A COMPARISON OF VARIOUS CULTURE ACTIVITY TESTS FOR DETERMINING THE RATE OF LACTIC ACID PRODUCTION BY CHEESE CULTURES

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Bachelor of Science

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Stillwater, Oklahoma

1948

Submitted to the Faculty of the Graduate School of
the Oklahoma Agricultural and Mechanical College
in Partial Fulfillment of the Requirements
for the Degree of
MASTER OF SCIENCE

1951

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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 guidance given throughout the research and during the preparation of this thesis.

The author is also indebted to R. E. Anderson for his help in running the cheesemaking processes.

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INTRODUCTION

Cheese cultures which appear satisfactory at the time of inoculation often fail to develop acid at a normal rate during the cheesemaking process. This situation is a source of intermittent trouble and expense to the cheese plant because it interferes with the plant routine and is a detriment to the quality of the finished cheese.

Various tests have been devised to determine the activity of cheese cultures and these tests have been employed in much research. However, since various investigators use different activity tests in evaluating cultures, it appears that a uniform test is needed so that the results of different research workers can be compared.

The work herein reported was undertaken in an attempt to establish which activity tests are practical and accurate for predicting the rate at which cheese cultures will produce acid during the cheesemaking process. This was determined by the extent the results of various tests were in agreement with the activity of cultures and the extent the results of various tests were in agreement with the rate of acid production during the cheesemaking process.

REVIEW OF LITERATURE

Hales (18) states that the primary functions of cheese cultures are:

1. To establish the desired kind of bacteria in milk.
2. To assure development of acid for proper coagulation of milk by the action of rennet.
3. To produce the desired rate of acid throughout the entire curd-making process.
4. To suppress the growth of undesirable bacteria.
5. To control the proper bacterial action during aging.

Other functions as cited by Wilster (50) are:

1. To break down insoluble casein.
2. To aid in the formation of brine-suluble calcium para-caseinate.
3. To aid in the expulsion of moisture from the curd particles.

Early investigations by Evans, Hastings, and Hart (15) and Knudsen (34) concluded that two or more organisms growing together can produce a greater amount of acidity than the total each can produce alone. Hammer and Bailey (20) stated that butter cultures are composed of a combination of S. lactis organisms and the associated organisms, S. citrovorus and S. paracitrovorus. The S. lactis organisms primarily ferment milk sugars to lactic acid while the S. citrovorus and S. paracitrovorus organisms produce volatile acid which is

responsible for the flavor and aroma of cultures. Further studies by Hammer (19) indicate that the volatile acid is produced from the citric acid of milk, while Michaelian and Hammer (38) concluded that lactic acid is not a source of the volatile acid. Cordes and Hammer (10) showed that the lactic acid produced from a pure culture of S. lactis grown in milk, is made up of approximately 95 per cent of the total acid produced. Of this, only 2 to 4 per cent was volatile acidity. When the associated bacteria were introduced, the volatile acidity rose to 10 to 15 per cent of the total acidity. Hammer (19) states that S. citrovorus produces only 0.25 per cent and S. paracitrovorus 0.39 to 0.77 per cent of the total lactic acid produced when the citric acid fermenting bacteria were added to a pure culture of S. lactis.

Hucker and Marquardt (27) secured desirable flavor in cheddar cheese using S. paracitrovorus alone or in conjuction with commercial starters. However, the same investigators found S. citrovorus had no effect upon the flavor of cheddar cheese.

Hansen, Bendixen, and Theophilus (21) concluded that cheese of inferior flavor and aroma and superior body and texture resulted with the use of a pure S. lactis culture as compared to a mixed culture.

Investigations by Kelly (33) indicated that the use of S. cremoris as a starter yielded little difference in flavor and aroma than S. lactis for cheesemaking.

Recent work by Beachboard (9) showed that pure cultures of

L. bulgaricus gave unsatisfactory acid production during the manufacture of cheddar cheese. L. bulgaricus in combination with 30 to 60 per cent commercial starters gave satisfactory acid development, but inferior flavor, aroma, body and texture of the cheese.

Cox and Whitehead (11) and Marshall (37) presented data showing that B. subtilis appeared to stimulate lactic acid production by S. lactis. Cox and Whitehead (11) found B. coli varied in its effect; two strains of staphylococci had a slight stimulating effect, and B. faecalis alkaligenes had a slight effect on lactic acid production. Nelson, Harriman, and Hammer (39) stated that 0.1 per cent abnormal milk retards acid development.

Baker and Hammer (8) concluded that milk from different animals made cultures of varying acidities, while different lots of milk from the same animal made cultures of approximately the same acidity. They also showed that milk with a high total solids content produced cultures of high acidity. Knudsen (34) stated that variations occur between subcultures grown in different lots of milk from individual cows. Pasteurized milk from various herds also shows differences. Horral, Elliker, and Kensler (26) stated that milk from individual herds varies from day to day in its ability to support growth of starter bacteria. They further stated that milk reconstituted from spray, non-fat, dry milk solids produced cultures and starters more constant in activity from day to day than did selected whole milk.

The results showed that with the use of reconstituted non-fat dry milk solids, the culture activity was relatively constant from day to day, with the lowest activity 0.40 per cent and the highest 0.48 per cent, while the culture activity on whole milk varied from 0.34 to 0.51 per cent. Horral et al stated (26), "It was believed that if mother cultures and starters could be carried in milk of the same composition over long periods of time, more uniformity in their activity could be obtained". They recommended the use of a 10 per cent non-fat dry milk solids medium for cultures. A study by Horral et al (26) showed that certain water supplies were found unsuitable for use in reconstituting milk for starters due to high solids content and toxic factors. Distilled water should be used whenever possible. Otherwise, the water should be tested to determine whether or not it provides a reconstituted milk suitable for cultures. Golding (17) believed that non-fat dry milk solids would ultimately become the medium for carrying both mother and bulk starters.

Whitehead and Cox (46) described "non-acid" milk as milk in which active lactic acid cultures did not develop acid at a normal rate.

Hunter and Whitehead (30) found that when milk containing inhibitory substances produced by growth of "non-acid" streptococci was used for culture propagation, it caused delayed coagulation , simulating starter failure caused by bacteriophage. Graphically, the authors described the development of two cultures in "non-acid" milk of different percentages. The results clearly indicated that one strain was more sensitive to

the inhibitory substance in "non-acid" milk than the second strain.

Knudsen (34) stated that the number of organisms in a starter reached maximum at coagulation. However, studies by Baker and Hammer (8) revealed that the maximum bacterial count was slightly beyond the point of coagulation; and as the ripening increased, the bacterial count decreased. Baker and Hammer (7) stated that prolonged overripening affected culture activity. Dahlberg and Ferris (12) found that aging starters generally slowed the development of acidity. However, Johns and Berard (32) concluded that prolonged overripening of starters to a greater extent than encountered in cheese factory practice, failed to slow down acid development or lower the final acidity reached. In a practical vat demonstration, an overripened portion of the starter worked slightly faster and produced cheese of a higher flavor than the control vat. The effect of overripening upon the proportion of milk-coagulating organisms was studied, and in two of the three starters the overripened portion contained a higher bacterial count than the normally ripened portion. After thirty days of repeated overripening the flavor of the overripened portion was superior to that of the normally ripened portion. Rice was quoted by Johns and Berard (32) as saying, "so long as overripening is avoided, a starter will produce acid normally at any period after the logarithmic period (of growth) is well established until maximum acidity is reached and no advantage is derived by cooling a starter as soon as coagulation occurs". Johns and Berard

commented by saying, "Our results also indicate that no disadvantage is derived by cooling a starter immediately after coagulation; they also suggest that over-ripening is unlikely to be responsible for the weakening of starters to which Rice refers."

Hales (18), in discussing practical cheesemaking conditions, stated that cultures regularly ripened to very low acidities became delicate and were easily lost. When overripened, they became slow. Due to sectional differences, a final ripening acidity was not given. However, it was recommended that cultures and starters be ripened to as low an acidity as possible with the cultures at highest activity. Angevine (2) stated that the titratable acidity of an active starter should be between 0.8 and 0.85 per cent.

Dahlberg and Ferris (12) found that lactic cultures, carried under excellent conditions, inoculated daily or every third day, were identical in appearance, flavor, and acid development. The results of starters incubated at 86°F. showed rapid acid development; at 100°F., slower acid development; and at 86°F. for two hours and then 100°F. for 6 hours, good acid development. Additional results indicated that incubation of cheese milk at 86°F. increased the acid production at the cooking temperatures; and cheese manufactured with starters transferred daily developed more flavor, better quality, in less time than when made with starters transferred every third day.

Sherman and Hodge (44) transferred cultures every 12 hours and 24 hours and determined that the total acidity produced in

7 days was less with the cultures transferred every 12 hours. The authors theorized that the fastest growing organisms were capable of producing the least total acid and that these were secured in the largest relative proportion by frequent transfers. Slow growth enabled an organism to better adapt itself to its environment and therefore they become more viable when exposed to adverse conditions. This research concluded with the hypothesis that slow growth was associated with greater acid production power among strains of the same genetic constitution.

Anderson and Meanwell (1) and Whitehead and Hunter (49) found that slow starters developed sooner with a light inoculation. Studies by Whitehead and Cox (47) showed that normal starter cultures were not affected by aeration of the milk; however, they may suddenly develop a sensitivity to aeration, and then may just as suddenly revert to the normal state. Evidence was brought forth on the possible mechanism of inhibiting the sensitive streptococci which tends to show that the action is connected with the oxygen-reduction by means of which the organisms obtain their growth energy.

Hood and Katznelson (23) and Doan (14) stated that penicillin, aureomycin, sulfamethazine, and streptomycin used in the treatment of mastitis and brucellosis in cows were in the milk for several milkings after treating such cows. Hood and Katznelson (23) found that penicillin and aureomycin completely inhibited acid development. Angevine (2) stated that antibiotics inhibit starter development more in summer than in winter. Doan (14) found that serious inhibition of starter

activity was caused by 0.1 unit of penicillin per 1 ml. of milk and partial arrest or slow acid developed with the use of 0.05 units per 1 ml. of milk. Ruehe (43) stated that 100 p.p.m. or more of penicillin prevented bacterial growth and acid production in cultures and 25 - 50 p.p.m. decreased acid production. Krienke (35) found that 1.0 per cent of the milk from the first milking of an aureomycin-treated udder mixed with 99 per cent normal milk showed a complete lack of acid development after 6 hours at 95°F. Pasteurization had no affect on the antibiotics studied. Autoclaving at 212°F. for 15 minutes reduced the toxicity somewhat. Hunter (29) reported that steaming culture milk for 1 hour decreased its penicillin content fifty per cent.

Doan (14) stated that the enzyme penicillinase was a positive antidote for penicillin; however, it was costly for commercial use. It has been reported by Doan (14) that starters tolerant to antibiotics were developed by continuous transfers into gradually increasing levels. He concluded that the procedure was lengthy and took experience to operate, and that the starters could lose their resistance in the absence of the antibiotic. The discarding of three milkings following treatment was suggested.

Davis and McClemont (13) found that mastitic milk would slow acid development of starters. Slow growth of S. lactis and S. cremoris were shown in mastitic milk, whereas normal milk samples supported growth of these organisms. Whitehead and Cox (46) observed that milk with a leucocyte count in

excess of 5,000,000 per ml. yielded a rennet curd in which normal amounts of acid could not be produced. Studies by Harrison and Dearden (22) disagreed with most investigators and concluded that mastic milk had no effect upon the rate of acid produced as certain strains of streptococci failed to grow at the cooking temperature. Prouty (42) found that in some samples pasteurization of milk at 65.5°C. to 68.5°C. for 30 minutes partially overcame the retarding influence of mastitic milk on the growth of S. lactis.

