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The Influence of Propagation Procedures on the Activity of Lactic Cultures

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CONTENTS

This bulletin reports the results of several years of research at the Oklahoma Agricultural Experiment Station to establish the influence of propagation procedures on the activity of cheese cultures. Although the results herein reported are primarily concerned with acid production in cheese making, much of the data can be applied to other lactic cultures especially butter cultures and commercial buttermilk.

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By H. C. Olson, R. F. Beachboard and F. D. Cohenour*

Rapid acid production is a desirable character of cultures used to make cheese. Slow cultures prolong the cheese making process and may be damaging to the quality of the finished cheese. Slow acid production is commonly caused by infection of the culture with bacteriophage or by the presence of antibiotics in the milk, but certain factors associated with the propagation procedures also may affect the rate of acid production.

The work here reported was undertaken in an effort to establish the influence on the activity of cultures of various factors concerned with their propagation.

REVIEW OF LITERATURE

The literature on the lactic cultures is rather extensive but there is comparatively little published material on the influence of factors concerned with the propagation on the rate of acid production by cheese cultures. Hammer and Babel (9**) presented a very complete review on the bacteriology of butter cultures.

Baker and Hammer (3) found that butter cultures produced higher acidities in high total solids milk than they did in milk having low total solids.

There appears to be some disagreement as to the influence of pasteurization exposures on the rates of acid production in milk by lactic cultures. Hammer and Baker (10) found that essentially the same rate of growth of starter organisms occurred in milk heated to 160°F. for 30 minutes as when a higher temperature was used for this period. Babel (2) found that lactic culture produced acid most rapidly in sterilized milk (15 pounds pressure for 15 minutes) compared to the rates of acid production by the cultures grown in milk heated

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** Numbers found in parenthesis indicate references to be found on page 28

at 160°F. and at 185°F. for 30 minutes. Milk sterilized at 15 pounds pressure for 25 minutes was less favorable for acid production than that sterilized for only 15 minutes. More rapid acid production occurred in milk pasteurized for 30 minutes at 160°F. than in milk pasteurized at 185°F. for the same period. Foster (5) showed that lactic cultures produced acid more rapidly in milk sterilized at 115°C. (239°F.) for 15 minutes than in milk heated to 80°C. (176°F.) for ten minutes. However, the periods of incubation used (21 hours and 45 hours) are much longer than those commonly employed for determining culture activity. Golding *et al* (7) found that acid production during six hours at 86°F. was more rapid in milk pasteurized at 165°F. for 30 minutes than in the same milk heated to 195°F., steamed, or sterilized for the same period of time. Their data showed that as the temperature of heating increased the rate of acid production decreased. Both Babel (2) and Foster (5) found that sterilization for periods longer than 15 minutes results in decreased rates of acid production.

Hammer and Baker (10) found that heavy inoculation rates did not increase the maximum amount of acid produced by starters. Babel (2) recommended a relatively heavy (1.0 per cent) rate of inoculation for routine propagation. Toens and Hammer (13) reported that rapid coagulation (6 to 8 hours) induced by heavy inoculation produced better cultures than did slow coagulation (16 to 20 hours).

Most authorities agree that incubation at 70°F. for 16 hours is satisfactory for cheese and butter cultures. Toens and Hammer (13) found that good starters can be produced over a wide range of incubation temperatures. They produced satisfactory cultures at 18°, 21° and 25°C. (64°, 70° and 77°F.) and somewhat poorer cultures at 30°C. (86°F.). Babel (2) reported no appreciable differences in the activity of cultures propagated at 86°F. for eight hours compared to those propagated at 72°F. for 14 to 16 hours.

Hammer and Baker (10) reported that over-ripening cultures by incubating at 70°F. for 30 hours of longer did not appreciably decrease the rate at which the starters coagulated milk. Golding *et al* (6) showed that the acidities of starters in the range usually found in the cheese factory had little effect on the rate of acid development in pasteurized skim milk incubated at 86°F. for eight hours.

Dahlberg and Ferris (4) reported slower acid development in cheese making by cultures transferred every third day compared to those transferred daily. Sherman and Hodge (12) noted that *S. lactic*

cultures transferred every 12 hours produced lower acidities than the original cultures.

Hammer and Baker (10) reported that holding starter cultures for considerable periods in ice water and at temperatures below freezing had no appreciable influence on the rates of coagulation. Golding *et al* (6) also reported that refrigeration for 24 hours at 31°F. did not change the activity of cultures.

General Methods

The following general methods were employed except in those experiments in which departures from these methods are indicated in the procedure for each:

ROUTINE PROPAGATION OF CULTURES

Grade A milk, either whole or skimmed, from the milking herd at Oklahoma A. & M. College was pasteurized in flowing steam in an autoclave for 30 minutes, cooled to 70° to 72°F. and then inoculated at the rate of about 0.1 per cent. The cultures were incubated at 70° to 72°F. for 16 hours and then placed in a cold room at 45° to 50°F. until the next transfer. The cultures were propagated daily or at intervals as long as a week, depending on the need for the cultures, but they were always propagated two or more times at daily intervals before being used in the manufacturing operations in order to insure rapid acid production.

THREE-HOUR ACTIVITY TEST

This test, which is similar to the one reported by Horrall and Elliker, (11) was run as follows: measured 10 ml. quantities of skim milk or reconstituted non-fat dry milk solids (10 gm. added to 100 ml. distilled water) were dispensed in rubber stoppered test tubes and pasteurized in flowing steam in an autoclave for 30 minutes. The milk was tempered to 98°F., inoculated with 0.3 ml. (3 per cent) of the culture, and incubated for three hours. At the end of the incubation period the milk was poured into a small Erlenmeyer flask and the tube rinsed with about 5 ml. of distilled water. The incubated milk and rinsings were titrated with 0.1N NaOH, using phenolphthalein as the indicator. The titration value was used as an indication of the activity of the culture.

