THE EFFECT OF VARIOUS FACTORS ON THE RATE OF PRODUCTION OF LACTIC ACID BY CHEESE CULTURES

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Bachelor of Science

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1939

Submitted to the Department of Dairying

Oklahoma Agricultural and Mechanical College

In Partial Fulfillment of the Requirements

for the Degree of

MASTER OF SCIENCE

1948

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ACKNOWLEDGMENT

The author wishes to take this opportunity to express his sincere appreciation to Dr. H. C. Olson for the many helpful suggestions and the guidance given throughout this study and during the preparation of this thesis.

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INTRODUCTION

Cheese cultures of S. lactis that fail to develop acid at a normal rate are a constant source of trouble and expense wherever cultures are used. Characteristically, many times cultures suddenly develop this defect, without warning or apparent reason, appearing to be satisfactory at the time it is used, then failing to develop the desired changes in the product being manufactured. These occurrences interfere with general plant routine, and if additional time is allowed to develop the acid in the product, its quality is impaired. If extremely slow acid development is encountered the product is often a total loss.

The rate of lactic acid development by cultures has been receiving considerable attention the past few years, probably because of the larger amounts of cheese and butter being manufactured from pasteurized milk, and the adoption of definite manufacturing schedules. These schedules assure certain degrees of acidity developed in a certain scheduled period of time, thus the cultures used must produce the acid in the required amounts consistently from day to day.

There are various causes of slow acid production by cultures. Some of these are rather easily found and corrected, while others are difficult if not impossible to detect.

It has been reported that in most cases, the period of slow acid production difficulty is not long, occurring for the most part in the latter part of the summer or the early fall.

The work herein reported was undertaken to determine the influence of the various factors upon the rate of acid production by cheese cultures.

REVIEW OF THE LITERATURE

Hammer (6) noted that S. lacticus is nearly always found in starters in large numbers, and that associated organisms make up only a very small percentage of the total flora. These associated organisms were known as S. citrovorus and S. paracitrovorus, and Hammer states that they are responsible for the volatile acidity in culture which is in turn responsible for the flavor and aroma of a culture. He also noted that in general S. citrovorus lives longer in old acid milk than the S. lacticus so there would seem to be little if any danger of the former being lost by the overripening of a starter, although the activity of both organisms may be seriously interfered with.

Kelly (14) compared the bacterial flora of various cheese cultures and stated that although in some cases the cheese made with S. cremoris as a starter culture had a better aroma and flavor during the making than similar cheese made with S. lactis cultures, little difference could be detected when the cheese was ready for consumption. He also stated that good cheese was made with pure S. lactis cultures which were acid producing organisms and develop no aroma. Kelly further states that equally as good raw milk cheese was made without starter, where S. lactis was the predominant organism, and that it appeared that acid production is the chief function of a starter, and that the starter has little direct action on the flavor and aroma of the cheese.

Evans et al (4) concluded that the action of two or more organisms growing together is not the sum of their individual actions when growing alone. When growing together, they may attack substances that neither can attack alone, or they may produce a larger quantity of acid than the sum of the quantities that either can produce alone. When added to pasteurized milk, the organisms of the "B. casei group" produce a sour taste in the cheese during the early part of the ripening period, and that no cheddar flavor is obtained in pasteurized milk cheese when the organisms of the "B. lactis acidi group" alone are used as starters.

Hansen et al (7) found that the flavor of cheese made with pure S. lactis cultures was almost equal to that of cheese made with mixed cultures, but the body and texture of the S. lactis cheese was superior to that of the cheese made with the mixed starters.

Hucker and Marquardt (11) concluded that S. paracitrovorus appears to have a decided effect upon the production of the characteristic flavor developed in cheddar cheese. This is particularly true in cases in which pasteurized milk is used for cheese making. S. citrovorus, when added to milk in large numbers previous to the cheese making showed no effect upon the flavor of the cheddar cheese. Other data presented by them showed that S. lactis, although producing a somewhat more acid flavor in some instances, produced cheese very similar to that produced with commercial starters which have, according to Orla-Jensen, S. cremoris as the predominating type of organism present. They state also that milk of a high quality to which chemically pure lactic acid had been added to give a final acid content of approximately 0.23 to 0.24 percent produced as high quality cheese as similar cheese made from milk with commercial starter added.

Eckles et al (3) state that immediately after milk is drawn from the cow, there appears to be a time during which there is no growth of bacteria, and apparently a decrease in the numbers. This germicidal period varies considerably in length, usually being shorter at higher temperatures but quite pronounced and more prolonged at lower temperatures. It varies in milk from different cows at different times, from various quarters of the udder and of fractions of milk from the same quarter. This property is destroyed by heating the milk to 150°F. to 176°F., for 30 minutes.

Nelson et al (15) found that milk from different animals make cultures of varying acidities, while different lots of milk from the same animals made cultures of approximately the same acidity. This study was made using milk from various sources in both raw and pasteurized condition for propagation of S. lactis cultures, and indicated that the source of the milk was not important as a cause of slow acid production under the usual circumstances. Wild strains of bacteria, either those naturally present in the milk or those gaining entrance through contamination from plant equipment, appeared unimportant as the cause of retarded acid development. Since neither the original milk nor the foreign organisms present caused as much difference in acid development as occurred between different cultures grown in the same lots of milk, slow acid production was apparently the result of some condition peculiar to the culture. By plate counts, the slow acid production was shown to be the result of comparatively slow growth of the organisms and not to contamination with bacteria or to disruption of the relationship between the normal culture species. Nelson et al found also, that in certain cases, slow development of acid has been attributed to the use of abnormal milk, and often in cheese cultures abnormal milk present in concentrations of only 0.1% interferes with acid development, and causes loss of vitality. Milk of low acidity due to mastitis should not be used for making culture. They also reported that leucocytes present in excess of five million per ml. prevents the development of normal acidities in milk. This defect however can be partially remedied by heating the milk to 50°C. for 30 seconds.

Hunter and Whitehead (12) found that if milk containing the inhibitory substances produced by growth of "non acid" streptococci is used for the propagation of starter, it can cause delayed coagulation, simulating a starter failure similar to bacteriophage. They also state that if milk which contains this substance is used for propagation of starter culture, it effects cultures differently according to the particular strains of streptococcus present, and that some strains are considerably less inhibited than others.

Harrison and Dearden (8) found that abnormal or mastitis milk has no effect upon the rate of acid production when compared with control samples of normal milk. The inability of certain S. lactis strains to grow at cooking temperatures appeared to be the cause of slowness.

Golding et al (5) found that the presence of a high solids content permits the development of a high titratable acidity due to the amount of buffer present in the high solids milk, and that therefore the use of low solids milk can cause much trouble in starter propagation. They also found that the optimum development of acidity for the three starters used in their work took place at about 86°F. and that the development of acidity at 60°F. and at 100°F. was insignificant during the eight hours of the test. The acidity of fresh starters in the range usually found in the cheese factory had little effect on the rate of acid development in skim milk, and that a cooking temperature of 102°F. greatly retarded the development of acidity and the longer the starter organisms were held at a scalding temperature (102°F.), the slower was their subsequent development of acidity when returned to 86°F.

