RESPONSES OF LACTIC CULTURES TO VARIOUS FRACTIONS AND TO HEAT TREATMENTS OF MILK

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1962

Submitted to the faculty of the Graduate School of
the Oklahoma State University
in partial fulfillment of the requirements
for the degree of
MASTER OF SCIENCE
May, 1963

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AND TO HEAT TREATMENTS OF MILK

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ACKNOWLEDGEMENTS

The author wishes to express his sincere appreciation to Dr. Harold C. Olson for his patient guidance and counsel during the course of this study and the preparation of this thesis. Appreciation is also expressed to other members of the Dairy Department for their advice and encouragement during this study.

Appreciation is expressed to Dr. Robert D. Morrison for his advice in the statistical analysis of the data.

The author wishes to express his appreciation to his wife, Blanche, and son, Stanley, Jr., for their patience and encouragement during this study.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

The rate of acid production by lactic starter cultures is very important in the dairy industry. Rapid acid production is essential in the manufacture of cheese in order to produce a high quality product and to lessen the chance of interference from bacteriophage. It is also desirable to have rapid acid production in the manufacture of other fermented milk products. Much research has been reported on factors affecting the rate of acid production by lactic cultures, such as the influence of stimulants, ripening time, incubation temperature, heat treatment of milk, and others. Several researchers have studied the effects of various heat treatments of milk, but the reports are somewhat conflicting as to the optimum heat treatment.

Very few reports have been published on the influence of the different milk fractions on the rate of acid production by lactic cultures. Additional research is needed to determine the stimulatory or inhibitory action of the milk fractions in order to modify milk to provide the best medium possible for growth of lactic cultures.

The objectives of the research reported herein were to study: (1) the effects of heat treatment of milk on the rate of acid production by lactic cultures and (2) the effects of various milk fractions on the rate of acid production by lactic cultures.

REVIEW OF LITERATURE

Effects of Heat Treatments of Milk on Culture Activity

Milk to be used for the propagation of lactic starter cultures must be heated to destroy most of the competing organisms, bacteriophage, and any heat labile growth inhibitors which might be present. Lactenins or growth inhibitors normally present in fresh, raw milk are inhibitory to many bacteria but they are destroyed by heat (4, 5, 8, 14, 20, 29, 30).

Compounds containing free sulfhydryl groups are produced in milk by certain temperature exposures (7, 12, 13, 14). These compounds, which contain the free sulfhydryl groups, are inhibitory to lactic starter cultures (13, 14).

There are conflicting reports as to which temperature exposures are best for milk to be used for the growth of lactic starter cultures. Ransom (25) recommended heating the milk to 190°F and holding it at that temperature for 20 to 30 minutes.

Babel (6) compared the ratio of acid production (amount of acid produced by lactic culture in a given time) of two cultures in 5 lots of milk heated at 145°, 160°, and 180°F for 30 minutes, respectively, and at 249°F for 15 and for 25 minutes, respectively. Unheated milk was also tested. He reported that the highest rate of acid production

occurred in the milk which had been heated at 249°F for 15 minutes. He found a variation in the response of the 2 cultures to the heat treatments. He reported that the raw milk was the poorest medium. The quality of the heated milk did not increase with each increase in heat exposure. There appeared to be certain zones of stimulation and inhibition of the activity of the cultures.

Foster (9) compared heat treatments of 239°F for 15 minutes (in the autoclave) and 176°F for 10 minutes of milk for lactic cultures. He used a 1% inoculation and incubated the cultures at 86°F or at 98.6°F, depending on their optima, using two strains of Streptococcus lactis. The cultures were titrated for acidity after 21 and 45 hours of incubation. He reported more acid production by the lactic cultures in the milk which had been heated at 239°F for 15 minutes than in that heated at 176°F for 10 minutes. He suggested three reasons why strong heating might have improved the milk as a lactic culture medium: (1) reduction of oxidation-reduction potential; (2) inactivation of a very heat resistant inhibitor; or (3) change in certain of the milk constituents to a form more readily available to the culture.

