk to stimulate acid production, pancreas extract was the most effective. By adding approximately 0.2% to milk for cheese making, the period required to make the cheese could be reduced approximately two and one-fourth hours. The pancreas extract had no detrimental effect on the quality of the finished cheese. Of the several amino acids and related compounds studied, guanylic acid in a concentration of 400 ppm stimulated acid production considerably and spermine in a concentration of 10 ppm was slightly stimulatory. L-ascorbic acid, L-glutamine, L-serine, and uracil showed no stimulatory effect,

while L-cysteine was inhibitory to the lactic culture.

The effect of amount of inoculum used in propagating mother culture was studied by propagating cultures through 10 successive transfers with inoculation rates of 0.1, 0.3, 1.0, 3.0, and 10.0%. The acidity tests indicated that an inoculation of 1.0% was the most desirable with 14 to 16 hours incubation at 70° to 72°F.

The influence of the depth of the milk layer on the rate of lactic acid production by cultures was studied by propagating the cultures in equal volumes of milk in shallow and in deep layers. The results indicated that better production of lactic acid resulted from the cultures grown in the containers in which the surface area was small. This led to the conclusion that, because Streptococcus lactis and Streptococcus cremoris are facultative anaerobes, the oxidation- reduction potential is more favorable when the milk has considerable depth and small surface area for the incorporation of oxygen.

Using inoculation rates of 5, 10, 20, and 40%, it was found that the period required for coagulation of milk decreased as the rate of inoculation increased. However, in making cottage cheese, it was found that when amounts greater than 10% were used, the body and texture of the cheese were inferior. From these results, it appeared that 10% of culture is the maximum that can be used.

The influence of the solids content of the milk on the rates of acid production and coagulation was studied with

reconstituted milk and with low solids milk fortified to different levels with non-fat dry milk solids. The results indicated that as the solids content increased, the period required for coagulation increased. The most desirable cheese resulted from the milk with 10 to 12% solids. Higher solids content in the milk prolonged the cheese making process excessively, while lower solids milk resulted in poorer quality cheese.

The cheese making process can be shortened considerably by ripening the milk to about 0.45% acidity, adding four times the normal amount of rennet and then adding acid after cutting to speed up the cooking process. The resulting cheese was of good quality except for an acid flavor which largely disappeared when the cheese was creamed. Without the addition of acid prior to cooking, the cheese matted excessively.

When four times the normal amount of rennet was used, the cheese wheyed off during storage subsequent to making. This defect was presumably due to continued action of the rennet extract enzymes. The coagulating enzyme can be destroyed by heating to 124°F. for 25 minutes or to 129°F. for 5 minutes.

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