Horrall and Elliker (10) state that investigations on slow acid production in cheddar cheese manufacture indicated that starter cultures varied in activity from day to day even though the milk used for the cultures was obtained from the same herd. They carried out trials to compare the uniformity and activity of S. lactis cheese cultures propagated in selected herd milk with that in reconstituted skim milk prepared from representative lots of high grade spray, non-fat dry milk solids. The reconstituted milk contained 10% milk solids. Cultures carried in the reconstituted skim milk were more uniform in activity from day to day than duplicate cultures carried in the selected herd milk. Both single and multiple strain mother cultures were propagated successfully in reconstituted skim milk for periods of as long as two years. The use of distilled water is recommended for use in reconstitution of the non-fat dry milk solids.

Dalberg and Ferris (2) found that when lactic starters were inoculated every day or every third day and carried under excellent conditions, their qualities were identical as judged by appearance, flavor and acid development. When the starters were incubated in milk at temperatures used in cheese making, there were slight differences in the starters. When incubated at 86°F. acid development was rapid and the same for both starters; at 100°F. acid development was very slow and at 86°F. for two hours followed by 100°F. for six hours the acid development was good and the same for both starters. Cheese manufactured with starters transferred daily developed more flavor of better quality than when made with starters transferred every third day. The data show that transfer of lactic cultures every third day as compared with daily transfer reduced the acid produced during the cheddar cheese making process, and incubation at 86°F. in the cheese milk increased the production of acid at the cooking temperatures.

Johns and Beard (13) found that severe and prolonged overripening of starters to an extent greater than usually encountered in cheese factory practices failed to slow down the rate of bacterial growth, or acid development, or to lower the final acidity reached. Starters were overripened for periods of from 24 hours up to two weeks. In a practical test, an overripened portion of a starter, worked slightly faster in the vat, and produced a cheese with a higher flavor score than the control starter. The effect of overripening upon the milk coagulating organisms varied considerably between three starters used in the study. In two of these, an overripened portion contained more of these organisms than the normally ripened portion. After 30 days of repeated overripening, the flavor of the overripened portions was judged superior to that of the normally ripened portions.

Babel (1) found that cultures which could produce acid normally at temperatures ranging from 98°F. to 102°F. would continue to produce it all of the way through the cheese manufacturing process, but that cultures which showed a slowing down in acid production at those temperatures seemed to produce acid normally up to the draining of the whey and after that point they slowed down.

METHODS

1. Routine Propagation of the Cultures

The cultures used in this work were standard commercial cultures taken from the Oklahoma A. & M. College culture collection, and were propagated daily as follows: Clean six ounce prescription bottles with molded screw caps, were filled with 100 ml. of skim milk. The bottles of milk were then pasteurized at a temperature of 210°F. for 30 minutes in flowing steam in an autoclave. They were then cooled in a water bath to a temperature of approximately 70°F., after which they were each inoculated with 6 drops of a mother culture, and placed in an incubator at a temperature of 70°F. for 16 hours. After incubation, they were placed in a cold room at a temperature of 50°F. until the next transfer.

2. Acidity of the Ripened Cultures

The acidity of the ripened cultures was determined by transferring 17.6 ml. of the ripened culture, using a 17.6 ml. milk pipette, into a small erlenmeyer flask. The pipette was then rinsed with 17.6 ml. of distilled water, and the flask titrated with .1N NaOH, using phenolphthalein as the indicator. The results were expressed as the per cent of acid calculated as lactic acid.

3. The Three Hour Activity Test

The three hour activity test was developed by Hlynka and Hood (9) and consists of the following: 10 ml. quantities of raw skim were dispensed into test tubes which were stoppered with loosely fitting rubber stoppers, heated to 210°F. in flowing steam for 30 minutes, and tempered to 98°F. They were then inoculated with three per cent mother culture, and then placed in a thermostatically controlled water bath which was ordinarily used for the methylene-blue test. This water bath was set at a temperature of 98°F. After being incubated for three hours, the tubes were removed and titrated with .1N NaOH, using phenolphthalein as the indicator. The results are expressed as the amount of .1N NaOH required to neutralize the acid in 10 ml. of skim milk after the inoculation and incubation.

4. The Seven Hour Activity Test

The purpose of this method is to similate the time and temperature used in cheese making. One hundred ml. portions of skim milk were dispensed into six ounce prescription bottles, pasteurized at 210°F. in flowing steam for 30 minutes in an autoclave, cooled in a water bath to a temperature of 88°F., and inoculated with one per cent of a mother culture. The bottles were then placed in an incubator at a temperature of 88°F. for seven hours. After incubation they were removed and titrated with .1N NaOH using phenolphthalein as the indicator.

EXPERIMENTAL

A. Sanitary Quality of the Milk

The influence of the quality of the culture milk used upon the activity of cheese cultures was studied by comparing the activities of cultures propagated daily in fresh milk with those of the same cultures propagated in the same milk after it had been allowed to develop a high bacterial count.

Mixed raw milk from the herd at the Oklahoma A. and M. College was pre-heated to a temperature of 95°F., separated and placed in a 2 liter Erlenmyer flask. The titratable acidity was determined, and plate counts were made according to the procedure outlined in Standard Methods for the Examination of Dairy Products, 8th Edition. (16) The plates were incubated at 89.6°F. (32°C.) Immediately after plating, a portion of the raw skim milk was dispensed in 100 ml. portions into 6 ounce prescription bottles with molded screw caps, and the milk was then pasteurized by heating in flowing steam, 210°F., for 30 minutes. After the heating, the container was placed in hot water, and cooled by running water to about 60°F. and then placed in a cold room at a temperature of 50°F. The remainder of the skim was then held at room temperature, about 80°F., for 8 hours. The acidity was again determined and the plate counts again made. The incubated portion of the milk was then dispensed into containers, pasteurized and cooled in the same manner as the fresh milk had been treated. The two lots of milk were then designated as, "fresh milk", and "high count milk". Both lots were then tempered in a water bath to 70°F. Ten active cheese cultures were selected and each inoculated into a bottle of fresh milk, and a bottle of high count milk using 6 drops of culture (about .3%) for each bottle. The inoculated bottles of milk were then incubated at 70°F.

for 16 hours in a thermostatically controlled incubator. At the end of the incubation period, the cultures were held in a cold room at 50°F. until the next transfer. In subsequent transfers those cultures grown in the fresh milk were propagated in fresh milk for a period of 7 days, and those cultures grown in high count milk were propagated in the high count milk for a period of 7 days. Each morning the activities of each freshly ripened culture in the two sets was determined by the three hour and the seven hour activity tests. The titratable acidities of the fresh, ripened cultures was also determined.

The influence of the quality of the milk used for daily propagations upon the activities of cheese cultures is shown in Table I. These results represent the averages obtained with ten cheese cultures propagated daily in fresh skim milk, and in the same milk after incubation for eight hours at room temperature. Both lots of milk were pasteurized in the usual manner prior to inoculation.