Golding et al (16) concluded that a cooking temperature of 102°F. greatly reduced the developing acidity; and the longer the period held at this temperature, the slower was the acid development when returned to 86°F. Further results indicated that the development of acidity at 60°F. and 100°F. was insignificant during an 8-hour period. Optimum acidity development was at 86°F. The results of Whitehead and Cox (47) agreed with Golding et al (17). Babel (4) determined the rate of acid production of cultures held at 86°F., 98°F., 101°F., and 104°F. All cultures produced acid slowly at 101°F. and 104°F. Six of the seven cultures produced slightly less acid when held at 86°F. for 7 hours than when held at 86°F. for 2 hours then 104°F. for 2 hours and then at 86°F. for 3 hours. In the cheesemaking process, a cooking temperature of 102°F. slightly retarded acid development with five of the seven cultures when compared to 100°F. A temperature of 104°F. appreciably retarded acid development compared to 100°F. All cultures and whey were examined for bacteriophage. Since none was observed, it was concluded that the decrease in acidity was due to the

temperature employed.

Whitehead and Hunter (48) concluded that organisms selected for active starters must have two properties: active acid formers at 20°C. to 30°C. and relatively unaffected in their own growth at 37°C. Harrison and Dearden (22) determined the rate of acid production of three cultures at temperatures of 22°, 28°, 30°, 40°, and 45°C. The first culture produced acid up to 37°C. but failed to produce acid at 40°C. The second and third cultures produced acid at 40°C. After incubation at 40°C., the first culture was incapable of growth at either 22° or 28°C. The second and third cultures were able to grow at 22°and 28°C. after 24 hours of incubation at 40°C. The use of cultures capable of normal growth at 40°C. was suggested. Golding et al (16) stated that the rate of acid development was due to the origin of the starter, the retarding effect of the scalding temperature, and the time the curd was held at the scalding temperature after cooking the curd. Horral et al (25) stated that high cooking temperatures, poor quality milk, bacteriophage, and toxic materials hindered activity development.

Nelson et al (39) demonstrated that the source of milk or extraneous matter was not the cause of a sudden type of slow acid production. When an inoculation of a slow culture into a fast culture did not slow acidity, it was concluded that slow acid production was apparently the result of a condition peculiar to the culture. Whitehead and Hunter (48) in New Zealand, Anderson and Meanwell (1) in England, and Babel (5) in the United States found that failure of some single-strain starters was due to bacteriophage. Hunter (29) and Whitehead and

Hunter (48) concluded that bacteriophage originated from the whey of cheese vats since bacteriophage with titers of 10^{-1} to 10^{-8} were shown in the whey of cheese vats normally producing acid. Whitehead and Hunter (49) found that bacteriophage in the air of commercial cheese factories came from whey separators. Some air-borne infections were so great that it was impossible to prevent infection of cultures for more than a few propagations. Nichols and Wolf (40) reported bacteriophage prevalent in England in May and August and widespread geographically.

Hunter (28) found that a heavy initial infection of bacteriophage caused lysis of organisms and cessation of acid development before the manufacturing process was completed; a light infection could have a noticeable effect upon the starter performance in the vat; and, an intermediate infection could have an effect on acid development in late stages of manufacturing. Anderson and Meanwell (1) reported that the inclusion of certain single-strain phaging starter cultures in a combination of starters would cause "pack up or slowness" in cheesemaking. Mixed cheese starters were also subject to bacteriophage failure. Whitehead and Hunter (48) found that bacteriophage could be reduced by an increase in inoculation from 0.2 to 1.0 - 1.6 per cent. The authors believed that a heavy inoculation eliminated the spontaneous bacteriophage appearance due to the rapid growth of streptococcus organisms by shortening the lag period of bacterial growth. Also, bacteriophage was reduced by an incubation temperature of 75°F. and the use of freshly heated and cooled milk, containing a minimum of dissolved air. Whitehead and Hunter (48) stated that it was impossible to

suggest why a prolonged lag period favored the appearance of bacteriophage.

Johns (31) stated that an entirely different bacterial strain should be substituted when phaging occurs. Nichols and Wolf(40) found:

1. Identical bacteriophage results were obtained from raw and pasteurized milk.
2. Bacteriophage outbreaks could not be correlated with the heat treatment of milk.
3. A certain bacteriophage race may not attack a certain streptococcus strain but it may affect the organism in the presence of its own homologous strain.

In further studies Nichols and Wolf (41) concluded that active bacteriophage would not usually survive a temperature of 75°C. for 7 1/2 minutes; some bacteriophage were not destroyed at 65° - 67°C. for 50 - 60 minutes; and most bacteriophage survived 70°C. for 10 - 15 minutes.

Whitehead and Cox (45) devised a culture activity test to simulate the cheesemaking process. The relative (not actual) amounts of acidity developed in milk from the same source by several starters were compared. This test involved the following: milk was inoculated with a 10 per cent culture, incubated at 100°F., rennet added, curd cut, and the increase in the whey acidity between 5 1/2 and 6 1/2 hours of incubation was compared. Whitehead and Cox (45) stated that, "a difference of more than 0.1 per cent lactic acid in the final reading can be taken as the true indication of a definite difference between two starters".

Hales (18) described the Spicer culture activity test which is a modification of the Whitehead and Cox test.

Johns and Berard (32) developed a culture activity test which involved the following: sterile skim milk was inoculated with a 1.0 per cent culture, and the samples were incubated at 86°F. for 2 hours and transferred to 102°F. for 4 hours. Acidity titrations were made hourly.

Babel (6) devised a culture activity test using a medium of sterile reconstituted milk and a 1.0 per cent inoculation. The samples were incubated at 86°F. for 8 hours and titrations were made at 2, 4, 6, and 8 hours of incubation. Babel (6) suggested that a curve be plotted to show culture activity.

Horral and Elliker (24) developed a rapid culture activity test which involved the following: reconstituted, non-fat dry milk solids were inoculated with a 3.0 per cent culture, incubated at 37.8°C. for 3 1/2 hours, and titrated.

Anderson and Meanwell (1) described their culture activity test as follows: a 1.0 per cent inoculation was added to 10 ml. of sterile milk, the samples were incubated at 30°C or 37°C. for 6 hours, and titrated.

Leber (36) devised a fast culture activity test using the reduction of resazurin as the end point in determining culture activity.

Golding (17) recommended a culture activity test based on the weighing of 1 gram of the culture and adding it to 100 grams of pasteurized milk, incubating for 6 hours at 86°F. and titrating.

METHODS

A. Propagation and Selection of Cultures.

The cultures used in these studies were obtained from the stock cultures carried at the Oklahoma A. and M. College Dairy Department. The cultures were propagated in fresh, skim milk, or occasionally in pasteurized-homogenized milk, obtained from the mixed herd supply of the Oklahoma A. and M. Dairy Department.

The mother cultures were propagated as follows: approximately 18 ml. of the milk were dispensed into 25 ml. screw-cap test tubes. The test tubes were pasteurized with flowing steam, in an autoclave, at 210°F. for 30 minutes and then cooled to 70°F. The tubes of pasteurized milk were inoculated with 1 drop (0.3 to 0.4 per cent) of mother culture, using a clean, sterile 1 ml. pipette or transfer tube. They were then placed in a thermostatically controlled incubator at 70°F. for 15 to 16 hours. At the end of the incubation period, the cultures were placed in a cold room maintained at 50°F. until the next transfer. The cultures were transferred at least four times weekly and always transferred on the day before trials were run.

The selection of cultures for use in the experimental work herein reported were made at weekly intervals, using the method of Horral and Elliker (25) to determine the rate of acid production of each culture. No observations were made on the flavor and aroma of the cultures.

The batch cultures used in the making of experimental cheese were propagated in the same manner as the mother cultures except that: 400 ml. of the milk were dispensed into 500 ml. Erlenmeyer flasks and the flasks covered with parchment paper fastened with a rubber band; also, a 1 ml. (0.25 per cent) inoculation of mother culture was used.

B. Determinations of Titratable Acidities.

In the investigations herein described, acidity determinations of the milk and whey were made by titrating 9 ml. portions with N/10 NaOH, until the first permanent pink color, using 4 drops of phenolphthalein as the indicator.

The acidity of the ripened cultures were determined by weighing 9 grams of a thoroughly shaken sample and titrating with N/10 NaOH, using phenolphthalein as the indicator.

C. Culture Activity Tests.

1. Babel Test.

The Babel (6) culture activity test given by the author is as follows: skim milk powder is reconstituted with 10 grams of powder per 100 ml. of distilled water and heated in flowing steam for 30 minutes. The reconstituted milk is cooled to 86°F. (30°C.), titrated for acidity and placed in flasks (100 ml. per flask). The 100 ml. of milk is inoculated with 1 per cent of the culture to be tested. Further titrations are made after 2, 4, 6, and 8 hours. No index for culture activity is given by the author.

2. Johns and Berard Test.

The culture activity test recommended by Johns and Berard (32) is as follows: fresh, sterile skim milk samples are

inoculated with 1 per cent of a ripened culture, shaken, incubated in a water bath for the first two hours at 86°F. and the following four hours in a 102°F. incubator. The transfer from the water bath to the incubator takes 1 1/2 hours to reach 102°F.

3. Anderson and Meanwell Test.

The Anderson and Meanwell (1) culture activity test is as follows: sterilized milk is inoculated with 1 per cent of the culture to be tested and duplicate test tubes are incubated for 6 hours at 30° or 37°C. The acidity is determined by titrating with N/9 NaOH using 1 ml. of 0.5 per cent phenolphthalein per 10 ml. as the indicator. No index for culture activity is given by the authors.

4. Horral and Elliker Test.

The medium for the Horral and Elliker (25) culture activity test is reconstituted high grade, spray, non-fat dry milk solids prepared at the rate of 10 per cent of the non-fat dry milk solids in distilled water and sterilized in an autoclave at 15 pounds pressure for ten minutes. Exactly 10 ml. of the sterile milk is pipetted with a clean, sterile 10 ml. pipette, into sterile screw-top test tubes and adjusted to 100°F. (37.8°C.). Each tube is then inoculated with 0.3 ml. (3 per cent) of the culture to be tested and incubated at 100°F. for 3 1/2 hours. Then, 5 ml. of distilled water is used to rinse the tube, and the contents are titrated until the first permanent pink color with N/10 NaOH using phenolphthalein as the indicator. The inoculation is at the same rate of speed so as to insure titration at exactly 3 1/2 hours of incubation for each test tube.

The authors suggest the following culture activity index.

Active acid production . . . . . above 0.35 per cent
Less active acid production. . . between 0.3-0.35 per cent
Little or no acid production . . below 0.3 per cent.

It should be noted that early investigations by Horral and Elliker (24) concluded that starter cultures above 0.4 per cent were active in the cheese vat, providing bacteriophage, overheating in the vat, poor milk quality, or other abnormal factors were elminated.

5. Golding Test.

The Golding (17) culture activity test as described by the author is as follows: 100 grams of freshly pasteurized whole milk is weighed into a clean, sterile, 250 ml. Erlenmeyer flask. The flask is covered with a clean, sterile 50 ml. beaker and placed in a water bath adjusted to 86°F. Then 1 gram of the culture to be tested is weighed into a clean, sterile 125 ml. Erlenmeyer flask. The milk is poured into the culture flask and returned to the larger flask three times. The 250 ml. flask is covered and placed in an 86°F. thermostatically controlled incubator for 6 hours.

At the end of the incubation period, 9 grams of the incubated milk is weighed out into a clean 125 ml. Erlenmeyer flask, and the percentage of acidity is determined by titrating with N/10 NaOH using phenolphthalein as the indicator. A gain of 0.3 per cent indicates an active culture.

6. Whitehead and Cox Test.

The Whitehead and Cox (45) culture activity test simulates the cheesemaking process in miniature. A pint of pasteurized milk is placed into a clean, sterile, large-mouth, quart fruit

jar and covered with a tight-fitting lid. An inoculation of 5 ml. of the culture to be tested is added to each jar. The inoculated milk is shaken and placed into a 100°F. thermostatically controlled water bath, and the level of the water is adjusted to above the level of the milk in the jars. After 1/2 hour, 1 ml. of active rennet is added to each jar and mixed well by inverting two or three times.