SEVEN-HOUR ACTIVITY TEST

Skim milk or reconstituted milk (10 gm. non-fat dry milk solids per 100 ml. distilled water) was dispensed in 100 ml. quantities in 6-ounce screw capped prescription bottles and pasteurized in flowing steam for 30 minutes. The bottles were cooled to 88°F., inoculated with one per cent of the cultures being tested, titrated for acidity and then incubated at 88°F. in a water bath for seven hours. The cultures were again titrated and the increase in acidity (calculated as lactic acid) during the seven-hour incubation was used as the index of the activity of each culture. The samples titrated at the end of the incubation period were weighed (18 gm.) because of the inaccuracies likely to be encountered in attempting to measure samples which had coagulated. The inoculation rate and the temperature and period of incubation were selected because they approximate those employed in cheese making.

CREATINE TEST (8)

Two ml. of culture were placed in a test tube, a small amount of creatine and two ml. of 40 per cent sodium hydroxide added, the mixture shaken and the rate and extent of appearance of red color observed.

STANDARD PLATE COUNTS (1)

Standard plate counts were run according to the method given in the 1948 edition of Standard Methods (1). The plates were incubated at 89.6°F. (32°C.) for 48 hours.

Experimental

SANITARY QUALITY OF THE MILK

The influence of the sanitary quality of culture milk on 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 developed a high bacterial count. Standard plate counts and titratable acidity were run on raw skim milk from the Oklahoma A. & M. College milking herd. A portion of the milk was dispensed in 6-ounce screwcapped perscription bottles, heated in flowing steam for 30 minutes, cooled in a water bath to about 60°F. and then placed in a cold room at 50°F. The remainder of the skim milk was allowed to incubate at 80°F. for six to eight hours to develop a high bacterial count and then treated in the same manner

as the fresh milk. After the high-count milk had been pasteurized and cooled, both lots were tempered to 70°F. Ten active cheese cultures were selected and each inoculated at the rate of about 0.4 per cent into a bottle of the pasteurized fresh milk and high-count milk. The inoculated bottles of milk were incubated at 70°F. for 16 hours and then held in a cold room at 50°F. until the next transfer. In seven subsequent daily transfers, the two sets of cultures were propagated in fresh and in high-count milk, respectively. Each day, at the completion of the 16-hour incubation, the activities of the two sets of cultures were determined by the three-hour and by the seven-hour culture activity tests. The titrable acidities of the mother cultures were also determined.

The results (Table I) indicate that the sanitary quality of the milk had no significant influence upon the activity of cheese cultures. With the three-hour activity test there appeared to be a slight advantage in favor of the cultures propagated in fresh milk in four of the seven propagations. With the seven-hour test the cultures grown in the fresh milk appeared to be slightly more active than those grown in the high-count milk in two, less active in one and the same in the remaining four of the seven propagations. The average percentage increase in titratable acidity in the seven-hour test was the same for the cultures propagated in the fresh and in the high-count milks.

WHOLE VERSUS SKIM MILK

The influence of the butterfat in milk on the activity of cheese cultures was determined by propagating cultures in whole milk and in skim milk from the same source and determining the activities by the three-hour activity test. The whole milk tested about 4.2 per cent butterfat and the skim milk about 0.01 per cent. Ten cheese cultures were used and two sets of the cultures, one in whole milk and the other in skim milk, were propagated daily for six days.

The results (Table 2) indicate that the butterfat has no influence on the activity of cheese cultures. In four of the six propagations, the cultures grown in skim milk averaged slightly more active than those grown in whole milk but the differences were slight and the averages for the six propagations were the same.

SOLIDS-NOT-FAT CONTENT

Trial 1—The influence on the activity of cultures of the solids-not-fat content of the milk used in propagating the cultures was studied by determining at weekly intervals the activity of cheese cultures prop-

agated daily in milk containing various levels of solids-not-fat. A good grade of non-fat dry milk solids was used in making the culture milk. The required amount was weighed out and mixed with distilled water in a Waring blendor, dispensed in culture bottles, pasteurized, and cooled in the usual manner. Five cultures were propagated daily in the milk with various levels of solids-not-fat and activity tests were run after seven, 14 and 21 propagations. The activity of each culture was determined by the seven-hour activity test, but through an error only nine grams of non-fat dry milk solids were used per 100 ml. water and as a result the titration values obtained are lower than they normally should be. In the first trial, three levels of solids-not-fat were used, namely, six grams, nine grams, and 12 grams per 100 ml. distilled water. These amounts correspond to approximately 5.66 per cent, 8.26 per cent and 10.71 per cent solids-not-fat in the reconstituted milk. The results (Table 3) indicate that the activity of cheese cultures increased as the solids-not-fat content of the milk used for propagating the mother culture increased and the differences were rather large.

Trial 2—These preliminary results suggested the use of much wider ranges in solids-not-fat content of milk used for propagating the cultures. Accordingly, a second experiment was performed in which six cultures were propagated daily in milk reconstituted at the rate of 6, 9, 12, 15 and 18 grams of non-fat dry milk solids per 100 ml. of water, respectively. These amounts correspond to approximately 5.66 per cent, 8.26 per cent, 10.71 per cent, 13.04 per cent and 15.25 per cent of solids-not-fat in the culture milk. The six cultures were propagated daily in the five lots of milk and activity tests were run after 7, 14, and 21 propagations, using the three-hour activity test. The results (Table 4) show conclusively that as the solids-not-fat content of the reconstituted milk increased there was an increase in the rate of acid production by the cultures. When the data in Table 4 were plotted on a graph, there was a practically straight line relationship between the solids-not-fat content of the culture milk and the titration values for activity. These results suggest that the activity of cheese cultures can be improved by adding extra solids-not-fat to the culture milk or by using a relatively high amount of milk solids-not-fat if reconstituted milk is used.