The results show that with each lot of milk there was an increase in titratable acidity during the eight hour incubation period and the average increase for the seven lots of milk was 0.019%.

There was a striking increase in bacterial content with each lot of milk during the incubation and the log average of the Standard Plate Counts for the fresh milk was 185,000, while that for the incubated milk was 26,000,000.

The results indicate that there were only slightly higher acidities on the ripened cultures from the fresh milk as compared to those from the incubated milk. In five of the seven comparisons, the acidities of the ripened culture were higher for the culture propagated in the fresh milk and in the other two comparisons, the acidities were higher with cultures which were propagated in the incubated milk. In no instances were the differences in acidities very great and the average difference for the seven propagations was only .005% in favor of the fresh milk.

Both the three hour and the seven hour activity tests indicate that there was no striking difference between the activities of cheese cultures propagated in fresh milk as compared to the same cultures propagated in milk which was incubated and which had developed a high bacterial count. With the three hour activity test, the cultures propagated in the fresh milk showed slightly more rapid acid development in four of the comparisons and slightly lower in the other three comparisons. The average difference in favor of the cultures propagated in the fresh milk was only .03 ml. of .1N NaOH required for the titration of ten ml. of skim milk inoculated with three per cent of culture, and incubated for three hours at 98°F.

With the seven hour activity test, the cultures propagated in the fresh milk showed slightly more rapid acid production in six out of seven comparisons and slower acid production in the other trial. However, the average difference in acid production in favor of the fresh milk cultures was only 0.04 ml. .1N NaOH required to neutralize the acid produced in ten ml. of skim milk inoculated with one per cent culture and incubated for seven hours at 88°F. The apparently descrepant results obtained on the sixth day are unexplainable.

Apparently there was no cumulative effect on the activity of the cultures resulting from their being propagated daily in fresh milk and in the same milk in which considerable numbers of bacteria were allowed to develop before being pasteurized preparatory to inoculation with the mother culture.

Table I

The Influence of the Quality of the Milk Used For Daily Propagations Upon the Activities of Cheese Cultures

Average for 10 Cultures With Each Type Milk

								Activity Tests			
		Acidity of Milk		Standard Plate Count		Acidity of Ripened Culture		Three Hour *		Seven Hour **	
Date of Transfer	Transfer Number	Fresh	High Count Milk	Fresh	High Count Milk	Fresh	High Count Milk	Fresh	High Count Milk	Fresh	High Count Milk
4-1-48	1	.175	.190	160,000	37,000,000	.8590	.8455	3.75	3.70	9.47	9.39
4-2-48	2	.170	.220	197,000	76,000,000	.9285	.9250	3.22	3.12	8.26	8.22
4-3-48	3	.170	.185	210,000	2,900,000	.9085	.8945	3.25	3.26	8.10	8.05
4-4-48	4	.170	.180	250,000	10,000,000	.9110	.9000	3.23	3.25	8.14	8.13
4-5-48	5	.175	.185	270,000	25,000,000	.8815	.8870	3.28	3.24	7.88	7.83
4-6-48	6	.175	.185	600,000	26,000,000	.9000	.9060	3.45	3.54	7.19	7.76
4-7-48	7	.175	.200	310,000	14,000,000	.7575	.7530	3.59	3.43	7.76	7.70
Average		.172	.191	log 185,000	log 26,000,000	.8780	.8730	3.39	3.36	8.11	8.15

* Ml. .1N NaOH required to neutralize the acid developed in 10 ml. skim milk

** Ml. .1N NaOH required to neutralize the acid developed in 10 ml. skim milk

B. The Influence of the Composition of the Milk Used for Propagation on the Activity of Cheese Cultures.

1. The Influence of Fat Content

The influence of the butter fat content of the culture milk used upon the activities of cheese cultures was studied by comparing the activities of the cultures propagated in whole milk with those of the same cultures propagated in the same milk after it had been skimmed.

Mixed raw milk from the herd at the Oklahoma A. and M. College was pre-heated to a temperature of 95°F., and skimmed with an electrically powered centrifugal separator. A sample of the whole milk was obtained from the separator supply tank, and a sample of the skim milk was collected immediately afterward from the skim milk spout of the separator. 10 ml. quantities of the two lots of milk were then dispensed into test tubes, loosely stoppered with rubber stoppers and then pasteurized at a temperature of 210°F., for thirty minutes in flowing steam, in an autoclave. Both lots were then tempered in a water bath to 98°F. Ten active cheese cultures were then selected, and used to inoculate ten tubes of each of the lots of milk, and the activities of the two sets were determined by the three hour activity test.

The influence of the butter fat content of the culture milk upon the activities of cheese cultures is shown in Table II, and summarized in Table III. These results represent the activities of ten cheese cultures which were inoculated into whole milk, and into the same milk after it had been skimmed. Both lots were pasteurized in the usual manner prior to inoculation.

The results indicate that the rate of acid production was slightly higher for the cultures grown in the skim milk as compared to those grown in the whole milk. In three of the five comparisons, the acidities were higher for the cultures which were inoculated into the whole milk, and in the other two comparisons, the acidities were higher with the cultures which were inoculated into the skim milk. In no instances are the differences in the acidities very great, and the average difference for the five trials was only .001 ml. in favor of the skim milk. Apparently there was no effect on the activity of the cultures resulting from their being inoculated into whole milk, and in the same milk which had been skimmed.

Table II

The Influence of the Butter Fat Content of the Culture Milk

Used Upon the Activities of Cheese Cultures

Culture No.	Trial 1		Trial 2		Trial 3		Trial 4		Trial 5	
	Whole	Skim	Whole	Skim	Whole	Skim	Whole	Skim	Whole	Skim
	Ml. .1N NaOH required to neutralize the acidity in 10 ml. skim milk inoculated with 3% culture and incubated for 3 hours at 98°F.									
5	4.7	4.0	3.5	3.3	3.9	3.7	3.7	3.3	3.5	3.1
6	3.7	3.7	4.3	4.5	5.2	5.6	3.6	3.6	3.0	3.5
7	3.6	3.6	3.8	3.4	4.1	4.2	3.7	3.4	3.6	3.4
9	3.8	3.7	3.6	3.6	4.2	4.2	3.6	3.4	3.5	3.4
11	4.0	4.2	3.9	4.0	5.2	5.6	4.2	4.2	3.1	3.1
12	4.1	3.7	3.4	3.3	4.6	5.2	4.0	4.1	3.2	3.2
16	3.3	3.3	3.3	3.1	4.1	5.0	4.1	3.9	3.4	3.6
19	3.5	3.6	3.2	3.1	4.1	4.6	2.9	3.0	2.5	2.6
21	3.2	3.3	3.2	3.2	4.0	4.2	3.8	3.6	2.8	2.8
25	3.4	3.3	3.0	2.9	5.1	5.6	4.6	4.3	3.3	3.3
Average	3.73	3.64	3.52	3.44	4.45	4.79	3.82	3.68	3.19	3.20

Table III

Summary of Results in Table II

Date	Trial No.	Fat Content-Whole	ml. of .1N NaOH required to neutralize the acid developed in 10 ml. milk. *	
			whole	skim
2-23-48	1	4.8%	3.73	3.64
2-26-48	2	4.5%	3.52	3.44
3-1-48	3	4.6%	4.45	4.79
3-4-48	4	4.2%	3.82	3.68
3-5-48	5	4.4%	3.19	3.20
Average		4.5%	3.74	3.75

* The Three Hour Activity Determination

2. The Influence of the Solids Not Fat Content

The influence of the solids-not-fat content of the culture milk used upon the activities of cheese cultures was studied by comparing the activities of cultures propagated daily in milk containing various levels of solids content.

a. Preliminary Study

The object of this preliminary study was to determine the influence of various levels of non-fat dry milk solids content of reconstituted milk on the activities of cheese cultures.