In 1 hour, the coagulated curd is cut into 1/4-inch squares with a long, clean, knife. The knife was rinsed in hot water between each jar. After two hours, all the whey is drained off, the curd incubated for two more hours, and all the whey drained again. Whey acidity is then determined by titrating 9 ml. of whey with N/10 NaOH, using phenolphthalein as the indicator. The curds are then incubated for an additional hour and the whey exuded is again titrated. The results are expressed as the per cent acid calculated as lactic acid. Whitehead and Cox (45) state, "a difference of more than 0.1 per cent of lactic acid in the final reading may be taken as a true indication of a definite difference between two starter cultures". Smaller differences can be ignored for they are within experimental error. From the two readings obtained with each sample, the relative (not actual) activities can be obtained.

A brief summary of the Whitehead and Cox test is as follows:

Time	Procedure
Start	Add 5 ml. culture inoculum to a pint of milk and place into 100°F. water bath.
30 minutes	Add 1 ml. active rennet to each jar. Shake.

1 1/2 hours	Cut curd into 1/4" squares.
3 1/2 hours	Drain all whey.
5 1/2 hours	Drain all whey. Titrate for first reading.
6 1/2 hours	Drain all whey. Titrate for final reading.

7. Spicer Activity Test.

The Spicer (18) culture activity test differs from the Whitehead and Cox test in three respects: a 500 ml. portion of pasteurized milk is used; the same amount of whey is withdrawn from each jar; and, a temperature of 86°F. is maintained for the first 2 hours and then 100°F. for 4 hours.

A brief summary of the test is as follows: add 5 ml. of culture to 500 ml. of pasteurized milk; 1 hour later, add 1 ml. of rennet; and cut the curd in 40 minutes. Drain an equal volume of whey from each jar and determine whey acidity at the first, third, fourth, and fifth hours. At the fifth hour, the most active culture produces the most acid.

8. Leber Test

The Leber (36) culture activity test is as follows: 10 grams of high quality non-fat dry milk solids are mixed with 90 ml. of distilled water at about 80°F. and 9 ml. are transferred to an 8 dram vial and immediately placed in a water bath at 98°F. Then, 1 ml. of the culture to be tested is added with a clean, sterile 1 ml. pipette and the skim milk is sucked into the pipette two or three times. Then, 1 ml. of 0.005 per cent resazurin solution is added to the vial, gently mixed, and the time recorded. The samples are observed for reduction

30 minutes after the dye is added and every 5 minutes thereafter. The resazurin is reduced when the original lilac color changes to a very pale pink of white color. The resazurin solution is prepared by adding one tablet of resazurin dye, certified by the Biological Staining Commission, to 200 ml. of gently boiling distilled water. A new dye is prepared every seven days.

Culture activity is as follows:

Excellent culture . if reduced in less than 35 minutes

Good culture. . . . if reduced in between 35 - 50 minutes

Fair culture. . . . if reduced in between 50 - 60 minutes

Poor culture. . . . if reduced in more than 60 minutes.

9. Litmus Milk Tests.

The litmus milk coagulation culture activity test involves the following procedure: 10 grams of high quality non-fat dry milk solids are mixed with 100 ml. of distilled water at room temperature, in a Waring Blender. Sufficient litmus solution is added to give the reconstituted milk a lavender color, and 10 ml. of the reconstituted skim milk is dispensed into clean test tubes, and steam sterilized.

In early studies, the test tubes were inoculated at room temperature but in most of the trials herein described the test tubes are placed in an ice-water bath and inoculated with 1 ml. (10 per cent), 0.5 ml. (5 per cent), 0.25 ml. (2 1/2 per cent), and 0.01 ml. (1 per cent) of the culture to be tested, using a clean sterile 1 ml. pipette calibrated in tenths. Half the test tubes are then tempered to 86°F. and the remainder to 100°F. and then placed respectively into 86°F. and 100°F.

thermostatically controlled water baths. At half-hour intervals, the test tubes are examined for litmus coagulation.

In order to obtain more uniform results and to facilitate handling, some of the culture activity tests were slightly modified so that the standard medium used in most of the culture activity tests herein described was high quality, spray, non-fat dry milk solids reconstituted at room temperature, in a Waring Blender, at the rate of 10 grams of solids plus 100 ml. of distilled water, and sterilized in an autoclave at 212°F., 10 to 15 pounds pressure for 15 minutes. In a few of the concluding trials, the reconstituted milk was steam pasteurized in an autoclave at 212°F. for 30 minutes.

In each instance where this reconstituted milk was not used, mention is made in the presentation of the experimental results.

Further modifications of the culture activity tests were as follows: in the Johns and Berard test, a temperature of 100°F. was maintained for the last four hours of incubation; in the Babel test, 10 ml. of the culture medium were measured into test tubes and inoculated with 1 per cent of the culture to be tested; in the Leber test, the culture was added after the dye was mixed with the milk and incubated at 100°F.; in the Anderson and Meanwell test, duplicate samples were not run, the incubation temperature chosen was 30°F., and 4 drops of a 0.5 per cent phenolphthalein indicator solution were used as the indicator.

D. Cheese Manufacturing Processes.

The types of cheese processed in these investigations were skim milk cheddar, whole milk cheddar, and cottage cheese. The cheddar cheese manufacturing process herein described was essentially the method of Wilson, recommended by Wilster (50), and the cottage cheese manufacturing process herein described was essentially the method advocated by Angevine (3). Plate I is a graphic outline of the Wilson method.

In the experimental work herein reported, small lots (15 pounds) of milk were employed in making cheddar and cottage cheese. The milk and skim milk used were procured from the Oklahoma A. and M. College creamery. For one trial, milk was obtained from a local creamery. One ten-gallon can of milk or skim milk was pasteurized in a Meyer-Blanke Nu-Processor starter pasteurizing vat at 143°- 145°F. for 30 minutes and immediately cooled to 60°F. The can was held overnight in a refrigerated room at 50°F.

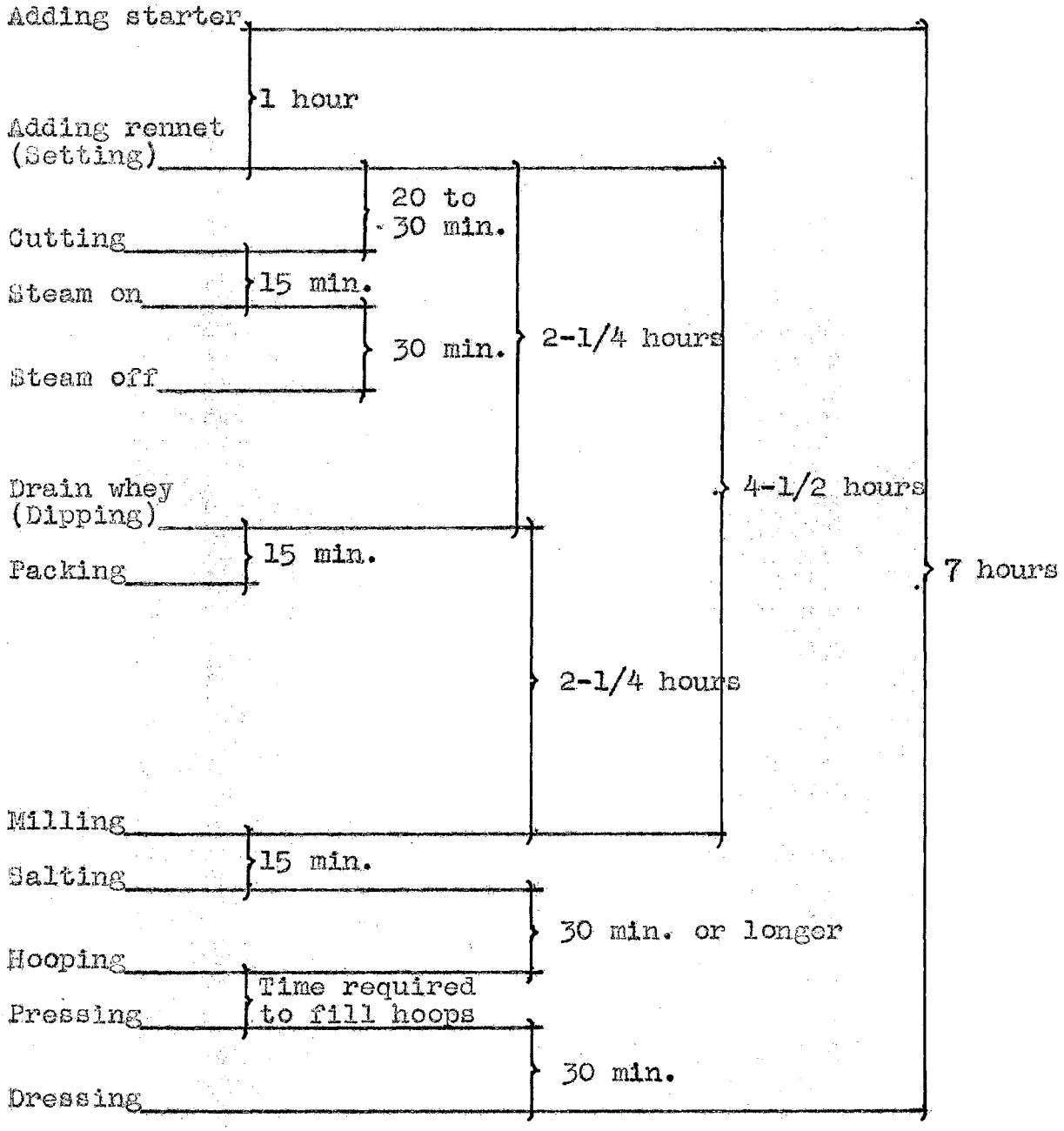
Fifteen pounds of milk were weighed into 12" x 12" x 12", clean, sterile, stainless steel containers and placed into a 60 gallon Angevine cheese vat. The vat was filled with water above the level of the milk in the containers and the temperature of the milk and of the water surrounding the containers was adjusted to 86° - 87°F.

1. Cheddar Cheese.

In making cheddar cheese 0.25 per cent of each culture to be tested was added to each container and stirred vigorously. In one hour, active rennet was added at the rate of 4 1/2 ounces per 1000 pounds of milk and stirred gently.

PLATE I

A Schematic Outline of the Manufacturing Method Used for Cheddar Cheese. [1]



1 G. H. Wilster, Practical Cheddar Cheese Manufacture and Cheese Technology, p. 144.

The coagulated curd was cut in 20 - 30 minutes with clean, sterile 1/4-inch knives. Whey acidity was determined immediately after cutting and at subsequent hourly intervals. After cutting, the curd was stirred gently at frequent intervals to prevent matting and to maintain a uniform temperature.

A cooking temperature of 98°F. was reached in 30 minutes. After cooking for 1 hour, the whey was drained as completely as possible and the curd piled to one side of the container. The drained curd was maintained between 90° - 96°F. throughout the cheddaring process. Acidity determinations were made on the whey at 1/2-hour intervals after the initial draining.

2. Cottage Cheese

The temperature of the milk and of the water surrounding the containers was adjusted to 90°F. Five per cent of the culture to be tested was added to each container and stirred vigorously. In one hour, Angevine coagulator was added at the rate of 25 ml. per 100 gallons of milk and thoroughly mixed with the milk. The curd was cut with 5/8-inch knives when the whey acidity reached approximately 0.5 per cent. Fifteen minutes after cutting cooking was started, and the curd was stirred gently at frequent intervals to prevent matting and to maintain a uniform temperature.

The temperature was then raised from 90°F. to 120°F. in approximately 45 minutes. This cooking temperature was maintained for 15 minutes, and the whey was then drained as completely as possible.

Acidity determinations were made at hourly intervals throughout the cheesemaking process. In the later part of the

cheesemaking process, slow cultures were transferred to another container and maintained at 90°F.