Green and Jezeski (13) reported a "stimulation-inhibition-stimulation-inhibition" cycle of the growth of lactic cultures in milk heated at various temperatures. The first zone of stimulation of the cultures was induced by a heat exposure of 143°F for 30 minutes to 161°F for 40 minutes. This zone

was followed by an inhibition zone induced by the heat exposure of 161°F for 40 minutes to 180°F for 10 minutes or 194°F for 1 to 45 minutes. The second zone of stimulation was observed in milk which had been heated at 194°F for 60 to 180 minutes or at 248°F for 15 to 30 minutes. The final zone of inhibition was observed in milk which had been heated in the autoclave (250°F) for a period greater than 30 minutes. They studied the effects of heat on the different milk proteins. The temperatures which produced the inhibition zones in milk for lactic cultures had little effect on casein but had a profound effect on the serum proteins. The serum proteins contain cysteine, which contains sulfhydryl (-SH) groups, while casein does not. Pure cysteine added to the milk before heating increased the intensity of the stimulation and inhibition cycle.

Green and Jezeski (14) gave the following explanations for their cycle of stimulation and inhibition of lactic cultures grown in milk which had been heated at various temperatures. The initial or first zone of stimulation was due to a variety of factors: (A) lowering of Eh, due to oxygen expulsion; (B) destruction of heat-labile inhibitors which are found in raw milk; (C) partial hydrolysis of protein and denaturing of serum proteins. The first zone of inhibition was due to an excess concentration of cysteine with an increase in toxic volatile sulfides. The final zone of inhibition was due to the production of more cysteine and volatile toxic sulfides due to the extreme heat denaturation

of the serum proteins.

Other workers have reported the production of sulfhydryl group-containing compounds in heated milk (7, 12). Gould (12) found that the production of free sulfhydryl groups in heated milk started at temperatures of 168.8°F to 172.4°F and became more intense as the temperature neared the boiling point of milk. The formation of sulfhydryl compounds was usually accompanied by the evolution of hydrogen sulfide.

Dill et al. (7) reported that sulfhydryl compounds were produced in skimmilk which had been heated by direct steam injection. They found peaks in the production of free sulfhydryl groups from skimmilk at temperature exposures of 220°F for 150 seconds, 260°F for 20 seconds, and 300°F for 2 seconds.

Hammer and Babel (15) suggested that the variations in acid production by starter cultures following different heat exposures was due to the effect of heat on the germicidal property of milk. They stated that a minimum heat exposure of 180°F for 30 minutes was desirable for milk intended for lactic starter cultures.

Other workers have reported on the presence and the characteristics of the bactericidal properties of milk (5, 20, 29, 30, 31). Auclair and Hirsch (5) reported the presence of two inhibitory substances in raw milk--lactenin-1 and lactenin-2. The pH and heat stabilities of the two lactenins varied. Lactenin-1 was destroyed by a heat exposure of 158°F for 20 minutes at pH 7.0. Lactenin-2

was destroyed completely by a heat exposure of 165.2°F for 20 minutes at pH 6.5 or 7.5. Lactenin-1 is a component of colostrum and lactenin-2 is a component of normal milk. A heat exposure of 136.4°F for 20 minutes left both the lactenins intact.

Jones and Little (20) reported that the natural inhibitor in raw milk originated in the udder of the cow. It was not destroyed by heating the milk at 143.6°F for 20 minutes, but it was completely inactivated when exposed to temperatures of 149°F or 158°F for 20 minutes. It deteriorates slowly when the milk is stored at 43°F.

Wilson and Rosenblum (30, 31) reported that lactenin was present in the whey fraction of milk. It was inactivated by temperature exposures of 176°F or higher, but survived the normal pasteurization treatment of milk. They suggest that lactenin might be an enzyme. Hemolytic streptococci were found to vary in their susceptibility to lactenin, some being sensitive and others resistant. Lactenin was found to be reversibly deactivated by sulfur-containing reducing compounds such as cysteine, glutathione, and thioglycolic acid. Excess amounts of thiamine inactivated the lactenin. They suggested that lactenin might have denied thiamine to the lactenin sensitive cells.

Stadhouders and Veringa (29) report that the inhibition of lactic streptococci and the prevention of inhibition of lactic streptococci by cysteine were related. Cysteine acted as an inhibitor in the presence of hydrogen

peroxide but formed a compound with milk peroxidase, in the absence of hydrogen peroxide, which was irreversible. Peroxidase sensitive variants of lactic streptococci, probably had an absolute requirement for free cysteine but the cysteine was complexed with peroxidase. Elliot (8) reported that milk peroxidase was inhibited by the presence of very small amounts of hydrogen sulfide. Hydrogen sulfide is produced along with the formation of free sulfhydryl groups in heated milk (12).