Three lots of reconstituted non-fat dry milk solids were prepared with distilled water, the lots containing six, nine and twelve per cent of the milk powder. The titratable acidity of the reconstituted milk was determined, and 100 ml. portions of the milk then dispensed into six ounce prescription bottles with molded screw caps. The milk was then pasteurized by heating to a temperature of about 210°F., in flowing steam for 30 minutes, then cooled in a water bath to 70°F. Five active cheese cultures were selected and each culture inoculated into a bottle of each of the three levels of total solids. The inoculated bottles of milk were then incubated at 70°F. for 16 hours in a thermostatically controlled incubator. At the end of the incubation period the cultures were removed and held in a cold room at 50°F. until the next transfer. In subsequent transfers each of the five cultures were propagated daily in the three lots of milk, namely six per cent, nine per cent and 12 per cent solids, for a period of 21 days. Every seven days, the activities of the cultures propagated in the three lots of milk were determined by the seven hour activity test.

The influence of the solids not fat content of milk used for propagating cheese cultures on the rates of acid production by the cultures is shown in Table IV. The results are expressed as the per cent increase in the acidity of reconstituted non-fat dry milk solids (9%) inoculated with one per cent culture and incubated for seven hours at 88°F.

The results indicate that as the solids-not-fat content increases, the rate of acid production by the cultures grown in it increases. After propagating one week, the cultures grown in the nine per cent solids milk developed .056% more acid during incubation in reconstituted (9%) milk at 88°F. for seven hours than did the culture propagated in milk containing six per cent solids-not-fat, while the cultures grown in the 12% milk developed .154% more acid during the seven hour incubation period at 88°F. than did the cultures propagated in the nine per cent milk. At the end of the second week, the cultures propagated in the nine per cent milk developed .058% more acid during the seven hour incubation period at 88°F., than did the cultures propagated in the six per cent milk, while the cultures propagated in the 12% milk developed .052% more acid during the seven hour incubation period at 88°F. than did the cultures propagated in the nine per cent milk. At the end of the third week, the cultures that were propagated in the nine per cent milk developed .044% more acid during the seven hour incubation period at 88°F. than did the cultures propagated in the six per cent milk, while the cultures propagated in the 12% milk developed .050% more acid during the seven hour incubation period at 88°F., than did the cultures propagated in the nine per cent milk. The averages of the three trials showed that the cultures propagated in the nine per cent milk had developed an average of .0526% more acid than these cultures propagated in the six per cent milk, and that the cultures propagated in the 12% milk developed an average of

.0854% more acid than those propagated in the nine per cent milk. These results show rather conclusively that increasing the non-fat dry milk solids content of the milk progressively increases the activities of the cultures carried therein, and that the higher the non-fat dry milk solids content in the milk, the more active are the cultures propagated.

Table IV

The Influence of the Solids Not Fat Content of Milk Used for Propagating Cheese Cultures on the Rates of Acid Production by the Cultures.

(The results are expressed as the % increase in the acidity of reconstituted non-fat dry milk solids (9%) inoculated with 1% culture and incubated seven hours at 88°F.)

Culture No.	Solids-not-fat content of reconstituted milk used for daily propagations		
	6%	9%	12%
	% increase in acidity during 7 hours at 88°F. after propagating for:		
	7 days		
1	.22	.35	.43
2	.39	.39	.50
3	.19	.36	.46
4	.42	.41	.51
5	.12	.11	.49
average	.268	.324	.478
	14 days		
1	.22	.32	.47
2	.32	.35	.37
3	.23	.29	.23
4	.19	.29	.34
5	.17	.17	.27
average	.226	.284	.336
	21 days		
1	.20	.28	.41
2	.27	.33	.39
3	.25	.33	.32
4	.24	.28	.32
5	.31	.27	.30
average	.254	.298	.348

b. The Influence of Wide Variations in Solids-not-Fat Content.

This work is a continuation of the preliminary study, the object of which was to study the effect of varying the amount of the non-fat dry milk solids content of the reconstituted milk upon the activities of cheese cultures. Since the preliminary results reported above showed that the solids-not-fat content of the milk used for propagating cultures had a profound influence on the rate of acid production by the cultures, it seemed advisable to repeat the experiment and use wider variations in the concentrations of solids-not-fat used.

Tekko Brand non-fat dry milk solids was reconstituted at the rate of 6, 9, 12, 15, and 18 per cent in distilled water. The five lots of reconstituted milk were then used for daily propagations of six cheese cultures, as in the previous experiment. The cultures were propagated for a period of 21 days, and the three hour activity test was run at regular intervals of seven days. The rate of acid production by the mother cultures propagated daily in fresh skim milk was also determined for comparison with the mother cultures propagated in the reconstituted milk. At the end of the 21 day period, one run was made to determine whether the effects noted during the 21 day period were cumulative, or whether the same effects could be noted by inoculating fresh mother culture into freshly reconstituted milk which had been pasteurized in the usual manner, at the same levels of non-fat dry milk solids content carried for the 21 day period. The three hour activity determination was run on this trial also.

The influence of the non-fat dry milk solids content of the milk used for daily propagations upon the activities of the cheese cultures is shown in Table V., and summarized in Table VI. These results represent the averages of six cheese cultures propagated daily in freshly reconstituted skim milk at levels of 6, 9, 12, 15 and 18 per cent solids-not-fat content. The results show that as the non-fat dry milk solids content is increased, the activities of the cheese cultures propagated therein also increased. The cultures propagated in the nine per cent milk showed an average increase of 0.54 ml. of .1N NaOH over the cultures propagated in six per cent milk. The cultures propagated in 12% milk showed an average increase of 0.32 ml. .1N NaOH over the cultures propagated in the nine per cent milk. The cultures propagated in 15% milk showed an average increase of 0.64 ml. .1N NaOH over the cultures propagated in the 12% milk. The cultures propagated in the 18% milk showed an average increase of 0.33 ml. of .1N NaOH over the cultures propagated in the 15% milk. The reliability of the results reported herein is established when one notes that the nine per cent milk which most nearly approximates normal skim milk, showed an average acidity of 3.22 ml. .1N NaOH developed in 10 ml. of skim milk, by the three hour acid method, while the mother cultures which were carried in normal skim milk showed an average acidity of 3.30 ml. .1N NaOH. The close similarity of these averages are therefore highly significant.