EXPERIMENTAL

A. Preliminary Comparisons of Various Culture Activity Tests.

Several preliminary trials were run with various culture activity tests in order to gain experience in running the tests, to work out a time schedule for the proper operation of each test, and to develop certain modifications which would eliminate some of the variables, especially in the medium used. These modifications of the tests as given by the authors are indicated in the section on methods. In the presentation of these results, small differences in titration values were disregarded as they were likely to be within experimental error.

The Babel, Johns and Berard, Anderson and Meanwell, Horral and Elliker, and Whitehead and Cox tests were employed. The results obtained are presented in Table I.

In Trial 1, the Babel, Johns and Berard, Anderson and Meanwell, and Horral and Elliker tests were compared using ten cultures. The results show that with each test, culture No. 8 was the most active and culture No. 1 was the least active. The results further show that with the Babel test, culture Nos. 4 and 13 were as active as No. 8 and culture No. 3 was as slow as No. 1. It should be noted that the Babel test was only titrated at the eighth hour of incubation, instead of at 2, 4, 6, and 8 hours of incubation, and the Johns and Berard test was only titrated at the sixth hour of incubation.

TABLE I

Preliminary Comparisons of Various Culture Activity Tests

Culture Number	Culture Activity Tests						
	Babel	Johns and Berard	Anderson and Meanwell	Horral and Elliker	Whitehead and Cox		
	Ml. of N/10 NaOH Required to Neutralize the Sample						
	Hours of Incubation						
	6	8	6	6	3½	5½	6½ Diff.
Trial 1 - September 29, 1950							
1		5.5	2.4	2.5	2.4		
2		7.4	6.0	3.7	2.4		
3		5.5	3.4	3.0	3.0		
4		7.5	4.4	4.3	3.3		
8		7.5	6.7	4.5	3.8		
11		5.8	6.2	4.3	3.2		
13		7.5	5.2	3.2	3.4		
17		6.8	4.7	3.8	3.3		
18		7.5	5.5	4.1	3.5		
20		6.0	4.4	3.3	3.2		
Trial 2 - October 3, 1950							
1				2.4	2.5	1.6	2.4 .8
2				2.3	2.4	1.2	1.3 .1
3				2.4	2.5	1.6	2.4 .8
4				2.3	2.3	1.9	2.4 .5
5				2.2	2.4	1.7	2.4 .7
6				2.1	2.2	1.0	1.2 .2
7				1.9	2.2	1.3	1.4 .1
8				1.9	2.2	1.3	1.4 .1
Trial 3 - October 5, 1950							
1	4.1		3.7	2.4	3.1		
2	6.3		4.5	2.8	3.0		
4	4.7		2.6	2.9	3.4		
5	5.8		3.5	2.6	2.6		
6	6.6		4.4	3.0	3.2		
8	6.2		5.1	3.5	3.0		
9	4.6		3.8	2.8	3.5		
10	5.5		5.0	3.6	3.6		
11	5.7		3.6	2.4	2.5		
18	6.7		4.8	3.2	3.8		

TABLE I, Cont'd

Culture Number	Culture Activity Tests							
	Babel		Johns and Berard	Anderson and Meanwell	Horral and Elliker	Whitehead and Cox		
	Ml. of N/10 NaOH Required to Neutralize the Sample							
	Hours of Incubation							
	6	8	6	6	3½	5½	6½	Diff.
Trial 4 - October 6, 1950								
1				3.0	3.4	2.0	2.2	.2
2				3.7	4.2	1.7	2.1	.4
4				3.4	3.7	1.7	1.9	.2
5				3.0	3.9	1.6	1.8	.2
6				3.6	3.9	1.6	1.7	.1
8				4.5	3.8	2.1	2.8	.7
9				3.0	3.3	1.8	1.9	.1
10				3.2	3.9	1.9	2.3	.4
11				3.0	3.5	1.8	1.9	.1
18				4.1	3.8	1.5	2.1	.6

These results show that with the Johns and Berard test there was a much wider range in titration values between the most active and the least active cultures than with the other tests.

In Trial 2, the Anderson and Meanwell, Horral and Elliker, and Whitehead and Cox tests were compared using 8 cultures. The results obtained with all three tests appeared to be in agreement as to the most active and the least active cultures. However, it should be observed that the results obtained with the Anderson and Meanwell and the Horral and Elliker tests are considerably lower than the results obtained in Trial 1, and also that the values obtained were much lower than expected for active cultures. The results of the Whitehead and Cox test indicate that culture Nos. 1, 3, and 5 were active, culture No. 4 appeared to be fairly active, while the remaining cultures appeared to be slow in activity. The results show that with the Whitehead and Cox test there was a much wider range in titration values between the most active and the least active culture than with the other tests.

In Trial 3, the Babel, Johns and Berard, Anderson and Meanwell, and Horral and Elliker tests were compared using ten cultures. The Babel and the Johns and Berard tests were titrated only at the sixth hour of incubation. From the results it appears that the most active culture by the various tests were as follows: Babel, Nos. 6 and 18; Johns and Berard, Nos. 8, 10, and 18; Anderson and Meanwell, Nos. 8 and 10; Horral and Elliker, Nos. 9, 10, and 18. The least active cultures were:

Babel, No. 1; Johns and Berard, No. 4; Anderson and Meanwell, Nos. 1 and 11; and Horral and Elliker, Nos. 5 and 11.

There was only a general agreement between the tests. The results show that with the Babel and Johns and Berard tests there was a much wider range in titration values between the most active and the least active cultures than with the other tests.

In Trial 4, the Anderson and Meanwell, Horral and Elliker, and Whitehead and Cox tests were compared using ten cultures. From these results it appeared that the most active cultures by the three tests were as follows: Anderson and Meanwell, Nos. 8 and 18; Horral and Elliker, Nos. 2, 5, 6, 8, 10, and 18; and Whitehead and Cox, Nos. 8, and 18. The least active cultures were: Anderson and Meanwell, Nos. 1, 5, 9, and 11; Horral and Elliker, Nos. 1, 9, and 11; Whitehead and Cox, Nos. 1, 4, 5, 6, 9, and 11.

From the results in Trial 4 it should be observed that cultures Nos. 8 and 18 appeared to be active, and Nos. 1, 9, and 11 slow by all three tests. It should be noted further that cultures Nos. 5 and 6 appeared to be active by the Horral and Elliker test but slow by the Whitehead and Cox test. The results in Trial 4 indicate that with the Johns and Berard test there was a much wider range in titration values between the most active and the least active culture than with the other tests.

Trials 3 and 4 were run on successive days using the same cultures in each trial. By comparing the results obtained in these two trials it was observed that the rate of acid

production varied from day to day. In the Anderson and Meanwell test, all cultures except No. 10, and in the Horral and Elliker test, all cultures except No. 9 were significantly more active in Trial 4 than in Trial 3. This apparent increase in the rate of acid production by the cultures in Trial 4 may have been due to the fact that the cultures used for inoculation in Trial 4 were ripened more than the cultures used for inoculation in Trial 3.

The results of the preliminary trials seem to indicate that the culture activity test showing the widest range between the most active culture and the least active culture were approximately in the following order: Johns and Berard, Babel (6 hours), Horral and Elliker, Anderson and Meanwell, and Whitehead and Cox. It should be noted that whey was titrated in the Whitehead and Cox test while milk was titrated in the other tests.

B. Comparison of Various Culture Activity Tests.

After running the preliminary trials reported in Section A, a series of twelve trials was undertaken using four or five cultures in each trial to compare the results obtained with the Babel, Johns and Berard, Anderson and Meanwell, Horral and Elliker, Golding, Whitehead and Cox, Spicer, and Leber culture activity tests. In some of the trials, certain tests were omitted. In Trials 1, 2, 3, and 4, cultures that appeared to be active by the Horral and Elliker rapid culture activity test were used while in the remaining trials, an attempt was made to select both active and slow cultures. Also, in the Babel test, in some instances, the titrations were made at the

6-hour interval; and in other instances, the titrations were made at the 6- and 8-hour intervals only. The results obtained are presented in Table II.

In Trial 1, the Babel, Johns and Berard, Anderson and Meanwell, and Horral and Elliker tests were compared using five cultures. The results indicate that with each test, No. 2 was the most active and No. 3 the least active culture. The results also show that with the Anderson and Meanwell test culture No. 5 was as slow as culture No. 3. Except for a slight variation in the Anderson and Meanwell test, the results of each test indicated that cultures Nos. 4 and 5 were approximately equal in activity. There was general agreement between the tests on the activity of the remaining cultures.

It should be noted that there was little difference between the most active and the least active cultures in each of the tests, except with the Horral and Elliker test where a difference of 0.9 ml. was obtained. It should be emphasized that in all of the experimental trials whey was titrated in the Whitehead and Cox test, while milk was titrated in the other tests.

The culture activity tests showing the widest range in titration values were as follows: Horral and Elliker, 0.9 ml.; Johns and Berard, 0.6 ml.; and Anderson and Meanwell, 0.4 ml. The results seem to indicate that with the Horral and Elliker test a significant difference between the most active and the least active cultures was obtained.

In Trial 2, the Babel (6 hours), Johns and Berard, Anderson and Meanwell, and Horral and Elliker tests were compared using five cultures. The results show that with each

TABLE II

A Comparison of Various Culture Activity Tests on Cheese Cultures

Culture Number	Culture Activity Tests																				
	Dabel					Johns and Berard						Anderson and Meanwell	Harral and Hilliker	Golding			Whitehead and Cox			Spicer	Leber
	Ml. of N/10 NaOH Required to Neutralize the Sample																				Rod. in Min.
	Hours of Incubation																				
	0	2	4	6	8	1	2	3	4	5	6	6	3½	0	6	Diff.	5½	6½	Diff.	6	
Trial 1 - October 10, 1950																					
1	1.8	2.3	2.3	4.7		2.1	2.2	2.2	2.4	2.6	2.9	3.0	3.4								
2	1.8	2.4	2.4	5.0		2.2	2.4	2.4	2.6	3.7	3.6	3.3	4.1								
3	1.8	2.2	2.3	4.4		2.2	2.3	2.4	2.6	2.7	2.8	2.9	3.2								
4	1.8	2.3	2.2	4.5		2.2	2.2	2.3	2.6	2.9	3.2	3.2	3.8								
5	1.8	2.2	2.2	4.5		2.1	2.2	2.3	2.6	2.9	3.3	2.9	3.7								
Trial 2 - October 11, 1950																					
1	1.7	1.8	2.1	3.2		1.7	1.8	2.1	2.5	3.4	3.8	2.5	2.5								
2	1.7	1.9	2.7	5.2		1.7	1.7	2.1	2.6	3.1	3.6	2.9	2.6								
3	1.7	1.8	2.3	3.5		1.7	1.8	1.9	2.3	2.7	2.9	2.5	3.0								
11	1.7	1.9	2.7	4.8		1.7	1.9	2.5	2.9	3.2	4.1	2.8	3.0								
13	1.7	2.2	2.4	5.6		1.7	1.9	2.3	3.1	3.3	5.2	3.1	3.1								
Trial 3 - October 12, 1950																					
1	1.7	2.1	3.3	6.6		1.8	2.1	2.5	3.5	4.5	6.0	3.4	3.4								
2	1.7	2.1	3.8	6.9		1.9	2.2	2.7	3.2	5.0	5.2	4.4	3.9								
11	1.7	2.1	3.5	6.7		1.8	2.1	2.9	3.1	4.4	5.4	3.6	3.6								
13	1.7	2.1	3.5	6.9		1.9	2.2	3.0	4.0	4.7	6.4	3.9	4.0								
16	1.7	1.9	2.9	4.8		1.8	2.1	2.4	2.7	3.0	3.9	2.9	3.7								