Trial 3—Since the activity determinations in the above experiment were made only after weekly intervals, it was thought that the increase in activity of the cultures may have been the result of a cumulative effect on the cultures of the environment to which they were exposed rather than to immediate response to the different levels of

milk solids used. To determine whether the response to various concentrates of solids-not-fat was cumulative or immediate, five cultures which had been propagated in the usual manner were inoculated into lots of reconstituted milk containing six grams, nine grams, 12 grams, 15 grams and 18 grams of milk solids-not-fat dissolved in 100 ml. of distilled water and the three-hour activity test was run on the cultures after they had incubated at 70°F. for 16 hours. The following average titration values for the five cultures propagated in milk with various levels of solids-not-fat were obtained: 6 grams SNF, 2.82 ml.; 9 grams SNF, 3.44 ml.; 12 grams SNF, 3.86 ml.; 15 grams SNF, 3.98 ml. and 18 grams SNF, 4.42 ml. These results compare favorably with those obtained on the cultures when tested for activity at weekly intervals and further emphasize the striking influence of the solids-not-fat content on the activity of cheese cultures.

HEAT TREATMENT OF THE MILK

There appears to be no standard temperature for pasteurization of milk for cheese cultures as some operators use temperatures of 165°F. for 30 minutes while others use higher temperatures, including sterilization exposures. The influence of the temperature used for pasteurizing the milk for the propagation of cultures was studied by propagating the cultures daily in milk that had been exposed to various temperatures and then determining the rates of acid production by the cultures.

Each day a lot of fresh skim milk was dispensed in 20 ml. portions into sterilized screw capped test tubes. Groups of five test tubes of the milk were then heated for 30 minutes in water baths at temperatures of 145°, 165°, 185° and 210°F. (boiling) and another group of five was sterilized in the autoclave at 250°F. (15 pounds pressure) for 20 minutes. After the heat treatment, the test tubes were cooled to 70°F. Five active cheese cultures were selected for inoculating the prepared culture milk at the rate of 0.5 per cent. After 16 hours incubation the activity of each culture was determined by the seven-hour test, using reconstituted non-fat dry milk solids dispensed in 20 ml. quantities in screw capped test tubes and heated in flowing steam for 30 minutes. Enough of the tubes of milk were prepared at the beginning of the experiment so that activity test could be run on six propagations of the cultures. These tubes of prepared milk were placed in frozen storage (about 0°F) until needed.

The five cultures were carried through seven propagations in the

lots of milk pasteurized at different temperatures and activity tests were run on all except the fifth propagation.

The results (Table 5) show that the cultures propagated in milk that had been pasteurized at 165°F. were more active than those propagated in milk heated to higher or lower temperatures. It appeared that the rate of acid production by the cultures decreased as the temperature used for heating the milk increased, except for the cultures propagated in the milk pasteurized at 145°F. which had the lowest average activity. However, one of the five cultures (Number 47) did not appear to be influenced by the heat treatment of the milk used for propagation. This culture also was very slow when propagated in the milk pasteurized at 145°F., presumably due to bacteriophage infection. It is possible that this bacteriophage infection may have influenced the activity of this culture in the other propagations. When culture Number 47 is excluded from the results, the heat treatments used for milk for the propagation of cultures, ranked from best to poorest, appeared to be: 165°, 185°, 145°, 210°F., and sterilized.

Observations were made daily on the flavor, odor and creatine tests for acetylmethlycarbinol plus biacetyl. The cultures propagated in the milk pasteurized at 145°F. generally were of very poor flavor and only one of the five cultures (Number 20) showed any considerable flavor development. No significant differences were noted in the flavor and odor produced by the cultures propagated in milk heated at 165°, 185°, 210° and 250°F. except for the expected differences in cooked flavor due to the intensity of heat treatment.

The general results indicate that for greatest activity, the milk used for bulk starter for cheese making should be pasteurized at 165°F. for 30 minutes rather than at a higher temperature. However, for mother cultures the milk should probably be pasteurized at 185°F. or higher in order to insure destruction of organisms in the milk which might cause trouble in subsequent propagations.

RATE OF INOCULATION

In the routine propagation of cultures in the laboratory at the Oklahoma Agricultural Experiment Station the cultures are usually inoculated at the rate of 0.3 to 0.5 per cent for incubation at 70°F. for 16 hours. Since small variations in the rate of inoculation appear to have no influence on the quality of the cultures from the standpoint of the acitvity or rate of acid production, it was thought that perhaps wide variations in the rates of inoculation might have some effect on

the cultures. Accordingly, three sets of five active cultures were propagated daily for six weeks with inoculation rates of 0.1, 0.4 and 1.6 per cent, respectively, and the seven-hour culture activity tests were run at weekly intervals. The results (Table 6) indicate that cultures propagated daily with 1.6 per cent inoculation were more active than cultures propagated with 0.1 per cent or 0.4 per cent inoculation. A comparison of the titration values obtained on the individual cultures at the weekly intervals revealed that the cultures propagated at the 0.1 per cent and the 0.4 per cent levels of inoculation ranked highest in five comparisons each while those propagated at the 1.6 per cent level ranked highest in 18.

These data indicate that the high rate of inoculation resulted in cultures that were generally more active than those inoculated at the normal (0.4 per cent) and low (0.1 per cent) levels.

INCUBATION TEMPERATURE

Since cheese making temperatures are generally considerably higher than the temperature commonly used for propagating cheese cultures, it was thought that if the cultures were incubated at higher temperatures than normal, the rate of acid production might be increased. Temperatures lower than normal might also be beneficial. Accordingly, the influence of the temperature of incubation of cheese cultures on their rates of acid production was determined by propagating daily at different temperatures and running activity tests at intervals. Each of three active cheese cultures was inoculated into four bottles of prepared skim milk and one bottle of each culture was incubated at 65°, 70°, 80° and 90°F., respectively. The cultures incubated at 65°F. were inoculated at the rate of 1.0 per cent and were incubated for 24 hours, while the cultures incubated at 70°, 80° and 90°F. were inoculated at the rate of 0.3 per cent and were incubated 16 hours. At the end of each week for a five-week period the activity of each culture was determined by the seven-hour activity test using reconstituted non-fat dry milk solids (nine grams in 100 ml. water) as the growth medium.