In order to determine whether the effects noted above were cumulative, or whether they would occur spontaneously whenever the non-fat dry milk solids content of the milk was increased, another trial was conducted similar to those above only the milk was freshly reconstituted, pasteurized in the usual manner, cooled in a water bath to 70°F. and inoculated with about .3% of the same six fresh mother cultures from the Oklahoma A. and M. College Collection. The following day, they were inoculated into skim milk which was pasteurized in the usual manner, tempered in a water bath to 98°F., and a three hour activity test was run. The results of this test are shown in Table VII. The culture grown in the nine per cent milk showed an average increase in acidity of 0.70 ml. .1N NaOH over those grown in the six per cent milk. The culture grown in the 12% milk showed an average increase of 0.45 ml. .1N NaOH over those grown in the nine per cent milk. The culture grown in the 15% milk showed an average decrease of 0.005 ml. .1N NaOH from that grown in the 12% milk. The culture grown in the 18% milk showed an average increase of 0.52 ml. .1N NaOH over that inoculated into the 15% milk. These results show conclusively that the effect of increasing the solids-not-fat content is not cumulative, and that an increase in the activity of cheese cultures can be caused spontaneously by increasing the solids-not-fat content of the milk into which the culture is to be inoculated. The cultures grown in the 18% milk has proved to be in all cases, the best level of solids-not-fat for the propagations of cheese cultures. From these results it appears that, for the most active cultures, it is desirable to grow cheese cultures in milk with a high solids-not-fat content.

Table V

The Influence of the Non-Fat Dry Milk Solids Content of the Milk Used For Daily Propagations Upon the Activities of Cheese Cultures

	Trial 1. Percent Total Solids						Trial 2. Percent Total Solids						Trial 3. Percent Total Solids					
	6%	9%	12%	15%	18%	M.C.	6%	9%	12%	15%	18%	M.C.	6%	9%	12%	15%	18%	M.C.
Culture No.	Ml. .1N NaOH required to neutralize the acidity in 10 ml. skim milk inoculated with 3% culture and incubated for 3 hours at 98°F.																	
5	2.8	3.1	3.9	4.8	5.0	3.0	2.9	3.4	3.3	3.5	4.8	3.2	2.5	2.9	3.2	3.5	3.7	2.6
6	2.7	3.4	3.4	4.4	4.3	3.4	2.6	3.4	3.4	4.5	4.7	3.2	2.9	3.4	4.0	4.2	4.8	3.6
11	2.8	3.4	3.8	4.2	4.8	3.3	2.6	3.0	3.4	4.3	4.7	3.3	2.6	3.4	3.8	4.3	5.1	3.5
21	2.8	3.2	3.3	4.2	4.8	3.4	2.6	3.0	3.3	3.9	4.4	3.4	2.6	2.9	3.7	3.9	4.3	2.8
23	2.7	3.2	3.8	4.5	4.8	3.5	2.7	3.0	3.5	4.0	3.6	3.5	2.8	3.3	2.8	4.6	5.1	3.4
25	2.5	3.3	3.5	4.3	4.4	3.5	2.7	3.2	3.4	3.7	4.0	3.4	2.5	3.6	4.4	4.5	4.0	3.5
Average	2.71	3.26	3.61	4.40	4.68	3.35	2.68	3.16	3.38	3.98	4.36	3.33	2.65	3.25	3.65	4.16	4.50	3.23

Table VI

Summary of Results in Table V

No. of daily propagations	ml. .1N NaOH required to neutralize the acidity in 10 ml. skim milk inoculated with 3% culture, and incubated for three hours at 98°F.					
	6%	9%	12%	15%	18%	Mother Cult.
7	2.71	3.26	3.61	4.40	4.68	3.35
14	2.68	3.16	3.38	3.98	4.36	3.33
21	2.65	3.25	3.65	4.16	4.50	3.23
Averages	2.68	3.22	3.54	4.18	4.51	3.30

Table VII

The Spontaneous Influence of Solids-Not-Fat Content of the Milk Used for Propagations Upon the Activity of Cheese Cultures

Culture No.	ml. .1N NaOH required to neutralize the acidity in 10 ml. skim milk inoculated with 3% culture and incubated for 3 hours at 98°F.					
	6%	9%	12%	15%	18%	Mother Culture
5	2.8	3.4	3.5	3.8	4.2	3.3
6	2.9	3.4	4.1	4.0	4.1	3.5
11	2.8	3.7	4.1	4.6	4.8	3.6
21	2.8	3.4	3.8	3.8	4.4	3.3
23	2.8	3.3	3.8	3.7	4.6	3.6
25	2.8	3.9	4.5	3.6	4.5	3.8
Average	2.81	3.51	3.96	3.91	4.43	3.51

C. The Influence of the Heat Treatment of the Milk Used.

1. Temperatures of Pasteurization

The influence of temperatures of pasteurization upon the activity of cheese cultures was studied by comparing the rates of acid production by cultures inoculated into skim milk pasteurized at various temperatures, ranging from 145°F., to 205°F., for 30 minutes.

Fresh, raw, skim milk from the Oklahoma A. and M. College Milk Plant was divided into four lots and each lot was dispensed into six ounce prescription bottles with molded screw caps and pasteurized for thirty minutes in a water bath at temperatures of 145°, 165°, 185° and 205°F., respectively. The four lots of milk were then cooled to approximately 60°F. and then dispensed in 10 ml. quantities in rubber stoppered test tubes. The four lots of tubes were then tempered in a water bath to a temperature of 98°F. Ten active cheese cultures were inoculated into 10 test tubes of each of the four lots of tempered milk, and the tubes then incubated at 98°F. for three hours. The 10 ml. quantities of milk were then titrated with .1N NaOH.

The influence of pasteurization at 145°, 165°, 185°, and 205°F. for 30 minutes on the rate of acid production by cheese cultures is shown in Table VIII and summarized in Table IX. The results obtained indicate that the average rate of acid production was highest for the cultures grown in the milk pasteurized at 165°F. and lowest for the cultures grown in the milk pasteurized at 145°F. In three of the five comparisons, the highest average acidities were obtained with the milk pasteurized at 205°F., and in the other two comparisons, with the milk pasteurized at 165°F. In three of five comparisons, the lowest average acidities were obtained in milk pasteurized at 145°F., while in one of the comparisons, it occurred in the milk pasteurized at 185°F., and in the remaining trial in milk pasteurized at 205°F.

It appears from results presented in Table VIII that pasteurization at temperatures of 165°F. or higher for 30 minutes is entirely satisfactory for cheese cultures. The data seem to indicate that pasteurization at 165°F. for 30 minutes gave the most consistent and highest average results but the differences between the average acidities obtained are in no instance very great and the additional protection afforded by pasteurization at temperatures above 165°F. may justify their use. The results further indicate that at 145°F. the acid production was slower than for any of the other pasteurization temperatures because the germicidal property, which inhibits bacterial development, presumably had not been destroyed.