Auclair (4) reported that lactenin found in normal milk was destroyed by hydrogen peroxide. Cysteine or other free sulfhydryl containing compounds only inhibited the lactenin and did not destroy it.

Effect of Milk Fractions on Culture Activity

There has been little work reported on the use of various fractions of milk as media for the growth of lactic starter cultures. Lightbody (21) used churned buttermilk in a comparison with skimmilk as a medium for the growth of lactic cultures. She reported that acid production was higher in the skimmilk than in the buttermilk, but that the final pH was about the same. Acid production in undiluted buttermilk was about the same as in skimmilk. The production of diacetyl was higher in the buttermilk. She concluded that buttermilk offered no great advantage as a medium for lactic starter cultures, but it could be used without having any harmful effects on the cultures.

Gillies (11) found strains of Streptococcus cremoris which were susceptible to inhibition by whole milk. These strains were more active when grown in the skimmilk fraction. The cream fraction was inhibitory to all strains of S. cremoris which were tested. This inhibition could be demonstrated by adding cream to skimmilk and the amount of inhibition depended on the amount of cream used.

Milk contains all or nearly all the substances necessary for optimum growth and acid production by lactic starter cultures, but not in forms that are readily available (10, 27). Stimulation of slow lactic cultures presumably occurs with the addition of any source of utilizable nitrogen, including protein breakdown products of faster cultures. Garvie and Mabbitt (10) showed that acid production by slow lactic cultures could be stimulated by the addition of peptides from casein hydrolyzed by acid or by enzyme.

Anderson and Elliker (2) reported that trypsinized skimmilk or peptonized milk, when added to reconstituted skimmilk in low concentrations, produced greater stimulation of lactic cultures than did the addition of individual amino acids. The trypsinized or peptonized milk contained many peptides. Anderson, Parker and Elliker (3) concluded that the peptide content of milk appeared to exert a greater effect on starter culture activity than did the protein content of milk. MacLeod and Gordon (22) also reported that certain peptides could be used by lactic

starter cultures.

The fat globule membrane of normal cow's milk contains a distinct type of protein, which does not appear to be related to casein and other milk proteins on a structural basis (16). It appears to contain all the essential amino acids.

Herald and Brunner (17) stated that the amino acids found in the fat globule membrane were: alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, serine, threonine, tryptophane, tyrosine, and valine. In addition to these Hare et al. (16) reported the presence of phenylalanine, proline, and glycine. They suggested that the variations in amino acid composition reported on the fat globule membrane might be due to the removal of various amounts of absorbed protein during the preparation of the membrane. They also reported that the fat globule membrane protein contained more arginine, phenylalanine, threonine, and glycine and less lysine, tryptophane, leucine, isoleucine and aspartic acid than any of the major proteins of milk.

Sandine, Speck and Aurand (26) found two stimulants for Lactobacillus casei and Streptococcus lactis in an extract from pancreas tissue. One of these stimulants was isolated and characterized as a peptide. This peptide contained lysine, aspartic acid, serine, glycine, glutamic acid, threonine, alanine, proline, valine, and probably both leucine and isoleucine.

Herald, Brunner and Bass (18) reported that the fat globule membrane protein contains proportionately more aluminum, calcium, copper, iron, magnesium, manganese, molybdenum, phosphorus, silver and zinc than did whole milk.

EXPERIMENTAL METHODS

Propagation of Cultures

The cultures used in the experiments reported herein were from the collection at Oklahoma State University. The cultures were originally from various commercial sources and contained the usual lactic acid producing organisms (*Streptococcus lactis* or *Streptococcus cremoris*) and *Leuconostoc* species. The stock cultures were maintained by adding two drops (about 1%) of culture to approximately 10 ml of sterile litmus milk contained in screw capped test tubes. Approximately 0.25 grams of calcium carbonate were added to each tube of litmus milk before sterilizing to partially neutralize the lactic acid produced. The inoculated tubes were immediately stored at 45°F and transferred at intervals of 3 to 4 weeks.

Before use in an experiment, the stock cultures were removed from storage, allowed to ripen 6 to 8 hours at room temperature (about 80°F) and then propagated in sterile litmus milk in stoppered test tubes. An inoculation of approximately 1% was used. After ripening at 72°F for 16 hours, the cultures were used immediately or stored at 45°F until used. Cultures that appeared to be very slow were not used in the experiments.