The results (Table 7) show that an incubation temperature of 70°F. was superior to either higher or lower temperatures for propagating cheese cultures. The data show that the activity of cheese cultures grown at 65°F. and at 80°F. were slightly less than those grown at 70°F., and that those grown at 90°F. had the slowest rates of acid production. It is apparent that the activity tests run at the end of two weeks propagation were rather low with all the cultures, especially

with those propagated at 90°F. When the titration values for this period are excluded, the same general conclusions can be drawn with regard to the influence of temperature of incubation on the activity of cheese cultures except that the average increase in acidity obtained with the cultures grown at 90°F. is considerably higher than the average for the five weekly determinations.

Observation were also made on the flavor and body of the cultures. The cultures propagated at 65°F. were generally lacking in flavor and there was a tendency for them to have a slight metallic flavor by the fifth week of propagation. The body of these cultures was generally weak. The cultures propagated at 70°F. had much better flavor than those grown at the other temperatures. The body of these cultures was much better than the body of the cultures grown at 65° and at 90°F. and was slightly better than that of the cultures grown at 80°F. The cultures grown at 80°F. had considerable flavor but tended to be coarser than those grown at 70°F. At 90°F. the cultures lacked the desirable flavor characteristics commonly associated with good cultures and the body was very coarse and chalky.

From the foregoing results it is apparent that an incubation temperature of 70° to 80°F. will give the best cultures from the standpoint of flavor, body and acid production.

INCUBATION PERIOD

There has been some disagreement over the influence of the degree of ripeness on the rate of acid production by cheese cultures. Some authorities state that over-ripening tends to make the cultures produce acid slowly, while others contend that moderate over-ripening has no effect.

The Influence on Acid Production in Milk—The effect of the ripeness of cultures on the rate of acid production was determined by inoculating cultures into bottles of prepared culture milk at two-hour intervals during a 12-hour period and incubating at 70°F. At the end of the incubation period the first bottles inoculated had incubated for 24 hours and the last bottles for only 12 hours. All the cultures were immediately submitted to the seven-hour activity test, using reconstituted non-fat dry milk solids as the incubation medium. Three trials were run, using nine cultures in each trial. The average increase in titratable acidity in the seven-hour activity test for the various incubation periods were as follows: 12 hours, 0.48 per cent; 14 hours, 0.49 per cent; 16

hours, 0.50 per cent; 18, 20, 22 and 24 hours, 0.51 per cent. The results show that there was an increase in activity of the cultures as the incubation period increased from 12 to 18 hours and that the activity remained constant from the 18-hour through the 24-hour periods.

These results indicate that, for the greatest activity, the cultures should be thoroughly ripe and that ripening for 24 hours is not detrimental to the rate of acid production by cheese cultures.

The Influence on Acid Production in Cheese Making—To further test the influence of the incubation period on the rate of acid production by a cheese culture, four lots of pasteurized milk cheddar cheese were made from a culture ripened for 12, 16, 20 and 24 hours at 70°F. The acidities on these bulk cultures were 0.76 per cent, 0.79 per cent, 0.81 per cent and 0.84 per cent, respectively. These four lots of cheese were made exactly the same except for the differences in degree of ripeness of the four lots of culture used. Observations were made on the titratable acidities at various stages of the manufacturing process.

The times required for the lots of cheese to reach milling acidities of 0.50 per cent were five hours and 35 minutes for the lots made from the cultures ripened for 12, 20 and 24 hours and five hours and 40 minutes for the culture ripened for 16 hours.

These results showed that the rates of acid production in the cheese were essentially the same, regardless of the differences in degree of ripeness of the cultures used. It should be noted that the culture ripened for only 12 hours was firmly coagulated and had a higher acidity (0.76 per cent) than was expected for such a short period of incubation but this happened to be an especially active culture and was apparently near its maximum development at the time it was used. The data emphasize that moderate over-ripening does not decrease the activity.

16-Hour and 24-Hour Incubation Periods—Since it appeared possible that persistent over-ripening in the daily propagation of cultures might affect the rate at which they produced acid, an experiment was set up in which cultures were incubated for 16 hours and for 24 hours for extended periods. Two sets of seven active cultures were propagated daily with one set incubated for 16 hours and the other for 24 hours at 70°F. At intervals during 18 weeks of prop-

agation the rates of acid production by the cultures were determined by the seven-hour activity test. Comparisons between the activities of the seven cultures propagated at 70°F. for 16 hours and for 24 hours daily for 18 weeks are shown in Table 8.

The results indicate that persistent over-ripening did not decrease the rate at which cheese cultures produce acid. In fact, it appeared that the cultures ripened for 24 hours were somewhat more active than those ripened for 16 hours because when the titration values of the individual cultures at each interval were compared it was found that those ripened for 24 hours were more active in 17 comparisons, less active in 15 and the same in 3 as compared to the cultures ripened for 16 hours.

The differences in rates of acid production by the cultures incubated for 16 hours and for 24 hours were generally small, as in about half of the comparisons of the titrations on the individual cultures (20 out of 42 comparisons) the differences in the amounts of acid produced in the seven-hour activity test were 0.01 per cent or less. However, in the remaining 22 comparisons in which the differences were 0.02 per cent or more, the cultures incubated for 24 hours were superior in 14 instances and those incubated for 16 hours in only eight.

Flavor observations indicated that the cultures incubated for the 24-hours period had more flavor but the flavor was coarser than that of the cultures incubated for 16 hours. No trouble with yeasts, molds or other contaminating organisms was encountered with either set of cultures. It is not recommended that culture be incubated for 24-hour periods but the above results emphasize that over-ripening is not damaging to the activity of cultures and may even be beneficial.