Table VIII

The Influence of Pasteurization of Culture Milk at 145°, 165°, 185°, and 205°F. Upon the Rate of Acid Production in the Milk

Culture No.	Trial 1.				Trial 2.				Trial 3.				Trial 4.				Trial 5.			
	145°	165°	185°	205°	145°	165°	185°	205°	145°	165°	185°	205°	145°	165°	185°	205°	145°	165°	185°	205°
	ml. .1N NaOH required to neutralize the acidity in 10 ml. skim milk inoculated with 3% culture and incubated for 3 hours at 98°F.																			
5	2.7	3.0	3.1	3.2	2.8	3.2	3.0	3.5	2.7	2.6	2.9	2.8	2.9	3.1	3.2	3.0	4.6	4.9	4.6	4.2
6	3.4	3.5	2.9	2.9	4.2	4.4	4.2	3.3	3.1	2.9	3.1	3.6	3.1	3.8	3.6	3.9	5.0	6.0	5.4	3.8
7	3.2	3.5	3.2	3.2	3.4	3.3	3.3	3.9	2.8	2.7	2.6	2.8	2.8	3.1	3.2	3.5	4.0	4.7	4.0	4.1
9	3.4	3.4	3.4	3.3	4.0	4.0	3.9	3.9	3.1	2.8	3.0	3.2	3.3	3.1	3.2	3.8	4.9	4.6	3.4	4.9
11	3.2	3.3	2.8	2.9	2.9	3.0	3.6	3.7	3.0	3.2	3.5	3.5	3.0	3.5	4.2	3.8	4.5	4.7	4.4	4.9
12	2.8	3.4	2.6	2.6	3.3	3.5	3.6	3.7	3.0	3.5	2.0	2.8	3.5	3.7	3.9	3.7	5.3	5.4	4.0	3.6
16	3.0	3.3	3.0	2.9	3.3	3.7	3.7	4.6	3.3	3.4	3.5	2.9	3.0	3.6	3.5	3.5	4.6	4.9	4.8	4.5
19	2.9	3.2	2.9	2.8	3.3	4.0	3.6	3.5	2.9	3.1	3.2	3.3	3.2	3.2	3.2	3.8	5.7	5.6	5.5	5.7
21	2.5	2.9	2.8	2.4	3.8	3.7	3.4	3.8	3.0	3.3	3.3	3.3	3.0	4.1	4.5	3.6	4.4	5.4	4.9	5.4
25	2.8	2.7	2.5	2.3	3.4	3.9	3.3	3.6	3.2	2.9	3.3	3.0	3.2	3.3	3.2	3.8	5.5	5.4	5.1	4.4
Average	2.99	3.22	2.92	2.85	3.44	3.67	3.56	3.75	3.01	3.04	3.04	3.12	3.10	3.45	3.57	3.64	4.85	5.16	4.61	4.55

Table IX

Summary of Results in Table VIII

	Ml. .1N NaOH required to neutralize the acid developed in approximately 10 ml. of skim milk by the Three Hour Activity Test			
Trial No.	145°F.	165°F.	185°F.	205°F.
1	2.99	3.22	2.92	2.85
2	3.44	3.67	3.56	3.75
3	3.01	3.04	3.04	3.12
4	3.10	3.45	3.57	3.64
5	4.85	5.16	4.61	4.55
Average	3.48	3.71	3.54	3.58

2. The Period of Exposure at 205°F.

The influence of the period of exposure of milk at 205°F. upon the rate of acid production by cheese cultures was studied by comparing the activities of the cultures inoculated into lots of fresh, skim milk which had been heated to a temperature of 205°F. for periods of 0, 5, 10, 20, and 40 minutes.

Fresh, raw skim milk from the Oklahoma A. and M. College Milk Plant was dispensed in 10 ml. portions into 50 sterile, rubber stoppered test tubes. One lot of 10 tubes was placed in the cooler at 50°F. until time for inoculation. The remaining 40 tubes were placed in a water bath at a temperature of 205°F. At the end of five minutes, one lot of 10 tubes was removed and cooled immediately in a water bath to 60°F. The remaining lots of 10 tubes each were similarly removed and cooled after 10, 20, and 40 minutes, respectively. The five lots of milk were then tempered to a temperature of 98°F., and each lot inoculated with 10 active cheese cultures, using three per cent inoculation. The inoculated milk was then incubated for three hours and the amount of .1N NaOH required to neutralize each 10 ml. portion of milk then determined.

The influence of the period of exposure of milk pasteurized at 205°F. upon the rate of acid production by cheese cultures is shown in Table X, and summarized in Table XI. The results indicate that the rate of acid production was highest for the cultures grown in the milk heated for five minutes at 205°F., and lowest in the unheated milk. This can be explained by the fact that the germicidal properties of the raw skim milk exercised an inhibitory effect over the action of the cheese cultures inoculated therein. The milk heated at 205°F. for five minutes required an average of 3.65 ml. of .1N NaOH to neutralize the acid, that heated for 10 minutes required 3.57 ml., the 20 and 40 minute lots required 3.47 ml. each, while the raw lot required 3.28 ml.

The five minute period of heating had the highest average acidity in all three trials, while the lowest average acidity occurred in two trials at 40 minutes and in the remaining trial at 20 minutes. After five minutes heating, the cultures showed progressively less acid production as the period of heating increased to 40 minutes, although the differences noted were not very great.

Table X

The Influence of the Period of Exposure of Milk at 205°F. upon the Rate of Production by Cheese Cultures

Culture No.	Trial 1					Trial 2					Trial 3				
	Period of Exposure in Minutes					Period of Exposure in Minutes					Period of Exposure in Minutes				
	0	5	10	20	40	0	5	10	20	40	0	5	10	20	40
	ml. .1N NaOH required to neutralize the acidity in 10 ml. skim milk inoculated with 3% culture and incubated for 3 hours at 98°F.														
5	3.3	3.7	3.4	3.4	3.4	3.0	3.3	3.3	3.4	3.5	3.0	3.4	3.1	3.2	3.3
6	3.6	4.2	3.3	3.9	3.7	3.1	3.2	3.5	3.9	3.5	2.9	3.8	3.8	3.1	3.4
7	3.7	3.5	3.1	3.7	3.5	2.8	3.1	3.0	3.2	3.1	3.0	3.2	3.2	3.4	3.4
9	3.7	3.4	3.5	3.5	3.5	3.0	3.2	3.2	3.4	3.3	3.0	3.0	3.1	3.2	3.3
11	4.0	4.8	4.4	4.0	4.0	3.2	3.6	3.6	3.8	3.5	3.1	3.5	3.6	3.4	3.5
12	4.0	4.9	4.7	3.5	3.5	3.3	3.6	3.5	3.3	3.3	3.1	3.5	3.6	3.4	3.4
16	3.0	4.1	4.4	3.5	3.7	3.0	3.3	3.4	2.6	3.7	2.9	3.1	3.0	2.9	3.0
19	4.1	4.2	4.0	4.0	4.0	3.2	3.6	3.4	3.6	3.6	3.2	3.4	3.4	3.4	3.4
21	3.9	4.0	4.3	4.0	4.2	3.4	3.6	3.5	3.6	3.6	3.1	3.6	3.3	3.5	3.1
25	3.5	4.5	4.5	3.9	3.8	3.3	3.8	3.5	3.0	2.6	3.2	3.4	3.6	3.5	3.5
Average	3.68	4.13	3.96	3.74	3.73	3.13	3.43	3.39	3.38	3.37	3.05	3.39	3.37	3.30	3.33