Reconstituted Products

Spray dried non-fat dry milk solids and spray dried buttermilk used in the experiments were reconstituted at the rate of 10 parts powder to 100 parts distilled water. This gave reconstituted products containing about 9.1% solids-not-fat. The reconstituted buttermilk and skimmilk were filtered to remove any undissolved powder.

Preparation of Milk Fractions

In each trial the various fractions tested came from the same lot of milk. The milk used was mixed herd milk from the Oklahoma State University dairy herd or from a local milk processing plant. Each lot of milk used was checked for antibiotics or other growth inhibitors, using the disc assay method with Bacillus subtilis as the test organism (20). Only milk free of growth inhibitors was used.

After a sample of whole milk was collected in an Erlenmeyer flask, the remainder of the milk was heated to 90-100°F and separated, using a small, electric, farm-type separator. The skimmilk was reseparated to remove as much of the fat as possible. The separator was disassembled, the bowl drained and the separator slime removed from inside the bowl with a spatula and placed in a screw capped vial.

Washed cream was prepared by diluting a portion of the fresh, uncooled cream with warm (about 100°F) tap water to about the original volume of the milk and reseparating. This process was repeated 3 more times, rinsing the separator

between each washing with warm water.

The cream portions were cooled immediately in an ice water bath to 40°F, held 2 hours, then churned using a one-gallon hand churn. The buttermilk and washed cream buttermilk were filtered through filter paper to remove small butter granules. In some trials the buttermilk was also centrifuged in a Babcock centrifuge for 10 minutes and the fatty material removed with an aspirator.

Buttermilk whey and skimmilk whey were prepared from reconstituted products. Rennet extract was added to the reconstituted skimmilk and buttermilk, warmed to about 100°F, at the rate of about 500 ppm. After coagulation the whey was filtered through filter paper and heated to 140°F to destroy the coagulating enzyme and then refiltered.

The washed cream buttermilk, the skimmilk whey and the buttermilk whey were all condensed to a solids content of more than 9%. This was accomplished by placing the material in a two liter balloon flask connected to a water jacketed condenser. The balloon flask containing the whey or buttermilk was placed in a hot water bath, an aspirator was connected and a vacuum of about 26 inches of mercury was drawn and maintained throughout the condensing process. The temperature of the water bath was never allowed to go above 135°F.

Normally the milk fractions were used within 12 hours from the time of obtaining the lot of milk to be tested. Each fraction was placed in a glass container and immediately placed in ice water (about 40°F) and held cold until used.

If the milk fractions were not to be used within 12 hours they were held in frozen storage.

The solids-not-fat level of each fraction was adjusted to about 9% with distilled water. This was done in order that combinations of the milk fractions with reconstituted or fresh skimmilk could be made and the resultant products would all have approximately the same solids-not-fat content as the skimmilk.

In the trials involving both raw and heated fractions, bacterial contamination and growth were kept at a minimum by using sterile equipment and by immediately cooling each fraction after it was obtained to about 40°F and holding cold until used.

All milk and milk fraction combinations were dispensed in measured 9 ml quantities into sterile, rubber stoppered test tubes before heating.

Heat Treatments

The tubes of milk or milk fractions exposed to heat treatments of less than 200°F were placed in a hot water bath (190-210°F), allowed to come up to the desired temperature, then placed in another water bath adjusted at that temperature and held for the desired holding time. Milk or milk fractions receiving heat exposures of 210°F were heated and held in the autoclave in free flowing steam without pressure. The media receiving heat exposures of 250°F were heated in the autoclave at 15 P.S.I. for the desired holding

time. All media were cooled immediately after heating in an ice water bath and held at 40°F until used.

Rate of Acid Production

The prepared tubes of milk and milk fractions were removed from the ice water bath and each inoculated with about 1% (2 drops) of active lactic culture. They were then tempered in a water bath to 72°F and incubated at this temperature in a thermostatically controlled incubator. After 8 hours of incubation the tubes were examined every 30 minutes for evidence of coagulation. When 2 or 3 tubes of a set inoculated with the same culture showed signs of coagulation, the entire set was removed and placed in an ice water bath to prevent further acid production. Since the rate of acid production decreases with decrease in pH, it was essential that the cultures be titrated for acidity soon after coagulation of the fastest cultures. Because of differences in activity among the cultures, the incubation periods usually ranged from 10 to 12 hours. No fixed period of incubation could be used because the faster cultures would be over-ripened and the slower cultures would be under-ripened. Differences in acidity which are apparent when the fastest culture first coagulates become increasingly smaller as ripening progresses until all cultures reach the same level of acidity. This would represent the pH level which inhibits further acid development.