TABLE II, Cont'd

Culture Number	Culture Activity Tests																				
	Babel					Johns and Berard						Anderson and Meanwell	Horral and Elliker	Golding			Whitehead and Cox			Spicer	Leber
	Ml. of N/10 NaOH Required to Neutralize the Sample																				Red. in Min.
	Hours of Incubation																				
	0	2	4	6	8	1	2	3	4	5	6	6	3½	0	6	Diff.	5½	6½	Diff.	6	
Trial 4 - October 16, 1950																					
1				4.3	7.4						3.9	2.5					1.3	1.9	0.6		
3				3.6	6.8						3.7	2.2					1.1	1.3	0.2		
11				3.3	4.9						3.2	2.2					1.1	1.6	0.5		
14				3.1	4.5						3.0	2.4					1.3	1.4	0.1		
Trial 5 - October 18, 1950																					
1	1.8	1.9	3.0	6.3	7.8	1.9	2.0	2.4	3.0		4.5		3.8	1.8	5.4	3.6	1.7	2.2	0.5		
2	1.8	1.9	2.4	5.4	7.1	1.9	2.0	2.3	3.0		4.6		3.8	1.8	4.9	3.1	2.1	2.5	0.4		
3	1.8	2.0	3.4	6.9	8.0	1.9	2.0	2.1	2.5		3.9		3.2	1.8	3.3	1.5	1.8	2.1	0.3		
13	1.8	2.0	2.9	6.1	7.6	1.9	2.0	2.3	3.1		4.9		4.1	1.8	5.7	3.9	2.0	2.8	0.8		
16	1.8	1.9	2.6	5.7	7.2	1.9	2.0	2.2	2.8		3.8		3.8	1.8	3.9	2.1	2.0	2.7	0.7		
Trial 6 - October 20, 1950																					
1	1.7	1.8	1.8	1.9	2.4	1.8	1.9	2.0	2.8	3.1	3.2		2.6	1.7	4.9	3.2	1.5	1.7	0.2		
2	1.7	1.8	2.1	4.7	6.1	1.8	1.9	2.0	2.8	3.9	4.7		3.2	1.7	5.9	4.2	1.9	2.5	0.6		
3	1.7	2.0	2.6	4.4	7.4	1.9	2.1	2.2	2.5	4.0	4.7		3.6	1.7	6.6	4.9	1.9	2.1	0.2		
13	1.7	2.0	2.7	6.6	7.7	1.8	2.0	2.1	2.4	3.2	4.1		3.6	1.7	6.1	4.4	2.0	2.5	0.5		
16	1.7	1.9	2.5	5.3	7.0	1.8	2.0	2.1	3.2	4.0	4.7		3.3	1.7	5.4	3.7	1.8	2.1	0.3		
Trial 7 - October 21, 1950																					
1				7.5	8.3						4.5		3.7	1.7	6.5	4.8					
2				6.9	7.9						6.0		4.7	1.7	6.1	4.4					
3				7.2	7.9						5.5		5.2	1.7	6.3	4.6					
6				5.8	7.5						3.8		4.7	1.7	6.0	4.3					

TABLE II, Cont'd

Culture Number	Culture Activity Tests																											Leber
	Babel					Johns and Berard							Anderson and Neamell	Horrel and Hillker	Golding				Whitehead and Cox				Spicer					
	Ml. of N/10 NaOH Required to Neutralize the Sample																											Red. in Min.
	Hours of Incubation																											
	0	2	4	6	8	1	2	3	4	5	6	6	3½	0	6	Diff.	5½	6½	Diff.	6								
Trial 8 - October 25, 1950																												
5	1.6	1.9	2.9	5.4	6.8	1.7	1.9	2.4	3.2	3.8	4.4		3.0	1.6	4.5	2.9	1.4	1.9	0.5									
9	1.6	1.9	2.5	5.2	6.5	1.7	1.9	2.2	3.0	3.8	4.7		3.1	1.6	4.4	2.8	1.4	1.9	0.5									
12	1.6	2.0	3.0	6.4	7.8	1.7	1.9	2.1	3.6	4.4	5.3		3.6	1.6	5.5	3.9	1.6	2.2	0.6									
17	1.6	2.0	2.9	6.4	7.7	1.7	2.0	2.5	3.4	4.1	5.1		3.0	1.6	3.2	1.6	1.4	1.5	0.1									
19	1.6	1.9	2.5	5.0	6.2	1.7	1.8	2.0	2.5	3.2	4.1		2.9	1.6	4.3	2.7	1.6	2.2	0.6									
Trial 9 - November 6, 1950																												
10	1.8	2.6	3.7	6.9	8.3	2.1	2.3	2.5	3.1	4.4	5.2		3.9	1.8	7.4	5.6	2.8	3.6	0.8									30
11	1.8	2.0	3.3	6.7	8.1	1.9	2.0	2.6	3.0	4.1	4.3		3.4	1.8	7.2	5.4	1.9	2.2	0.3									30
12	1.8	2.1	3.4	7.7	7.9	1.9	2.1	2.5	3.2	4.2	5.9		4.1	1.8	7.0	5.2	2.0	3.0	1.0									30
18	1.8	2.1	3.0	7.0	8.8	1.9	2.1	2.5	3.3	4.6	5.3		4.2	1.8	7.5	5.7	2.1	3.2	1.1									30
19	1.8	2.0	3.0	6.9	8.5	1.9	2.0	2.4	3.4	3.5	5.1		3.7	1.8	7.4	5.6	2.2	2.7	0.5									30
Trial 10 - November 7, 1950																												
3	1.6	2.0	3.3	6.0	7.6	1.7	1.9	2.3	3.1	3.9	4.0		3.4	1.6	6.2	4.6	1.6	1.8	0.2									23
4	1.6	1.7	3.5	6.3	7.7	1.7	1.9	2.2	3.4	4.1	4.8		3.9	1.6	6.5	4.9	0.9	1.6	0.7									37
5	1.6	1.8	3.3	5.9	7.2	1.7	1.8	2.3	3.0	3.1	3.7		3.3	1.6	5.5	3.9	1.7	2.3	0.6									37
20	1.6	1.8	3.0	5.9	6.9	1.7	1.8	1.9	2.4	2.7	3.5		3.6	1.6	6.4	4.8	1.3	1.6	0.3									55
26	1.6	1.8	2.7	5.6	6.9	1.7	1.8	2.3	2.9	3.7	4.6		3.6	1.6	6.3	4.7	1.7	1.8	0.1									60+

TABLE II, Cont'd

Culture Number	Culture Activity Tests																					
	Babel					Johns and Berard						Anderson and Meanwell	Horrel and Hilliker			Golding			Whitehead and Cox		Spicer	Leber
	Ml. of N/10 NaOH Required to Neutralize Sample																					Red. in Min.
	Hours of Incubation											6	3½	0	6	Diff.	5½	6½	Diff.	6		
	0	2	4	6	8	1	2	3	4	5	6											
Trial 11 - November 9, 1950																						
2	1.5	1.8	3.1	6.2	7.8	1.6	1.8	2.4	3.3	4.9	5.6		3.7	1.5	3.7	2.2						
5	1.5	2.0	2.7	4.9	6.2	1.6	2.1	2.5	2.8	3.0	3.7		3.4	1.5	5.8	4.3						
8	1.5	2.0	3.7	7.5	8.1	1.7	2.1	3.0	4.3	5.3	6.6		4.2	1.5	5.7	4.2						
11	1.5	2.1	3.2	6.6	7.9	1.7	2.0	2.5	3.2	4.2	4.5		3.3	1.5	4.9	3.4						
16	1.5	2.0	3.3	6.9	8.0	1.6	2.1	2.6	3.2	4.2	4.5		3.7	1.5	5.2	3.7						
Trial 12 - November 28, 1950																						
10													3.9								3.8	15
15													3.5								2.5	16
24													3.8								2.8	17
27													3.7								2.7	18
28													3.6								2.6	19

of the tests, No. 13 was the most active while No. 1 was the least active culture, except in the Johns and Berard test which showed that No. 1 was fairly active while No. 3 was the least active culture. Otherwise, the results of the tests generally agreed on culture activity in the range between the most active and the least active culture. The difference in titratable acidity between the most active and the least active cultures for each of the tests was as follows: Babel (6 hours), 2.4 ml.; Johns and Berard, 2.3 ml.; Anderson and Meanwell, 0.6 ml.; and Horral and Elliker, 0.6 ml. These results seem to indicate that with the Babel (6 hours) and the Johns and Berard tests there was the widest range in titration values between the most active and the least active cultures.

In Trial 3, the Babel (6 hours), Johns and Berard, Anderson and Meanwell, and Horral and Elliker tests were compared using five cultures. The results show that with the Babel (6 hours), Johns and Berard, and Horral and Elliker tests No. 13 appeared to be the most active culture while in the Anderson and Meanwell test No. 2 appeared to be the most active culture. It should be pointed out that in the Babel (6 hours) and Horral and Elliker tests culture No. 2 seemed equally as active as culture No. 13. The least active culture was No. 16 in the Babel (6 hours), Johns and Berard, and Anderson and Meanwell tests, while No. 1 was the least active in the Horral and Elliker test. There was general agreement between the tests on the activity of the remaining cultures. The difference in titratable acidity between the most active and the least active cultures for each

of the tests was as follows: Johns and Berard, 2.5 ml.; Babel (6 hours), 2.1 ml.; Anderson and Meanwell, 1.5 ml.; and Horral Elliker, 0.6 ml. These results seem to indicate that with the Johns and Berard test there was the widest range in titration values between the most active and the least active cultures.

In Trial 4, the Babel (6 hours), Johns and Berard, Anderson and Meanwell and Whitehead and Cox tests were compared using four cultures. The results indicate that with each test No. 1 was the most active culture and No. 14 was the least active culture, except the results of the Anderson and Meanwell test showed that Nos. 3 and 11 were the least active cultures. The results indicated that in the Whitehead and Cox test culture No. 11 was also as active as culture No. 1. There was general agreement between tests on the activity of the remaining cultures.

In Trial 4 the low results in the Anderson and Meanwell, Johns and Berard, and Horral and Elliker tests should be noted and compared to the significantly higher titration values obtained with the same cultures in Trial 3.

The difference in titratable acidity between the most active and the least active cultures for each of the tests was as follows: Babel (8 hours), 2.9 ml.; Babel (6 hours), 1.2 ml.; Johns and Berard, 0.9 ml.; Anderson and Meanwell, 0.3 ml.; and Whitehead and Cox, 0.5 ml. These results seem to indicate that with the Babel (8 hours) test there was the widest range in titration values between the most active and the least active cultures. The results in Trial 1 seem to further indicate that the rise in acidity between the 6- and 8-hour incubation periods was greater with the active cultures than with the slow cultures.

At this time, evidence is given that either the 6- or 8-hour incubation period for the Babel test would be a good index to culture activity.

In Trial 5, the Babel (6 and 8 hours), Johns and Berard, Horral and Elliker, Golding, and Whitehead and Cox culture activity tests were compared using five cultures. The results show that with the Johns and Berard, Horral and Elliker, Golding, and Whitehead and Cox tests, No. 13 appeared to be the most active culture, while in the Babel (6 and 8 hours) test, No. 3 appeared to be the most active culture; but in the Johns and Berard, Horral and Elliker, Golding, and Whitehead and Cox tests, No. 3 was the least active culture. In the Johns and Berard test, No. 16 was as slow as No. 3.

From Trial 5, it appears that the activity of the cultures with each test was as follows: Babel (6 hours), Nos. 1, 3, and 13, active and Nos. 3 and 16 fairly active; Johns and Berard, Nos. 1, 2, and 13, active and Nos. 3 and 16 fairly active; Horral and Elliker, Nos. 1, 2, 13, and 16, active, and No. 3 fairly active; Golding, Nos. 1, 2, and 13, active, No. 16, fairly active, and No. 3 slow; Whitehead and Cox, Nos. 13 and 16, active, Nos. 1 and 2 fairly active, and No. 3, slow. Except for the slight variances above, the tests were generally in agreement.