Table XI

Summary of Results in Table X

Trial No.	Average ml. .1N NaOH required to neutralize the acid developed in 10 ml. skim milk by the The Three Hour Activity Test.				
	0 min.	5 min.	10 min.	20 min.	40 min.
1	3.68	4.13	3.96	3.74	3.73
2	3.13	3.43	3.39	3.38	3.37
3	3.05	3.39	3.37	3.30	3.33
Average	3.28	3.65	3.57	3.47	3.47

D. The Influence of Temperature of Incubation.

1. Comparison Between 70° and 90°F.

Since cheese making temperatures usually range from a setting temperature of 86°F., to a cooking temperature of 102°F., it seemed logical that cultures carried in this temperature range would produce acid more rapidly than those carried at 70°F. An experiment was set up to compare the 70°F. temperature of incubation with an incubation temperature of 90°F.

Fresh, raw skim milk from the Oklahoma A. and M. College Milk Plant was divided into three lots, and 100 ml. quantities of each of the three lots dispensed into six ounce prescription bottles, with molded screw caps. Two of the lots were pasteurized in the usual manner, and cooled in a water bath, while the other lot was sterilized at fifteen pounds pressure for 20 minutes, and then cooled in a water bath. One lot of the pasteurized milk and the sterilized lot were tempered in the water bath to 90°F. and the remaining lot was tempered to 70°F. Eight active cheese cultures were selected from the Oklahoma A. and M. culture collection, and each culture was inoculated into a bottle of each of the two lots of pasteurized and into the lot of sterilized milk, using .3% inoculation. After inoculation, the two lots of milk tempered to 90°F., and the remaining lot tempered to 70°F. were incubated in thermostatically controlled incubators at 90°F., and 70°F., respectively, for 16 hours. At the end of the incubation period, the cultures were removed and held in a cold room at 50°F. until the next transfer. The three lots of milk were prepared and propagated daily in this manner for a period of seven days, and the comparative amounts of acid produced during seven hours of incubation at 88°F. in pasteurized skim milk inoculated with one per cent culture then determined.

The influence of incubation at 70°F. as compared with 90°F. on the rate of acid production by cheese cultures is shown in Table XII. The results show that the mother cultures that were propagated in pasteurized milk at 70°F., had an average acidity of 0.9087%, while those propagated at 90°F. had 0.7788% for the pasteurized lot, and 0.8825% for the sterilized lot of milk. The seven hour activity test shows similar results in that the cultures propagated at 70°F., developed an average acidity of 0.8625% during seven hours at 88°F., the pasteurized lot propagated at 90°F. 0.6962%, and the sterilized lot propagated at 90°F. 0.7075% acid. The mother cultures were scored for flavor after the seven days of propagation and were characterized as follows:

Culture No.	Propagated at 90°F. in pasteurized milk	Propagated at 90°F. in sterilized milk	Propagated at 70°F. in pasteurized milk
3	flat-coarse	green	flat-mild
5	very coarse	flat	flat
6	coarse	flat-coarse	mild-green
8	distinct off	" "	fine flavor
12	" "	distinct off	" "
14	" "	flat	" "
21	" "	very flat	" "
25	" "	distinct off	" "

From the results obtained above, it is evident that the cheese cultures propagated at 70°F. are more active than those propagated at 90°F. The flavor scores also indicate that 70°F. is a better temperature for incubation than 90°F. because in a majority of cases, a distinct off flavor developed in the cultures incubated at 90°F., which suggests that this temperature allowed the development of undesirable bacteria in the culture.

2. Comparison between 70°F., and 80°F.

Since the results given above show that a 70°F. temperature of incubation was definitely superior to 90°F., it seemed advisable to compare the 70°F. temperature with 80°F. This experiment was repeated exactly as the experiment presented above in Part 1, except that comparisons were made between 70°F., and 80°F. temperatures of incubation rather than between 70°F. and 90°F.

The cultures were propagated for 14 more days, and the seven hour activity test was run at the end of each seven days.

The influence of incubation cheese cultures at 70°F., and at 80°F. is shown in Table XIII and summarized in Table XIV. The pasteurized milk which was incubated at 80°F. had an average acidity for the 14 days of 0.8781% as compared with the sterilized milk at 80°F., which had an average acidity of 0.9045%. Thus the sterilized lot showed an average increase in acidity of 0.0264% over the pasteurized lot at 80°F. The cultures incubated at 70°F. showed an average acidity of 0.9158% which is greater than the sterilized lot at 80°F., and which is also more acid development than the pasteurized lot at 80°F. The taste tests indicated no difference as far as the flavor quality of the 70°F. cultures, and the lot which had been propagated at 80°F.

The results indicate that there is only slight difference between the two temperatures of incubation as far as the activity, and the flavor characteristics of the two lots of cultures are concerned. In no case was the difference in acidities very great, and the differences are thus considered insignificant.

Table XII

Rate of Acid Production by Cheese Cultures After Propagation
For One Week at 70°F., and at 90°F.

	Conditions for Daily Propagation					
	70°F.	90°F.		70°F.	90°F	
	% acid in mother cultures:			% acid in skim milk inoculated with 1% cult. and incubated 7 hours at 88°F.		
Culture No.	Pasteurized Milk	Past. Milk	Sterilized Milk	Past. Milk	Past. Milk	Sterilized Milk
3	.900	.670	.850	.800	.730	.790
5	.950	.750	.870	.920	.770	.830
6	.810	.790	.870	.850	.770	.830
8	.840	.735	.880	.860	.750	.810
12	.920	.930	1.000	.870	.890	.910
14	.940	.725	.780	.860	.360	.450
21	.930	.780	.880	.850	.510	.450
25	.980	.850	.930	.890	.790	.590
Average	.9087	.7787	.8825	.8625	.6962	.7075

Table XIII

Rate of Acid Production by Cheese Cultures After Propagation For One Week at 70°F. and 80°F.

	Trial 1.			Trial 2.		
	Incubation Temperature			Incubation Temperature		
	70°F.	80°F.		70°F.	80°F.	
	Past. Milk	Past. Milk	Sterilized Milk	Past. Milk	Past. Milk	Sterilized Milk
Culture No.	% acid in skim milk inoculated with 1% culture and incubated 7 hours at 88°F.					
3	.900	.890	.935	1.040	.880	.870
5	.935	.945	.985	.970	.890	.890
6	.840	.850	.860	.900	.880	.830
8	.840	.835	.870	.870	.920	.860
12	.920	.900	1.030	.880	.920	.870
14	.950	.900	.945	.890	.780	.830
21	.970	.945	1.010	.940	.600	.730
25	.880	.975	1.040	.900	.930	.930
Average	.9043	.9050	.9593	.9237	.8500	.8512

Table XIV

Summary of Results in Table XIII.