The entire contents of each tube were titrated with 0.1N

NaOH, using phenolphthalein as the indicator. The results were expressed as the ml of 0.1N NaOH required to neutralize to a light pink end point.

Statistical Analysis

An analysis of variance procedure was used to test for interaction between cultures and treatments (23). For this analysis the error degrees of freedom were equal to trial x culture ≠ trial x treatment ≠ trial x culture x treatment.

To compare the rate of acid production in each of the milk fractions with that in skimmilk or reconstituted skimmilk, an analysis was used to compare each treatment mean (average titration value of each milk fraction) with the control mean (average titration value of skimmilk). Dunnett's procedure (28) for comparing all means with a control was used. The mean titration values were considered as a set of p treatments plus a control. Error degrees of freedom was obtained by multiplying culture degrees of freedom by treatment degrees of freedom. Dunnett's t value was extracted from a table of t values for one-sided comparisons between p treatment means and a control. The significant difference (d') was calculated from the following formula:

$$d' = (\text{Dunnett's t}) \times S_{\bar{d}}, \text{ where } S_{\bar{d}} = \sqrt{\frac{2(\text{Error Mean Square})}{\text{No. of observations}}}$$

The difference between the control mean titration value and each of the milk fractions mean titration value was compared to the significance difference (d') as a measure of significance.

Duncan's new multiple-range test (28) was used to analyze the differences between mean titration values of cultures grown in milk exposed to various heat treatments. Significant studentized ranges (SSR) were extracted from a table of studentized ranges for the new multiple range test. The SSR were each multiplied by $S_{\bar{x}} = \sqrt{\frac{\text{Error mean square}}{\text{No. of observations}}}$ to obtain least significant ranges (LSR). The mean titration values were ranked from lowest to highest and all possible differences were determined; these were then compared to the appropriate LSR as a measure of significance.

RESULTS AND DISCUSSION

Effects of Heat Treatments of Milk

Skimmilk, skimmilk plus whole milk, and reconstituted non-fat milk were used as media to compare the effects of heat treatments of milk on the rates of acid production by lactic cultures.

Test for Interaction. In order to test for interaction between the cultures and the heat treatments, two replicate trials were conducted involving twelve cultures and five heat treatments. The heat exposures used were 143°F, 160°F, 180°F, and 210°F for 30 minutes and 250°F for 15 minutes. The analysis of the results is shown in Table I.

TABLE I

TEST FOR INTERACTION BETWEEN CULTURES AND HEAT TREATMENTS

Source	d.f.	S.S.	M.S.	F*
Total	119	107.91	.90	
Lots of Milk	1	.43	.43	
Cultures	11	11.84	1.07	1.52
Treatments	4	10.32	2.58	3.68
Treat. x Cultures	44	43.78	.99	1.41
Error	59	41.54	.70	

*F value required for treatments alone= 3.65 at the 1% level.
*F value required for significance of treatment x culture interaction= 1.36 at the 10% level.

These results indicate that there was some interaction between the heat treatments and the cultures but this interaction was significant only at the 10% level. On the other hand the effects of the heat treatments alone were highly significant ($P < .01$).

Skimmilk. Two trials were conducted in which the rates of acid production by lactic cultures were compared in lots of skimmilk which had been heated at 143°F, 180°F, and 210°F for 30 minutes and at 250°F for 15 minutes. Ten observations were made with 8 cultures. A summary of the results is presented in Table II.

These results show some differences in rates of acid production due to the heat treatments. The rate of acid production was highest in the skimmilk which had been heated at 143°F, second highest in that heated at 250°F, third highest in that heated at 180°F, and lowest in that heated at 210°F. The difference in the rates of acid production in skimmilk heated at 143°F and in that heated at 210°F was significant ($P < .05$), while the differences among the remaining lots of milk were not significant at the 5% level.