The results in Trial 5 indicate that in the Babel test, the increase in titratable acidity between the sixth and eighth hours of incubation was less for the active cultures and the difference in titration value between the most active and the

least active cultures was greater at the sixth hour of incubation than at the eighth hour of incubation. The difference in titratable acidity between the most active and least active cultures was as follows: Golding, 2.4 ml.; Babel (6 hours), 1.5 ml.; Babel (8 hours), 0.9 ml.; Horral and Elliker, 0.9 ml; Johns and Berard, 0.8 ml.; and Whitehead and Cox, 0.4 ml. These results seem to indicate that with the Golding test there was the widest range in titration values between the most active and the least active cultures.

In Trial 6, the Babel (6 and 8 hours), Johns and Berard, Horral and Elliker, Golding, and the Whitehead and Cox tests were compared using the same five cultures as in Trial 5. From these results it appears that the most active cultures by the culture activity tests were as follows: Babel (6 and 8 hours), No. 13; Johns and Berard, Nos. 2, 3, and 16; Horral and Elliker, Nos. 3 and 13; Golding, Nos. 3 and 13; and Whitehead and Cox, Nos. 2 and 13. The results indicate that with each test, No. 1 was the least active culture. However, it should be noted that in the results of the Whitehead and Cox test, Nos. 3 and 16 were also as slow as culture No. 1. There was only a general agreement among the tests.

It should be further noted that with the Babel test cultures Nos. 3 and 16 showed a pronounced increase in acidity between the sixth and eighth hour titration periods.

The difference in titratable acidity between the most active and the least active culture for each of the culture activity tests was as follows: Babel (8 hours), 5.3 ml.; Babel (6 hours), 3.4 ml.; Golding, 1.7 ml.; Johns and Berard, 1.5 ml.; Horral

and Elliker, 1.0 ml.; and Whitehead and Cox, 0.4 ml. These results indicate that with the Babel (8 hours) test there was the widest range in titration values between the most active and the least active cultures.

In Trial 7, the Babel (6 and 8 hours), Johns and Berard, Horral and Elliker, and Golding tests were compared using four cultures. From the results in Trial 7, it appears that the most active cultures were as follows: Babel (6 and 8 hours), No. 11 Johns and Berard, No. 2; Horral and Elliker, No. 3; and Golding, Nos. 1 and 3. In general, the results of the tests indicated that cultures Nos. 1 and 3 were the most active cultures. The results in Trial 7 further indicate that with each test except the Horral and Elliker test, No. 6 was the least active culture. The results of the Horral and Elliker test indicate that No. 1 was as slow as No. 6. Except for the slight variances above, the tests were in general agreement on the activity of the four cultures analyzed.

The difference in titratable acidity between the most active and the least active cultures for each of the culture activity tests was as follows: Johns and Berard, 2.2 ml.; Babel (6 hours), 1.9 ml.; Horral and Elliker, 1.5 ml.; Babel (8 hours), 0.8 ml.; and Golding, 0.5 ml. The results in Trial 7 indicate that with the Johns and Berard and Babel (6 hours) test there was the widest range between the most active and the least active cultures. The rise in titratable acidity between the sixth and eighth hours of incubation was greater for the slower cultures with the Babel test.

In Trial 8, the Babel (6 and 8 hours), Johns and Berard, Horral and Elliker, Golding, and Whitehead and Cox tests were compared using five cultures. The results indicate that in each test No. 12 was the most active culture and the results also show that in the Babel (6 and 8 hours) No. 17, and in the Whitehead and Cox Nos. 5, 9, and 19 were approximately equal in activity to No. 12. The results further indicate that in each test, except the Whitehead and Cox test, No. 19 was the least active culture. The tests generally agreed in the activity of the remaining cultures.

From Trial 9, it appears that the activity of the cultures with each test was as follows: Babel (6 and 8 hours), Nos. 5, 9, and 19, active, Nos. 12 and 17, fairly active; Johns and Berard, Nos. 12 and 17, active, Nos. 5, 9, and 19, fairly active; Horral and Elliker, No. 12 active, Nos. 5, 9, 17, and 19, fairly active; Golding, No. 12, active, Nos. 5, 9, and 19, fairly active, and No. 17 slow; Whitehead and Cox, Nos. 5, 9, 12 and 19, active, and No. 17, slow.

The differences in titratable acidity between the most active and the least active culture for each of the culture activity tests was as follows: Golding, 2.3 ml.; Babel (8 hours), 1.6 ml.; Babel (6 hours), 1.4 ml.; Johns and Berard, 1.2 ml.; Horral and Elliker, 0.7 ml.; and Whitehead and Cox, 0.5 ml.

The results in Trial 8 indicate that the Golding test showed the widest range between the most active and the least active cultures. It should be noted that the Babel (6 and 8 hours) and the Johns and Berard tests were approximately equal

in showing the widest range between the most active and the least active cultures.

The rise in titratable acidity in the Babel test between the sixth and eighth hours of incubation was approximately the same for the fast and slow cultures.

In Trial 9, the Babel (6 and 8 hours), Johns and Berard, Horral and Elliker, Golding, Whitehead and Cox, and Leber tests were compared using five cultures. The results indicated that with the Babel (8 hours), Golding, and Whitehead and Cox tests, No. 18, and in the Babel (6 hours) and Johns and Berard, No. 12 were the most active cultures. However, it should be noted that in the results of the Johns and Berard test, culture No. 18 was more active than culture No. 12 after the first five hours of incubation but tested lower on the sixth hour of incubation. This discrepancy could have been caused by a rapid increase in activity of culture No. 12 or a slow increase in activity of culture No. 18 between the fifth and sixth hours of incubation.

From Trial 9, it appears that the activity of the cultures with each test was as follows: Babel (6 and 8 hours), Nos. 10, 11, 12, 18, and 19, active; Johns and Berard, Nos. 10, 12, 18, and 19, active, and No. 11, fairly active; Horral and Elliker, Nos. 10, 12, 18, and 19, active, No. 11, fairly active; Golding, Nos. 10, 11, 12, 18, and 19, active; Whitehead and Cox, Nos. 10, 12, and 18, active, Nos. 11 and 19, fairly active. In general, except for slight variations, the tests agreed on culture activity.

In Trial 9, the results of the Leber test indicated that all of the cultures showed excellent activity, in that each culture was reduced in less than 30 minutes. In the Babel test, it is noted that except for the most active culture, the increase in titratable acidity between the sixth and eighth hours of incubation were approximately the same for all the cultures.

It was further noted that the difference in titratable acidity between the most active and the least active culture for each culture activity test, except the Johns and Berard test, showed the narrowest range in Trial 9 of any of the trials to date. These differences in the tests were as follows: Johns and Berard, 1.6 ml.; Babel (6 hours), 1.0 ml.; Babel (8 hours), 0.9 ml.; Horral and Elliker, 0.8 ml.; Whitehead and Cox, 0.8 ml.; and Golding, 0.5 ml. These results indicate that with the Babel and Johns and Berard tests, there was the widest range in titration values between the most active and the least active cultures.

In Trial 10, the Babel (6 and 8 hours), Johns and Berard, Horral and Elliker, Golding, Whitehead and Cox, and Leber tests were compared using five cultures. The results indicate that with each test No. 4 was the most active culture while results of the Babel (8 hours) test showed that No. 3, the Golding test Nos. 20 and 26, and the Whitehead and Cox test, No. 5, to be as active as culture No. 4. The results of the Babel (6 and 8 hours), Whitehead and Cox, and Leber tests indicated No. 26; the Horral and Elliker and Golding tests, No. 5; and the Johns and Berard test No. 20, as the least active cultures.

The results of the tests in the remaining cultures were somewhat in disagreement as to culture activity. This difference might have been due to the narrow range of titratable values between the most active and the least active cultures.

In Trial 10, the difference in titratable acidity between the most active and the least active cultures was as follows: Johns and Berard, 1.3 ml.; Golding, 1.0 ml.; Babel (8 hours), 0.8 ml.; Babel (6 hours), 0.7 ml.; Horral and Elliker, 0.6 ml.; and Whitehead and Cox, 0.6 ml. It should be noted that with the Johns and Berard test there was the widest range between the most active and the least active cultures. Since the Leber activity test was based on culture reduction time in minutes, the difference in the range between the most active and the least active cultures was not compared with the other tests.

It should be noted that in Trial 10 and in succeeding trials, the cultures with the Leber test were examined for reduction after 10 minutes and every 5 minutes thereafter.

In Trial 11, the Babel (6 and 8 hours), Johns and Berard, Horral and Elliker, and Golding tests were compared using five cultures. The results indicated that with each test, No. 8 was the most active culture and except for the Golding test, No. 5 was the least active culture. It should be noted that with the Babel (8 hours) test, cultures Nos. 2, 11, and 16 were approximately as active as No. 8, and in the Golding test, No. 5 was approximately as active as No. 8. However the results further indicate that in the Horral and Elliker test No. 11 and in the Golding test, No. 2 were as inactive as No. 5.

These tests varied slightly in their agreement on the activity of the remaining cultures.

The culture activity tests with the greatest difference in titratable acidity between the most active and the least active cultures were in the following order: Johns and Berard, 2.9 ml.; Golding, 2.1 ml.; Babel (6 hours), 2.0 ml.; Babel (8 hours), 1.9 ml.; and Horral and Elliker, 0.9 ml. These results indicate that with the Johns and Berard and Babel (6 hours) tests there was the widest range between the most active and the least active cultures.

It should be noted that in Trial 11, as in Trials 6, 7, and 10, with the Babel test the increase in acidity between the sixth and eighth hours was greater for the cultures that showed less activity at the sixth hour of incubation. However, in Trial 5, the increase in acidity between the sixth and eighth hours of incubation was greater for the cultures that showed more activity at the sixth hour of incubation.

In Trial 12, the Horral and Elliker, Spicer, and Leber tests were compared using five cultures. The results indicated that with each test No. 1 was the most active culture. However, it should be observed that these three tests indicated that all the cultures were active. There was no significant differentiation between cultures since the range between the most active and the least active cultures was extremely narrow.

A summary of the comparison of various culture activity tests on cheese cultures is presented in Table III.

In order to average the differences in titration values

TABLE III

A Summary of the Comparison of Various Culture Activity Tests on Cheese Cultures

Culture Number	Culture Activity Tests								
	Babel		Johns and Berard	Anderson and Meanwell	Horral and Elliker		Golding	Whitehead and Cox	Leber
	Rank (6 Hr.)	Rank (8 Hr.)	Rank	Rank	Rank	Rating*	Rank Rating**	Rank	Rank Rating***
Trial 1 - October 10, 1950									
1	2		4	3	4	LA			
2	1		1	1	1	A			
3	5		5	5	5	LA			
4	3		3	2	2	A			
5	3		2	4	3	A			
Trial 2 - October 11, 1950									
1	5		3	4	5	S			
2	2		4	2	4	S			
3	4		5	4	2	LA			
11	3		2	3	2	LA			
13	1		1	1	1	LA			
Trial 3 - October 12, 1950									
1	4		2	4	5	LA			
2	1		4	1	2	A			
11	3		3	3	4	A			
13	1		1	2	1	A			
16	5		5	5	3	A			
Trial 4 - October 16, 1950									
1	1	1	1	1				1	
3	2	2	2	3				4	
11	3	3	3	3				2	
14	4	4	4	2				3	

TABLE III, Cont'd

Culture Number	Culture Activity Tests									
	Babel		Johns and Berard	Anderson and Meanwell	Horral and Elliker		Golding		Whitehead and Cox	Leber
	Rank (6 Hr.)	Rank (8 Hr.)	Rank	Rank	Rank	Rating*	Rank	Rating**	Rank	Rank Rating***
					Trial 5 - October 18, 1950					
1	2	2	3		2	A	2	A	4	
2	5	5	2		2	A	3	A	3	
3	1	1	4		5	LA	5	I	5	
13	3	3	1		1	A	1	A	1	
16	4	4	5		2	A	4	I	2	
					Trial 6 - October 20, 1950					
1	5	5	5		5	S	5	A	5	
2	3	4	1		4	LA	3	A	1	
3	4	2	1		1	A	1	A	3	
13	1	1	4		1	A	2	A	1	
16	2	3	1		3	LA	4	A	3	
					Trial 7 - October 21, 1950					
1	1	1	3		4	A	1	A		
2	3	2	1		2	A	3	A		
3	2	2	2		1	A	2	A		
6	4	4	4		2	A	4	A		
					Trial 8 - October 25, 1950					
5	3	3	4		3	LA	2	I	3	
9	4	4	3		2	LA	3	I	3	
12	1	1	1		1	A	1	A	1	
17	1	2	2		3	LA	5	I	5	
19	5	5	5		5	S	4	I	1	