TWICE DAILY PROPAGATION

Trial 1—Five Percent Inoculation for the Eight-Hour Period—It was thought that perhaps transferring twice daily would eliminate the lag phase common to cultures during the early stages of incubation and therefore maintain the cultures in a more active condition than those transferred once daily. To test this theory, two sets of ten cheese cultures were used. One set was propagated daily in skim milk using a 0.4 per cent inoculation, incubating 16 hours at 70°F., and then storing in a cold room at about 45°F. until the next transfer. The other set was propagated in like manner except that after the 16 hours of incubation, the cultures were transferred into prepared skim milk, using

a five per cent inoculation and incubating for about eight hours at 88°F., after which they were again transferred for the next 16-hour incubation period. The activity of each culture was checked daily after the 16-hour incubation period by the three-hour activity test.

The results (Table 9) indicate that the cultures propagated once daily produced acid slightly more rapidly than those propagated twice daily.

Trial 2—Various Inoculation Rates for the Eight-Hour Period—Another trial was run the same as above except that the amount of inoculation used for the propagation at 88°F. was varied from 0.40 per cent to 6.4 per cent. In this trial four cultures were used and they were propagated for four days using the once daily and twice daily propagations, respectively, for the set of the cultures. The influence of amount of inoculation used for this short incubation period (about eight hours) on the activity of cheese cultures propagated twice daily is shown in Table 10. Data on cultures propagated once daily are also shown. The data indicate that the rate of acid production of the cultures increased as the rate of inoculation used for the eight-hour propagation increased. However, even with an inoculation rate of approximately 6.4 per cent for the eight-hour propagation when the culture were propagated twice daily, the cultures were not as active as those propagated once daily.

From these data it appeared that there was no benefit from twice daily propagation of cheese cultures compared to once daily transfer.

TEMPERATURE OF STORAGE BETWEEN PROPAGATIONS

Many plants chill the cultures in ice water as soon as they are ripe and hold them at this temperature (32°F.) until the next propagation while others place the cultures in cold rooms at 40° to 50°F. In order to determine the influence of the temperature of storage between propagation on the activities of cheese cultures, two sets of ten active cheese cultures were propagated daily for six days. After each incubation period of 16 hours at 70°F., one set of cultures was held in ice water and the other set in a cold room at about 45°F. until the next transfer. The three-hour activity tests were run daily on each set of cultures. The average titration values for the ten cultures for six propagations are shown in Table 11.

The results show that there appeared to be no advantage in holding the cultures in ice water between propagations compared to holding them in a cold room at about 45°F. Although the cultures held in ice

water had a slightly higher average titration value for the six propagations, the data show that the cultures held at 45°F. had the higher average value with four of the six propagations.

PERIOD AND TEMPERATURE OF STORAGE

Since it is sometimes desirable to hold cultures for varying periods between transfers, trials were run to determine the influence of storage conditions on the activities of cheese cultures.

Trial 1—Influence of Storage at 50°F. on Acid Production in Milk—In this preliminary trial, three active cultures were transferred daily and the ripened cultures placed in cold storage at 50°F. At the end of nine days the cultures ranging in age from one to nine days were tested for activity with the seven-hour activity test. All the cultures were then inoculated into prepared culture milk and after 16 hours incubation at 70°F. the activities again determined.

It is apparent from the results in Table 12 that the cultures stored at 50°F. were much more active after they had been carried through one propagation than they were when taken out of storage. The cultures showed no significant decreases in activity during the first five days of storage but after the fifth day they were noticeably slower.

Trial 2—Influence of Storage at Various Temperatures on Acid Production in Milk—Since the above results indicated that cultures could be held at 50°F. or five days with no appreciable decrease in activity, it was thought that perhaps they could be held at lower temperatures for considerably longer periods. Accordingly, two cultures were used in two trials in which 20 transfers of each culture were propagated in the usual manner and, after ripening, five containers of each were stored at 0° to —10°, 32°, 40°, and 50°F. At the end of one, three, seven and nine days storage one bottle of each culture was removed from each lot stored at the four temperatures indicated and the activity of each culture determined by the seven-hour activity test. The cultures were then propagated in the regular manner and activity tests again run after they had ripened.

The results presented in Table 13 indicate that frozen storage had a depressing effect on the acid production rates by cheese cultures and that this effect was still evident after the cultures had been carried through one propagation. It is probable that after further propagations the cultures would have become active again. It appeared that storage at 40° and at 50°F. maintained the cultures in a more active

condition than did storage at 32°F. The cultures stored at 40° and at 50°F. appeared to maintain these activities during the first five days of storage after which there seemed to be a slight decline. The results seem to justify the conclusion that cultures can be stored for five days at 40° or at 50°F. without loss of activity.

Trial 3—Influence of Storage at 50°F. on Acid Production in Cheese Making—In order to determine the influence of storage period of cultures on the rates of acid production during cheese making, three trials were run in which cultures propagated from mother cultures stored at 50°F. for from zero to seven days were used in making cheddar cheese. An active culture was propagated daily and on alternate days the ripened culture was stored at 50°F. so that at the end of the seventh day there were cultures on hand that were zero (fresh), one, three, five and seven days old. These were inoculated into prepared culture milk and after ripening at 70°F. for 16 hours each culture was used in making cheddar cheese. In each trial the five vats of pasteurized milk cheddar cheese were made from the same lot of milk and other factors concerned with manufacturing procedure were maintained as nearly alike as possible so that the only variable was the storage period of the cultures used to prepare the bulk cultures for the cheese. Titratable acidity determinations were made at frequent intervals during the manufacturing process and plotted on graphs in order to determine as accurately as possible the period required from adding the starter to reaching a milling acidity of 0.5 per cent. The data are shown in Table 14. In Trial 1, the culture appeared to become progressively slower as the storage period lengthened beyond three days while in Trials 2 and 3 the storage period of the mother cultures appeared to have no influence on the rate of acid development in the cheese. When the titratable acidity values obtained with the five lots in Trial 1 were plotted in a curve, it was found that the starters propagated from the fresh culture (zero days) and the culture stored for one day produced more acid during the early stages of the cheese making process than the cultures stored for longer periods. It is probable that bacteriophage was present in the milk used and that the cultures which were very active were able to outgrow the bacteriophage while the less active cultures were considerably slowed up by it. The general results indicate that the activity of cheese culture is not greatly impaired by storing the mother cultures for seven days at 50°F.