Based on the results from the above preliminary trials two additional trials were run using heat treatments of 143°F, 160°F, 180°F, and 210°F for 30 minutes, respectively, and of 250°F for 15 minutes. Twenty-eight observations with 15 cultures were made in these 2 trials. A summary of the results is given in Table III.

TABLE II

RATES OF ACID PRODUCTION BY LACTIC CULTURES IN SKIMMILK SUBJECTED TO VARIOUS HEAT EXPOSURES (143°F, 180°F, 210°F, and 250°F)

10 observations with 8 cultures in 2 trials				
Heat Treatment	Mean Titration Values[1] ml	Differences between means	Significance LSR[2]	P < .05
210°F for 30 min.	5.02	143°-210° = .75	.58	/
180°F for 30 min.	5.37	143°-180° = .40	.56	-
250°F for 15 min.	5.54	143°-250° = .23	.54	-
143°F for 30 min.	5.77	250°-210° = .52	.56	-
		250°-180° = .17	.54	-
		180°-210° = .35	.54	-

[1]Ranked from lowest to highest
[2]Least significant range = difference required for significance

TABLE III

RATES OF ACID PRODUCTION BY LACTIC CULTURES IN SKIMMILK SUBJECTED TO VARIOUS HEAT EXPOSURES (143°F, 160°F, 180°F, 210°F, and 250°F)

28 observations with 15 cultures in 2 trials

Heat Treatment	Mean Titration Values[1] ml	Differences between means	Significance LSR[2]	P < .05	LSR[2]	P < .01
210°F for 30 min.	6.03	160°-210° = 0.86	.47	≠	.60	≠
180°F for 30 min.	6.18	160°-180° = 0.71	.46	≠	.59	≠
143°F for 30 min.	6.40	160°-143° = 0.49	.44	≠	.57	-
250°F for 15 min.	6.50	160°-250° = 0.39	.42	-	.55	-
160°F for 30 min.	6.89	250°-210° = 0.47	.46	≠	.59	-
		250°-180° = 0.32	.44	-	.57	-
		250°-143° = 0.10	.42	-	.55	-
		143°-210° = 0.37	.44	-	.57	-
		143°-180° = 0.22	.42	-	.55	-
		180°-210° = 0.15	.42	-	.55	-

[1]Ranked from lowest to highest
[2]Least significant range = difference required for significance

The rate of acid production was highest in the milk heated at 160°F followed by that in milk heated at 250°F, 143°F, 180°F, and 210°F, respectively. The differences in the rates of acid production in milk heated at 160°F compared to that in milk heated at 180°F and in that heated at 210°F were highly significant ($P < .01$). The rates of acid production were significantly higher in milk heated at 160°F compared to that in milk heated at 143°F and in milk heated at 210°F ($P < .05$). The differences in rates of acid production in the milk receiving the other heat treatments were not significant ($P > .05$).

Since a wide range of heat exposures are used for heat treatments of milk for the propagation and use of lactic cultures in various cultured products, a trial was conducted to determine the influence of such treatments on the rates of acid production. Portions of a lot of skimmilk were heated at 143°F, 160°F, 180°F, and 200°F for exposure periods of 0, 5, 10, 20, and 40 minutes. Additional lots of skimmilk were heated to 150°F, 170°F, 190°F, and 210°F with no holding time. Four cultures were used in this trial. The results are shown in Figures 1 and 2.

Figure 1, which depicts the rates of acid production in skimmilk heated to various temperatures with no holding time, indicates that the rate of acid production increased as the heat treatment increased up to 180°F and then decreased with further increase in the heat treatment. The increase in the rates of acid production in the milk heated