TABLE XII, Cont'd

Culture Number	Culture Activity Tests											
	Babel		Johns and Berard	Anderson and Meanwell	Norral and Elliker		Golding		Whitehead and Cox	Leber		
	Rank (6 Hr.)	Rank (8 Hr.)	Rank	Rank	Rank	Rating*	Rank	Rating**	Rank	Rank	Rating***	
Trial 9 - November 6, 1950												
10	3	3	3		3	A	2	A	1	1	E	
11	4	4	5		5	LA	4	A	5	1	E	
12	1	5	1		1	A	5	A	3	1	E	
18	2	1	2		1	A	1	A	2	1	E	
19	3	2	4		4	A	2	A	4	1	E	
Trial 10 - November 7, 1950												
3	2	2	3		4	LA	4	A	3	1	E	
4	1	1	1		1	A	1	A	5	2	G	
5	3	3	4		5	LA	5	A	1	2	G	
20	3	4	5		2	S	2	A	4	4	F	
26	5	4	2		2	A	3	A	1	5	P	
Trial 11 - November 9, 1950												
2	4	4	2		2	A	5	I				
5	5	5	5		4	LA	1	A				
8	1	1	1		1	A	2	A				
11	2	3	3		5	LA	4	A				
16	3	2	3		2	A	3	A				

* A - Active (over .35%).
LA - Less Active (between .30 - .35%).
S - Slow (under .30%).

**A - Active (gain over .30%).
I - Inactive (gain less .30%).

*** E - Excellent (reduction in less than 35 minutes).
G - Good (reduction between 35-50 minutes).
F - Fair (reduction between 50-60 minutes).
P - Poor (reduction in excess of 60 minutes).

between the most active and the least active cultures reported in Section B, Trials 5, 6, 8, 9, and 10 were selected for evaluation as these trials contained a full comparison of all the various culture activity tests. The results obtained are presented in Table IV.

From these results it should be noted that the average difference in titration values between the most active and the least active cultures with each of the culture activity tests was as follows: Babel(6 hours), 1.6 ml.; Babel (8 hours), 1.9 ml.; Johns and Berard, 1.28 ml.; Horral and Elliker, .8 ml.; Golding, 1.58 ml.; and Whitehead and Cox, .56 ml. It should be emphasized that whey was titrated in the Whitehead and Cox test while milk was titrated in the other tests.

It should be further observed that in Trial 6, the Babel (6 and 8 hours) test showed an abnormal difference between the most active and the least active cultures. This discrepancy was due to the inactivity of culture No. 1 in the Babel test. If the results in Trial 6 were omitted, the average difference in titration values between the most active and the least active cultures with each of the culture activity tests would be as follows: Babel (6 hours), 1.15 ml.; Babel (8 hours), 1.05 ml.; Johns and Berard, 1.22 ml.; Horral and Elliker, .75 ml.; Golding, 1.55 ml.; and Whitehead and Cox, .6 ml.

From the foregoing data, the results of the Golding test showed the widest difference in titration values between the most active and the least active cultures, while the results of the Johns and Berard and Babel tests showed the next widest

TABLE IV

Average Differences in Titration Values
Between the Most Active and the Least Active Cultures
With Various Culture Activity Tests.

Trial	Culture Activity Tests					
	Bebel		Johns and Berard	Horral and Elliker	Golding	Whitehead and Cox
	Hours of Incubation					
	6	8	6	3½	6	6½
5	1.50	0.90	0.80	0.90	2.40	0.50
6	3.40	5.30	1.50	1.00	1.70	0.40
8	1.40	1.60	1.20	0.70	2.30	0.50
9	1.00	0.90	1.60	0.80	0.50	0.80
10	0.70	0.80	1.30	0.60	1.00	0.60
Ave.	1.60	1.90	1.28	0.80	1.58	0.56
Ave. Omitting Trial 6	1.15	1.05	1.22	0.75	1.55	0.60

range and were approximately equal in titration values. The results of the Horral and Elliker test showed a small difference, and the results of the Whitehead and Cox test showed the smallest difference in titration values between the most active and the least active cultures.

C. Comparison of Various Culture Activity Tests With The Rate of Acid Production in Cheesemaking.

Since the results in Sections A and B on various culture activity tests did not contain a standard of comparison, a series of seven trials was initiated in which the rate of acid production in the cheesemaking process was used as a standard to evaluate the efficiency of various culture activity tests. The activity of each culture was judged by the rate of acid produced by it during the cheesemaking process as compared to the rate normally expected.

In four of the seven trials, cheddar cheese was manufactured, and in the remaining three trials cottage cheese was manufactured, as outlined in the section under methods. Small lots (15 pounds) of milk were employed in these studies. An attempt was made to select active, fairly active, and slow cultures for each trial by the Horral and Elliker rapid culture activity test and by the performance of the cultures in preceding trials.

On the day previous to the running of each trial, small lots (400 ml.) of skim milk were prepared by heating in an autoclave to 212°F. with flowing steam for 30 minutes. Usually, nine lots of pasteurized skim milk were inoculated with 0.25 per cent of the various cultures to be tested and incubated at

70°F. This light inoculation rate was used so that after approximately twelve hours of incubation, the partially ripened cultures could be titrated and cultures representing various degrees of activity selected for use. The cultures were left at room temperature, and at the time the cheese milk was inoculated the cultures were again titrated, so as to obtain the acidity of the fully ripened cultures.

1. Cheddar Cheese.

In the manufacture of cheddar cheese, the time schedule as advocated by Wilster (50) was followed regardless of the acidity in the cheese milk. A milling acidity of 0.5 per cent or more six hours from the time the cheese milk was inoculated was considered to be indicative of an active culture. Those cultures producing slightly less than 0.5 per cent acid were considered to be fairly active, and those producing much less than 0.5 per cent acid were considered to be slow or inactive.

The Babel, Johns and Berard, Horral and Elliker, and Leber culture activity tests were compared in four trials, using cultures of various degrees of activity, with the rate of acid produced during the cheddar cheesemaking process. The results obtained in four trials are presented in Table V.

In Trial 1, skim milk was used to make cheddar cheese. The results of the Babel, Johns and Berard, Horral and Elliker, and Leber tests agree with the results in the rate of acid produced in cheesemaking, in that Nos. 22 and 25 were active cultures and Nos. 15 and 27 were slow cultures. The results of the four culture activity tests agreed among themselves.

TABLE V

A Comparison of Various Culture Activity Tests
With the Rate of Acid Production
During the Cheddar Cheesemaking Process.

Culture Number	Culture Activity Tests					Acid Production in Cheese Manufacture	
	Babel		Johns and Berard	Horral and Elliker	Leber		
	Ml. of N/10 NaOH Required to Neutralize the Sample				Red. in Min.	Rank	Rating
	Hours of Incubation						
	6	8	6	3½			
Trial 1 - November 17, 1950							
15	5.6	7.5	3.8	3.1	58	3	Slow
22	7.8	8.9	6.8	5.1	32	1	Active
25	7.7	8.7	6.7	4.6	47	2	Active
27	2.8	5.4	2.7	2.3	60+	4	Slow
Trial 2 - November 22, 1950							
1	5.6	7.7	6.4	4.5		1	Active
16	5.6	7.5	6.1	4.4		5	Slow
22	6.8	7.8	6.5	5.3		2	Active
25	6.6	7.7	6.5	4.4		3	Fairly active
27	3.4	6.1	3.4	2.6		4	Slow
Trial 3 - December 1, 1950							
1	6.2	7.5	6.2	4.3	15	1	Active
6	3.9	5.6	4.0	3.2	24	4	Slow
11	5.9	8.3	5.0	3.6	23	5	Slow
24	4.5	7.3	4.8	3.2	22	3	Slow
25	5.8	7.5	5.8	4.0	11	2	Fairly active
Trial 4 - December 6, 1950							
1	6.4	7.9	6.0	4.6	17	1	Active
6	4.5	6.9	4.0	3.2	18	5	Slow
15	4.0	6.6	3.6	3.0	35	4	Slow
21	5.3	7.3	5.5	4.4	11	2	Active
25	7.1	8.8	6.4	5.1	10	3	Fairly active

It appeared that, of all the tests employed in Trial 1, the Johns and Berard test came closest to agreeing with the results of the rate of acid production in cheesemaking.

In Trial 2, the results of the Babel, Johns and Berard, and Horral and Elliker tests agree with the results in the rate of acid produced in cheesemaking, in that cultures Nos. 1 and 22 were active, No. 25, fairly active, and No. 27, slow; however, they differ from the rate of acid production, in that culture No. 16 was active with all of the tests and slow in the rate of acid produced in cheesemaking. The results of the activity tests generally agreed among themselves. From the results in Trial 2, it appears that the Johns and Berard test and then the Babel test gave the best index of culture activity.

In Trial 3, the results of the Babel, Johns and Berard, and Horral and Elliker tests agree with the results of the rate of acid produced in cheesemaking, in that culture No. 1 was active, No. 25, fairly active, and No. 6, slow; however, they differ in that with the Babel and Johns and Berard tests cultures Nos. 11 and 24 were fairly active but were slow in the rate of acid production in cheesemaking. The results of the Horral and Elliker test agree with the results of the rate of acid produced in cheesemaking, in that cultures Nos. 1 and 25 were active cultures; however, they differ in that cultures Nos. 6, 11, and 24 were fairly active with the Horral and Elliker test but slow in the rate of acid produced in cheesemaking. The results of the Leber test indicate that all the cultures were active, whereas only two of the five cultures

were active in the rate of acid produced in cheesemaking. The results of the activity tests generally agreed among themselves.

From the results obtained in Trial 3, it appears that the Johns and Berard and then the Babel test gave the best index of culture activity.

In Trial 4, the results of the Babel and Johns and Berard tests agree with results of the rate of acid production in cheesemaking, in that cultures Nos. 1 and 21 were active and cultures Nos. 6 and 15 were slow; however, they differ in that culture No. 25 was very active with the Babel and Johns and Berard tests, as well as the Horral and Elliker and Leber tests, but only fairly active in the rate of acid production in cheesemaking. The results of the Horral and Elliker and Leber tests agree with the Babel and Johns and Berard tests and with the rate of acid production in cheesemaking, in that cultures Nos. 1 and 21 were active, but differ in that the rate of acid production in cheesemaking indicated that cultures Nos. 6 and 15 were slow and culture No. 25, fairly active. The results of the Horral and Elliker and Leber tests show that cultures Nos. 6 and 15 were fairly active and culture No. 25 was very active.

From the results obtained in Trials 1 through 4, it appears that the Johns and Berard test gave the best index to culture activity.

2. Cottage Cheese.

The cottage cheese manufacturing process was essentially the method recommended by Angevine (3). The curd was cut when the acidity of the whey was 0.5 per cent. At the 4 1/2 or 5

hour interval, when the lots containing fast cultures were cut and before cooking was started, the lots containing slow cultures were transferred to another vat of water for further incubation at 90°F. Titrations were made at hourly intervals.

The Babel, Johns and Berard, Horral and Elliker, and Leber culture activity tests were compared in three trials using cultures of varying degrees of activity with the rate of acid produced during the cottage cheesemaking process. The results obtained are presented in Table VI.