Trial 4—Influence of Period and Temperature of Storage on Acid Production in Cheese Making—In another experiment the influence of the period and temperature of storage of mother cultures on the rate of acid production in cheesemaking was determined by storing a culture

at temperatures ranging from below freezing to 50°F. for various periods and making cheese from bulk cultures prepared from the stored mother cultures. An active culture was inoculated into 12 bottles of prepared culture milk and after incubation at 70°F. for 16 hours, three bottles each were stored at 0° —10°, 32°, 40° and 50°F. After one day of storage a bottle of culture stored at each of the four temperatures employed was removed and used to prepare bulk culture. On the following day, four vats of pasteurized milk cheese were made from the freshly ripened bulk cultures. The four lots of cheese were made from the same lot of pasteurized milk and all other factors concerned with the cheese making process were kept as nearly uniform as possible except for the culture used. The cheese making procedure was repeated with the cultures after storage for three days and for five days, but obviously a different lot of pasteurized milk was used for each day's make. Titratable acidity determinations were made at frequent intervals in the cheese making process and the data plotted on a graph to determine the approximate period required from inoculation of the milk to reaching a milling acidity of 0.5 per cent.

The results shown in Table 15 indicate that the temperature of storage during five days had no great influence on the activity of a cheese culture but it did appear that the best acid development was obtained with the cultures stored at 40°F., and the poorest with those stored at 32°F. The failure of the bulk culture prepared from the culture stored at 32°F. for five days to coagulate was possibly due to the storage conditions slowing up the culture to the extent that it became vulnerable to the action of bacteriophage. These results suggest that the best storage temperature for several days is about 40°F.

STORAGE WITH CALCIUM CARBONATE ADDED TO THE CULTURE MEDIUM

Since calcium carbonate is commonly used as an aid in maintaining the activity of cultures during shipment from commerical concerns to dairy plants, it seemed logical that this material could be used to extend the period of storage of cultures in dairy plants. An experiment was run in which an active culture was inoculated into seven bottles containing about 20 ml. of ordinary prepared culture milk and into bottles of the same milk with about one gram of calcium carbonate added. After incubation at 70°F. for 16 hours the cultures were stored at 45° to 50°F. A bottle of each culture, one with and one without calcium carbonate, was removed from storage at intervals, propagated once in the usual manner, and then inoculated into milk for bulk cultures.

These bulk cultures and one prepared from the mother culture propagated daily were used to make three lots of cottage cheese. The three lots of cheese were cut at the same time and the whey acidity immediately after cutting was used as the measure of the activity of each culture. The bulk cultures propagated from the stored cultures were tested for activity by the seven-hour activity test at intervals throughout the storage period.

The results presented in Table 16 show that the culture stored without calcium carbonate became too slow for cheese making after five days of storage, while that with calcium carbonate added was still suitable for cheese making at the end of 13 days. Comparisons between the culture stored with calcium carbonate added and the same culture transferred daily show that the stored culture was just as active as that propagated daily and even appeared to be slightly more active at the seven, nine, 11 and 13 day intervals. These results confirmed the previous findings that cultures can be stored for about five days at 40° to 50°F. without impairing of activity but that beyond this period they decrease in activity. The results show further that calcium carbonate added to the culture milk greatly lengthens the period of storage during which the cultures maintain active acid production.

Summary and Conclusions

1. Cultures grown in milk that had a very high average bacterial content before pasteurization were as active as those grown in milk that had a much lower average count.

2. The butterfat in milk had no influence on the activity of cheese cultures.

3. The solids-not-fat content of the milk use in propagating cultures had a relatively great influence on the activity of the cultures. As the solid-not-fat content increased, the rate of acid production by the cultures also increased, in practically a straight line relationship. In propagating cultures, it appears advisable to use milk of a high solid-not-fat content, or if reconstituted milk is used, the solid content should be high (10 to 12 per cent).

4. In comparisons of the activity of cultures propagated in milk exposed to various heat treatments in the preparation of the culture milk, it was found that the most active cultures were those propagated in the milk that had been pasteurized at 165°F. for 30 minutes. More intense heat treatment of the milk resulted in less active cultures. Pas-

teurization of the milk at 145°F. for 30 minutes resulted in relatively slow cultures. From the results it appeared that a pasteurization of 165°F. for 30 minutes would be the most desirable for the preparation of milk for bulk cultures from the standpoints of activity and flavor development but more intense heat treatment may be necessary for milk for mother cultures in order to insure destruction of heat-resistant types of bacteria which might be damaging to the cultures.

5. The data on rate of inoculation indicate that a high rate (1.6 per cent) resulted in more active cultures than a low (0.1 per cent) or moderate (0.4 per cent) rate of inoculation.

6. Incubation at 70°F. is more favorable for the propagation of active cultures than incubation at either higher (80° and 90°F.) or lower (65°F.) temperatures. Good cultures can be produced at 80°F. but those propagated at 70°F. were generally better in flavor and body.

7. The activity of cultures increases as the incubation period is increased from 12 to 18 hours and then remains constant through the 24-hour period. This showed that over-ripening by prolonging the incubation period did not decrease the rate of acid production by cheese cultures.