Trial No.	Mother Cult.-70°F.	Pasteurized-80°F.	Sterilized-80°F.
1 7 days	.9043	.9050	.9593
2 14 days	.9273	.8512	.8500
Averages	.9158	.8781	.9045

E. Influence of Chilling.

Since many plants commonly cool the cultures as soon as they are ripened even if they are to be used the same day, an experiment was set up to determine the influence of this chilling upon the rate of acid production by the cheese cultures. This was accomplished by holding the freshly ripened cultures for one hour and for five hours at temperatures of 70°F., and of 32°F., and comparing their activities with those of the fresh mother cultures.

Fresh raw skim milk was obtained from the Oklahoma A. and M. College Milk Plant, dispensed in 100 ml. portions into 10 six ounce prescription bottles with molded screw caps, pasteurized in the usual manner and cooled to 70°F. in a water bath. After cooling the bottles were inoculated with 10 active cheese cultures. Each of these lots of inoculated milk was then dispensed in 10 ml. portions into five sterile test tubes, stoppered with rubber stoppers, and placed in a thermostatically controlled incubator at 70°F. for 16 hours. Two of the sets of tubes were allowed to remain in the 70° incubator, and the other three sets were removed. Immediately upon removal from the incubator, one set of cultures were tested for activity by the three hour activity test, and the other two sets were placed in ice water at 32°F. At the end of one hour, a set of tubes were removed from the ice water and another from the 70°F. incubator, and tested for activity by the three hour activity test. At the end of five hours the remaining two sets were removed from the 70°F., and the 32°F. holding temperatures, and tested for activity by the three hour activity test.

The influence of chilling ripened cultures for one hour and for five hours is shown in Table XV, and summarized in Table XVI. The results indicate that, in general, chilling the cultures to a temperature of 32°F. immediately after ripening, maintained the cultures in a more active condition than when the cultures were not cooled during the holding from one to five hours. The averages of the three trials shown in Table XVI indicate that the cultures held for one hour at 32°F. were slightly more active than the same cultures when fresh. The cultures held at 32°F. for five hours were significantly less active than the fresh cultures, or those held at 32°F. for one hour.

The cultures held for one hour at 70°F. subsequent to the 16 hour ripening period appeared to be significantly less active than the fresh cultures, while those held for five hours at 70°F. produced more acid than those held for one hour, but less acid than the fresh cultures.

These results indicate that in general the cultures should be cooled after thorough ripening unless they are to be used immediately.

Table XV

The Influence of Chilling Upon the Rate of Acid Production by Cheese Cultures

Culture No.	Trial No. 1					Trial No. 2					Trial No. 3				
	70°F.			32°F.		70°F.			32°F.		70°F.			32°F.	
	Fresh	Held 1 hour	Held 5 hours	Held 1 hour	Held 5 hours	Fresh	Held 1 hour	Held 5 hours	Held 1 hour	Held 5 hours	Fresh	Held 1 hour	Held 5 hours	Held 1 hour	Held 5 hours
	ml. .1N NaOH required to neutralize the acid in 10 ml. of skim milk inoculated with 3 ml. of culture and incubated for 3 hours at 98°F.														
5	3.1	3.2	3.4	3.2	3.2	3.2	3.0	3.3	3.3	3.2	3.4	3.2	3.3	3.0	3.2
6	3.4	2.9	2.7	3.1	3.0	4.3	3.5	3.5	3.9	3.6	3.7	3.1	3.8	3.5	3.1
7	3.1	3.1	3.0	2.9	2.6	3.3	3.1	2.9	3.2	2.9	3.3	2.7	3.2	3.0	3.3
9	3.4	3.5	3.2	3.4	3.4	3.7	3.5	3.5	3.7	3.5	3.3	3.0	3.7	3.1	3.5
11	3.8	3.2	3.7	3.6	3.4	3.5	3.2	3.9	3.9	3.5	3.6	3.1	3.6	3.9	3.5
12	3.6	3.7	3.2	3.5	3.3	4.2	3.8	3.7	4.4	3.7	3.4	2.9	3.1	3.3	3.9
16	3.6	3.4	3.3	3.6	3.2	4.1	3.1	3.8	4.0	3.6	3.4	2.8	3.5	4.3	4.1
19	3.1	3.6	3.0	3.4	3.3	3.5	2.9	3.6	3.6	3.9	3.1	3.2	3.8	3.4	3.5
21	3.2	3.0	3.2	3.5	3.6	4.2	3.6	3.5	4.5	3.7	3.3	3.8	3.7	3.4	3.2
25	3.4	2.8	3.1	3.0	3.0	3.3	3.4	3.6	3.5	3.3	3.4	3.2	3.2	3.4	3.2
Average	3.37	3.24	3.18	3.32	3.20	3.73	3.31	3.53	3.80	3.49	3.39	3.10	3.49	3.43	3.45

Table XVI

Summary of Results in Table XV

Trial No.	ml. .1N NaOH required to neutralize the acid developed in 10 ml. skim milk by the Three Hour Activity Test				
	70°F.			32°F.	
	Fresh	Held 1 hour	Held 5 hours	Held 1 hour	Held 5 hours
1	3.37	3.24	3.18	3.32	3.20
2	3.73	3.31	3.53	3.80	3.49
3	3.39	3.10	3.49	3.43	3.45
Average	3.49	3.21	3.40	3.51	3.38

CONCLUSIONS

1. There is no striking difference between the activities of cheese cultures propagated in fresh milk as compared to the same cultures propagated in milk which was incubated and which had developed a high bacterial count.

2. The rate of acid production is slightly higher for cheese cultures grown in skim milk, as compared to those grown in whole milk, however in no instances are the differences in activities very great.

3. Increasing the non-fat milk solids content of the milk progressively increases the activities of cheese cultures propagated therein.

4. Pasteurization of milk for cultures at temperatures of 165°F. for 30 minutes appeared to be as satisfactory as higher exposures, but the additional protection given by pasteurization at higher temperatures may justify the use of higher temperatures.

5. Milk pasteurized at 145°F. for 30 minutes produced acid more slowly than that pasteurized at 165°F. or higher; the slower acid production in milk pasteurized at 145°F. for 30 minutes probably was due to the fact that the germicidal property of the milk was not destroyed.

6. Cheese cultures propagated daily at 70°F. produced acid in milk more rapidly than those propagated at 90°F.

7. Cheese cultures propagated for one week in pasteurized milk at an incubation temperature of 90°F. developed off flavors, as did cultures which were propagated in sterilized milk at 90°F.

8. Cheese cultures propagated in pasteurized milk at an incubation temperature of 80°F. were equally as good both in flavor characteristics and in acid development as the same cultures propagated at 70°F.

9. In general cheese cultures should be cooled after thorough ripening, unless they are to be used immediately.

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