In Trial 1, the results of the Babel, Johns and Berard, Horral and Elliker, and Leber tests agree with the results in the rate of acid produced in cheesemaking, in that culture No. 24 was active, but differ in that in each of the tests the results indicate that culture No. 28 was slow, whereas the rate of acid produced in cheesemaking indicated that it was active. The results of the Babel and Johns and Berard tests agree with the rate of acid produced in cheesemaking, in that culture No. 15 was slow; however, the results of the Horral and Elliker test shows culture No. 15 to be fairly active and the Leber test shows culture No. 15 to be active. The results of each test indicate that culture No. 10 was active, whereas the rate of acid produced in cheesemaking showed it to be fairly active. It should be observed that the largest discrepancy in Trial 1 was with culture No. 28, for the results of the Horral and Elliker test showed it to be slow, the Babel and Johns and Berard, fairly active, and the acid produced in cheesemaking showed it to be active. The tests agree among themselves, and,

TABLE VI

A Comparison of Various Culture Activity Tests
With the Rate of Acid Production
During the Cottage Cheesemaking Process.

Culture Number	Culture Activity Tests					Acid Production in Cheese Manufacture	
	Babel	Johns and Berard	Horral and Elliker	Leber			
	Ml. of N/10 NaOH Required to Neutralize the Sample				Red. in Min.		
	Hours of Incubation					Rank	Rating
	6	8	6	3½			
Trial 1 - November 15, 1950							
10	7.1	7.8	6.0	4.4	23	3	Fairly active
15	4.7	6.9	4.1	3.2	24	4	Slow
24	7.0	7.6	5.6	3.9	23	1	Active
28	4.7	6.8	5.0	2.7	40	2	Active
Trial 2 - December 13, 1950							
1	7.2	8.2	6.1	3.6	20	1	Active
11	7.1	8.1	4.6	3.8	23	4	Slow
15	6.1	7.7	4.3	3.0	39	5	Slow
24	7.5	8.3	6.7	3.5	21	3	Active
25	7.8	8.5	7.1	3.2	20	2	Active
Trial 3 - December 15, 1950							
1	6.5	7.4	6.1	4.1	20	1	Active
15	5.9	7.2	4.9	3.2	29	4	Slow
16	5.5	6.0	4.5	3.5	36	5	Slow
24	7.0	7.5	6.6	4.4	21	3	Active
25	7.5	7.9	7.0	4.3	18	2	Active

except for culture No. 28, they generally agree with the rate of acid produced in cheesemaking. From the results obtained in Trial 1, it appears that the Babel and Johns and Berard tests gave the best index to culture activity.

In Trial 2, the results of the Babel, Johns and Berard, Horral and Elliker, and Leber tests agree with the results in the rate of acid produced in cheesemaking in that cultures Nos. 1, 24, and 25 were active, but differ in that cultures Nos. 11 and 15 were slow in the rate of acid produced in cheesemaking and active by the Babel and Leber and fairly active by the Johns and Berard tests. From Trial 2, it appears that the culture activity tests generally agreed among themselves, and that the Johns and Berard test gave the best index of culture activity.

In Trial 3, the Babel and Johns and Berard tests agree with the rate of acid produced in cheesemaking, in that cultures Nos. 1, 24, and 25 were active and cultures Nos. 15 and 16 were slow. The Horral and Elliker and Leber tests also agreed with the rate of acid produced in cheesemaking, in that cultures Nos. 1, 24, and 25 were active, but differed in that the results of the tests indicated that cultures Nos. 15 and 16 were active, whereas they were slow in the rate of acid produced in cheesemaking.

From the results obtained in Trials 1, 2, and 3, it appears that the Johns and Berard and Babel tests gave the best index of culture activity.

D. Relationship of the Acidity of Ripened Cultures to the Rate of Acid Production in Cheesemaking.

In commercial manufacturing practices, occasionally a ripened culture containing a high per cent of titratable acidity fails to develop acid at a normal rate, and occasionally a ripened culture containing a low per cent of titratable acidity develops somewhat faster than a culture containing a high per cent of titratable acidity.

A comparison was made in a series of six trials with four or five cultures in each trial to determine the relationship of the acidity of ripened cultures to culture activity and to the rate of acid produced during the cheesemaking process. The results obtained are presented in Table VII.

In Trial 1, the results generally agree that the two cultures showing the highest, and the two cultures showing the lowest, per cent of titratable acidity in the ripened cultures became the two most active and the two least active cultures in the culture activity tests and in the rate of acid produced in cheesemaking.

In Trial 2, the results appeared to show general agreement in that those cultures which had a high titratable acidity in the ripened culture showed a high degree of activity in the culture activity tests and in the rate of acid produced in cheesemaking.

In Trial 3, the results did not agree with Trials 1 and 2. Although cultures Nos. 25 and 11 indicated a high titratable acidity in the ripened culture, culture No. 25 showed more activity in the culture activity tests, and significantly more activity in the rate of acid produced in cheesemaking than No. 11. The per cent of titratable acidity in the ripened culture appeared to be an excellent indication of culture activity and

TABLE VII

A Comparison of the Acidity of Ripened Cultures to Culture Activity and the Rate of Acid Production in Cheesemaking.

Culture Number	Ripened Culture		Culture Activity Rank	Acid Production in Cheese Rank
	% Titratable Acidity	Rank		
	Trial 1 - November 17, 1950			
15	.38	3	3	3
22	.75	2	1	1
25	.82	1	2	2
27	.34	4	4	4
	Trial 2 - November 22, 1950			
1	.84	1	1	1
16	.70	4	4	5
22	.79	3	2	2
25	.83	2	3	3
27	.60	5	5	4
	Trial 3 - December 1, 1950			
1	.68	3	1	1
6	.54	4	5	4
11	.80	2	3	5
24	.52	5	4	3
25	.83	1	2	2
	Trial 4 - December 6, 1950			
1	.85	2	3	1
6	.55	5	4	5
15	.58	4	5	4
21	.80	3	2	2
25	.91	1	1	3
	Trial 5 - December 13, 1950			
1	.75	3	3	1
11	.79	2	4	4
15	.68	5	5	5
24	.71	4	2	3
25	.80	1	1	2
	Trial 6 - December 15, 1950			
1	.76	3	3	1
15	.70	5	4	4
16	.72	4	5	5
24	.82	2	2	3
25	.84	1	1	2

* Average rank of all tests run on date of trial.

the rate of acid produced in cheesemaking with cultures Nos. 6 and 25.

In Trial 4, the two ripened cultures containing a low per cent of titratable acidity ranked low in the culture activity tests and in the acid produced during cheesemaking. It should be noted, however, that the ripened culture containing the highest per cent of titratable acidity was the most active in the culture activity tests and one of the slowest acid producers during cheesemaking.

In Trial 5, the results appeared to show general agreement in that those cultures showing a high titratable acidity in the ripened culture usually showed a high degree of activity in the culture activity tests and in the rate of acid produced during the cheesemaking process. However, it should be observed that whereas culture No. 1 ranked third in the per cent of titratable acidity and in the results of the culture activity tests, it placed highest of the five cultures in the rate of acid produced during the cheesemaking process. Also, whereas culture No. 11 ranked second in the per cent of titratable acidity, it placed fourth in the ranking of culture activity, and fourth in the rate of acid produced during the cheesemaking process.

In Trial 6, except for culture No.1, the results appeared to show general agreement, in that those cultures showing a high titratable acidity in the ripened culture usually showed a high degree of activity in the culture activity tests and in the rate of acid produced during the cheesemaking process.

The results in Trials 1 through 6 appear to indicate that the per cent of titratable acidity in the ripened culture was indicative of culture activity and the rate of acid produced in the cheesemaking process. A high titratable acidity in the ripened culture generally indicated an active culture according to the culture activity tests and by the rate of acid produced in cheesemaking. However, the culture with the highest titratable acidity in the ripened culture did not always produce the most active culture according to the culture activity tests or by the rate of acid produced in cheesemaking.

E. The Use of Litmus Milk as a Culture Activity Test.

Since litmus milk is commonly used in dairy bacteriology to determine the rate of reduction and coagulation of various organisms, and since milk coagulates within a narrow pH range, it was thought that litmus milk might be used as a simple and accurate test to determine culture activity. With this thought in mind, a study was undertaken to determine the relationship of the rate of litmus milk coagulation to the rate of acid production in cheesemaking by various cultures.

The litmus milk was prepared and the test run as outlined in the section under methods. Inoculations of 1, 2 1/2, 5, and 10 per cent of each culture to be tested were run. Since the data indicated that the 1 per cent and 10 per cent inoculation rates appeared to show little correlation between the coagulation of litmus milk and the rate of acid produced in cheesemaking the data was omitted.

The relationship between the rate of litmus milk coagulation and the rate of acid production in cheesemaking using 2 1/2 and 5 per cent (86°F. and 100°F.) inoculations are presented in Table VIII.

In Trials 1 and 2, inoculations were made at room temperature, but in Trials 3, 4, and 5, the test tubes of milk were iced before and during inoculation in order to prevent bacterial growth before tempering to the desired incubation temperature.

In Trials 1, 2, 4, and 5, there was a close agreement between the rate of litmus milk coagulation using either 2 1/2 or 5 per cent inoculation at 100°F. with the rate of acid production in cheesemaking, while at 86°F. there was only a fairly close agreement.

In Trial 3, there was only a general agreement in inoculation temperatures and rates of inoculation between the rate of litmus milk coagulation and the rate of acid production in cheesemaking.

TABLE VIII

A Comparison of the Litmus Milk Coagulation Test to the Rate of Acid Production in Cheesemaking.

Culture Number	Hours Required to Coagulate Litmus Milk				Acid Production in Cheese Manufacture	
	Inoculation					
	5 Per Cent		2½ Per Cent			
	Incubation Temperature					
	86°F.	100°F.	86°F.	100°F.	Rank	Rating
Trial 1 - November 17, 1950						
15	4	7½+		7½	3	Slow
22	4	4	4	5	1	Active
25	4	5	4½	6	2	Active
27	5	7½+		7½+	4	Slow
Trial 2 - November 22, 1950						
1	3½	3½	4½	4	1	Active
16	4½	5	6½	7½	5	Slow
22	3½	3½	5	4½	2	Active
25	4	4	4½	5½	3	Fairly active
27	5½	5½	6½	7½	4	Slow
Trial 3 - December 1, 1950						
1	4	3½	4½	4½	1	Active
6	4	6	6	7	4	Slow
11	4	4	4½	5½	5	Slow
24	4½	5	5	6	3	Slow
25	4	4	5	6½	2	Fairly active
Trial 4 - December 6, 1950						
1	4	3½	4½	5	1	Active
6	6½	4½	6½	6½	5	Slow
15	7	6	7	7+	4	Slow
21	4½	3½	5	5	2	Active
25	4	3	4½	4½	3	Fairly active
Trial 5 - December 15, 1950						
1	4½	4	5	5	1	Active
15	4½	5	5½	6	4	Slow
16	4	5	5	6½	5	Slow
24	4	4	5	5	3	Active
25	3½	4½	4½	5½	2	Active

CONCLUSIONS

1. The Johns and Berard and Babel tests appeared to be more accurate than the Anderson and Meanwell, Horral and Elliker, Whitehead and Cox, Golding, and Leber culture activity tests in determining culture activity.

2. The Johns and Berard test was slightly more accurate than the Babel test.

3. The Babel test required less equipment and work than the Johns and Berard test.

4. The Johns and Berard and Babel tests can be simplified by titrating only at the sixth hour of incubation.

5. The Horral and Elliker test involved little work, is rapid, but only fairly accurate in determining culture activity.

6. The Anderson and Meanwell test was generally accurate in determining culture activity.

7. The Whitehead and Cox and Golding tests appeared to be accurate in determining culture activity but both involved considerable time and effort to perform.

8. The Leber test gave the most rapid results of all the tests and was easy to perform; however, it gave the poorest index of culture activity of all the tests compared.

9. An activity test which involves the inoculation of sterile litmus milk with either 2 1/2 or 5 per cent of the culture to be tested, incubated at 100°F., and observed regularly for coagulation proved to be an easily performed, rapid, and fairly accurate test for culture activity.

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THESIS TITLE: A Comparison of Various Culture Activity Tests for Determining the Rate of Lactic Acid Production by Cheese Cultures

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