8. Twice daily transfer of cultures, involving one propagation at 70°F. for 16 hours and another at 88°F. for seven to eight hours does not increase their activity but rather appeared to result in cultures somewhat slower than those propagated once daily.

9. Cultures can be stored at 40°F. to 50°F. for five days or longer without decreasing their rates of acid production in cheese making. These temperatures seemed to be more favorable for storage than lower temperatures (32° or 0° to -10°F.)

10. The addition of a small amount of calcium carbonate to the containers of milk in which cultures are propagated greatly prolongs the period during which cultures will remain active in storage at 40° to 50°F.

11. Before being used in cheese making cultures should be carried through at least one propagation after removal from storage.

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Sanitary Quality of Milk

TABLE 1—Titration values of cultures propagated in fresh and in high count milk. (Three- and seven-hour tests; averages of 10 cultures.)

Propagation no.	Titratable acidities on:		Standard plate counts on		Average acidities of mother cultures propagated in:		Titration Values			
							Fresh milk		High-count milk	
	Fresh milk	High-count milk	Fresh milk	High-count milk	Fresh milk	High-count milk	3-hour test	7-hour test	3-hour test	7-hour test
	%	%			%	%	ml.	%	ml.	%
1	0.18	0.19	160,000	37,000,000	0.86	0.85	3.75	0.62	3.70	0.62
2	0.17	0.22	197,000	76,000,000	0.93	0.93	3.22	0.52	3.12	0.51
3	0.17	0.19	210,000	2,900,000	0.91	0.89	3.25	0.50	3.26	0.50
4	0.17	0.18	250,000	10,000,000	0.91	0.90	3.23	0.51	3.25	0.51
5	0.18	0.19	270,000	25,000,000	0.88	0.89	3.28	0.49	3.24	0.48
6	0.18	0.19	600,000	26,000,000	0.90	0.91	3.45	0.43	3.54	0.47
7	0.18	0.20	310,000	14,000,000	0.76	0.75	3.59	0.47	3.43	0.47
Avg.	0.18	0.19	261,000	18,500,000	0.88	0.87	3.40	0.51	3.36	0.51

Butterfat Content

TABLE 2—Activity of cheese cultures propagated in whole and in skim milk. (Three-hour test; averages of 10 cultures.)

	Titration value for successive propagations:						
	Prop. No. 1	Prop. No. 2	Prop. No. 3	Prop. No. 4	Prop. No. 5	Prop. No. 6	Avg.
	ml.	ml.	ml.	ml.	ml.	ml	ml.
Whole milk	3.65	3.82	3.58	3.84	3.61	4.21	3.79
Skim milk	3.76	3.83	3.67	3.93	3.47	4.10	3.79

Solids Not Fat

TABLE 3—Activity of cheese cultures propagated in reconstituted milk containing various amounts of solids-not-fat (Experiment I). (Seven-hour test; averages of five cultures.)

Solids-not-fat per 100 ml. water	Increase in titratable acidity after propagating daily for:			
	1 week	2 weeks	3 weeks	Average
gm.	%	%	%	%
6	0.27	0.23	0.25	0.25
9	0.32	0.25	0.30	0.29
12	0.48	0.34	0.35	0.39

Solids Not Fat

TABLE 4—Titration values of cheese cultures propagated in reconstituted milk containing various amounts of solids-not-fat. (Three-hour test; averages of six cultures.)

Solids-not-fat per 100 ml. water	Titration values after propagating daily for:			
	1 week	2 weeks	3 weeks	Average
gm.	ml.	ml.	ml.	ml.
6	2.71	2.68	2.65	2.68
9	3.26	3.16	3.25	3.22
12	3.61	3.38	3.65	3.55
15	4.40	3.98	4.16	4.18
18	4.68	4.36	4.50	4.51

Heat Treatment

TABLE 5—Activity of cheese cultures propagated in milk treated with various heat exposures. (Seven-hour test; averages of five cultures.)

Heat treatment	Culture Number: 23	29	47	52	62	Avg. All Cultures	Avg. Excl. No. 47
	%	%	%	%	%	%	%
145°F. for 30 min.	0.44	0.53	0.17	0.56	0.50	0.44	0.51
165°F. for 30 min.	0.51	0.51	0.56	0.60	0.56	0.55	0.55
185°F. for 30 min.	0.49	0.49	0.54	0.55	0.54	0.52	0.52
210°F. for 30 min.	0.48	0.50	0.55	0.51	0.51	0.51	0.50
250°F. for 20 min.	0.47	0.49	0.57	0.50	0.43	0.49	0.47

Rate of Inoculation

TABLE 6—Activity of cheese cultures propagated daily with various rates of inoculation. (Seven-hour test; averages of five cultures.)

Rate of inoculation	Increase in titratable acidity after propagating daily for: 1 week	2 weeks	3 weeks	4 weeks	5 weeks	6 weeks	Avg.
%	%	%	%	%	%	%	%
0.1	0.36	0.53	0.52	0.53	0.52	0.50	0.49
0.4	0.52	0.56	0.53	0.52	0.45	0.53	0.52
1.6	0.60	0.58	0.53	0.52	0.52	0.53	0.55

Incubation Temperature

TABLE 7—Activity of cheese cultures propagated daily at various incubation temperatures. (Seven-hour test; averages of three cultures.)

Temperature of incubation	Increase in titratable acidity after propagating daily for: 1 week	2 weeks	3 weeks	4 weeks	5 weeks	Avg.	Avg. second week	Excl.
	%	%	%	%	%	%	%	
65° F.	0.35	0.37	0.49	0.50	0.55	0.45	0.47	
70° F.	0.41	0.37	0.51	0.53	0.54	0.47	0.50	
80° F.	0.40	0.35	0.47	0.51	0.51	0.45	0.47	
90° F.	0.39	0.18	0.42	0.49	0.49	0.39	0.45	

Period of Incubation

TABLE 8—Activity of cheese cultures propagated daily for 16 hours and 24 hours at 70° F. (Seven-hour test; averages of seven cultures.)

Incubation period	Increase in titratable acidity after propagating daily for:						
	1 week	3 weeks	4 weeks	5 weeks	10 weeks	18 weeks	Avg.
	%	%	%	%	%	%	%
16 hrs.	0.60	0.62	0.54	0.50	0.44	0.49	0.53
24 hrs	0.61	0.61	0.54	0.49	0.46	0.49	0.53

Twice Daily Propagation

TABLE 9—Activity of cheese cultures propagated once- and twice-daily. (Three-hour test; Averages of 10 cultures.)

Frequency of propagation	Titration values for successive propagations				
	Prop. No. 1	Prop. No. 2	Prop. No. 3	Prop. No. 4	Avg.
	ml.	ml.	ml.	ml.	ml.
Once daily	3.16	2.82	3.33	3.33	3.16
Twice daily	3.03	2.85	3.26	3.26	3.10

Twice Daily Propagation With Variations in Inoculation Rate

TABLE 10—Activity of cultures propagated once daily compared to those propagated twice daily with different rates of inoculation for the eight-hour period. (Three-hour test; averages of four cultures.)

Rate of inoculation	Titration values				
	Prop. No. 1	Prop. No. 2	Prop. No. 3	Prop. No. 4	Avg.
%	ml.	ml.	ml.	ml.	ml.
		Propagated Once Daily			
0.40	4.10	3.64	3.70	4.08	3.88
		Propagated Twice Daily			
0.40	3.89	3.63	3.63	3.54	3.67
0.80	4.04	3.58	3.68	3.58	3.72
1.60	4.10**	3.58**	3.70**	3.57**	3.74**
3.20	3.99	3.68	3.74	3.58	3.75
6.40	4.24	3.64	3.75	3.69	3.83

** Average for three cultures only, as culture No. 2 became infected with bacteriophage at the 1.60 per cent rate of inoculation.

Storage Temperatures

TABLE 11—Activity of cultures stored at 45° F. and at 32° F. between propagations. (Three hour test, average of ten cultures)

Storage temperatures	Titration values for successive propagations						
	Prop. No. 1	Prop. No. 2	Prop. No. 3	Prop. No. 4	Prop. No. 5	Prop. No. 6	Avg.
	ml.	ml.	ml.	ml.	ml.	ml.	ml.
45° F. (cold room)	3.76	3.83	3.67	3.93	3.47	4.10	3.79
32° F. (ice water)	3.74	3.84	3.63	3.92	3.76	3.93	3.80

Storage Period

TABLE 12—Activity of cultures stored for various periods at 50° F. (Seven-hour test; averages of three cultures.)

Period Stored	Inoculated from:	
	Stored culture	Culture after one propagation
days	%	%
1	0.43	0.56
2	0.46	0.56
3	0.41	0.57
4	0.38	0.53
5	0.40	0.55
6	0.27	0.50
7	0.15	0.44
8	0.07	0.42
9	0.05	0.49

Storage Temperature and Period

TABLE 13—Activity of cheese cultures stored at different temperatures for various periods. (Seven-hour test; averages of two trials with two cultures each.)

Holding temperature	Increase in titratable acidity after propagation for:				
	1 day	3 days	5 days	7 days	9 days
	%	%	%	%	%
Inoculation from stored cultures					
0° to -10°F.	0.28	0.12	0.17	0.09	0.05
32°F.	0.41	0.38	0.31	0.23	0.15
40°F.	0.42	0.41	0.42	0.42	0.33
50°F.	0.44	0.40	0.43	0.42	0.28
Inoculation from cultures after one propagation					
0° to -10°F.	0.40	0.29	0.48	0.40	0.19
32°F.	0.43	0.47	0.50	0.46	0.25
40°F.	0.41	0.46	0.50	0.45	0.39
50°F.	0.41	0.46	0.50	0.44	0.41

TABLE 14—Time required to develop 0.5 per cent milling acidity in cheddar cheese made with cultures propagated from cultures stored at 50° F. for various periods.

Days stored	Trial No. 1	Trial No. 2	Trial No. 3	Avg.
	hrs:min	hrs:min	hrs:min	hrs:min
0 (fresh	5:15	5:30	5:20	5:22
1	5:15	5:35	5:20	5:23
3	5:45	5:10	5:20	5:25
5	5:45	5:10	5:20	5:25
7	*	5:10	5:20	

* Very slow acid development. Milled in 5 hrs. 50 min. at an acidity of 0.31 per cent.

TABLE 15—Time required to develop 0.5 per cent milling acidity in cheddar cheese made with cultures propagated from cultures stored at various temperatures.

Storage temperature	Period of storage		
	1 day	3 days	5 days
	hrs: min	hrs: min	hrs: min
0° to -10 F.	5:05	6:05	5:20
32° F.	5:05	5:15	*
40° F.	5:05	5:05	5:25
50° F.	5:05	5:25	5:25

* Bulk culture prepared from stored culture failed to coagulate.

Calcium Carbonate

TABLE 16—Activity and rates of acid production in cheese making for cultures stored for varying periods at 45° to 50° F. with and without calcium carbonate added.

Storage conditions	Period stored (days)					
	1	5	7	9	11	13
	%	%	%	%	%	%
Activity test on Bulk Cultures (Seven-hour test)						
Nothing added		0.46	**			
$CaCO_3$ added		0.46	0.45		0.49	0.53
Control culture*		0.46	0.45		0.42	0.51
Whey acidity at cutting						
Nothing added	0.44	0.53	**			
$CaCO_3$ added	0.46	0.50	0.52	0.52	0.54	0.52
Control culture*	0.46	0.50	0.50	0.52	0.53	0.50

* No storage; culture propagated daily.
** Culture very slow; failed to coagulate the milk for bulk culture.