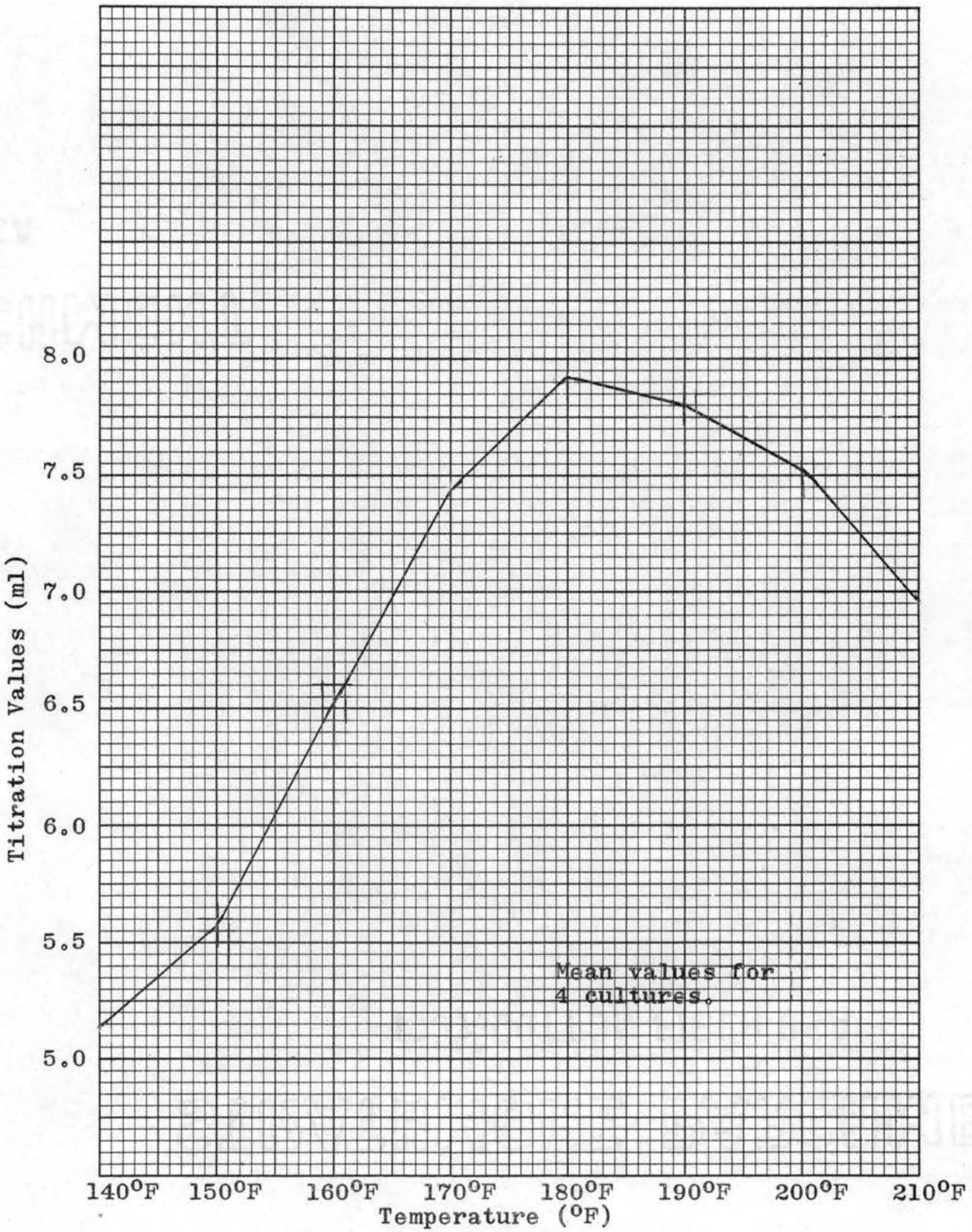

Figure 1. Rate of acid production by lactic cultures in skimmilk subjected to various heat exposures. (No Holding Time)

up to 180°F and the decrease in that heated to temperatures greater than 180°F was similar to responses of lactic cultures to heat reported by Green and Jezeski (14). They attributed the initial increase in the rates of acid production to the destruction of the bactericidal property native to milk, and to the partial heat denaturation of milk proteins. The subsequent decrease was attributed to the production of toxic sulfhydryl-containing compounds in the milk which had received the more severe heat treatments.

Figure 2 shows that the rates of acid production in skimmilk heated at 140°F were lower at all exposure times than those in the skimmilk receiving the other heat treatments. This was to be expected since the bactericidal property native to milk is not destroyed at this temperature (29, 30). The rates of acid production in the skimmilk heated at 160°F increased with each increase in exposure time. This appears to be largely due to the destruction of the bactericidal property of the milk, since it is destroyed by these heat exposures (29). The high rate of acid production which occurred in skimmilk heated at 180°F was essentially the same in the lots held for 0, 5 and 10 minutes and then declined with the lots held for 20 and for 40 minutes, respectively. The decline in the rate of acid production was probably due to toxic compounds induced by the heating. The rate of acid production in the skimmilk heated at 200°F was much less in the lot held for 5 minutes and considerably less in that held for

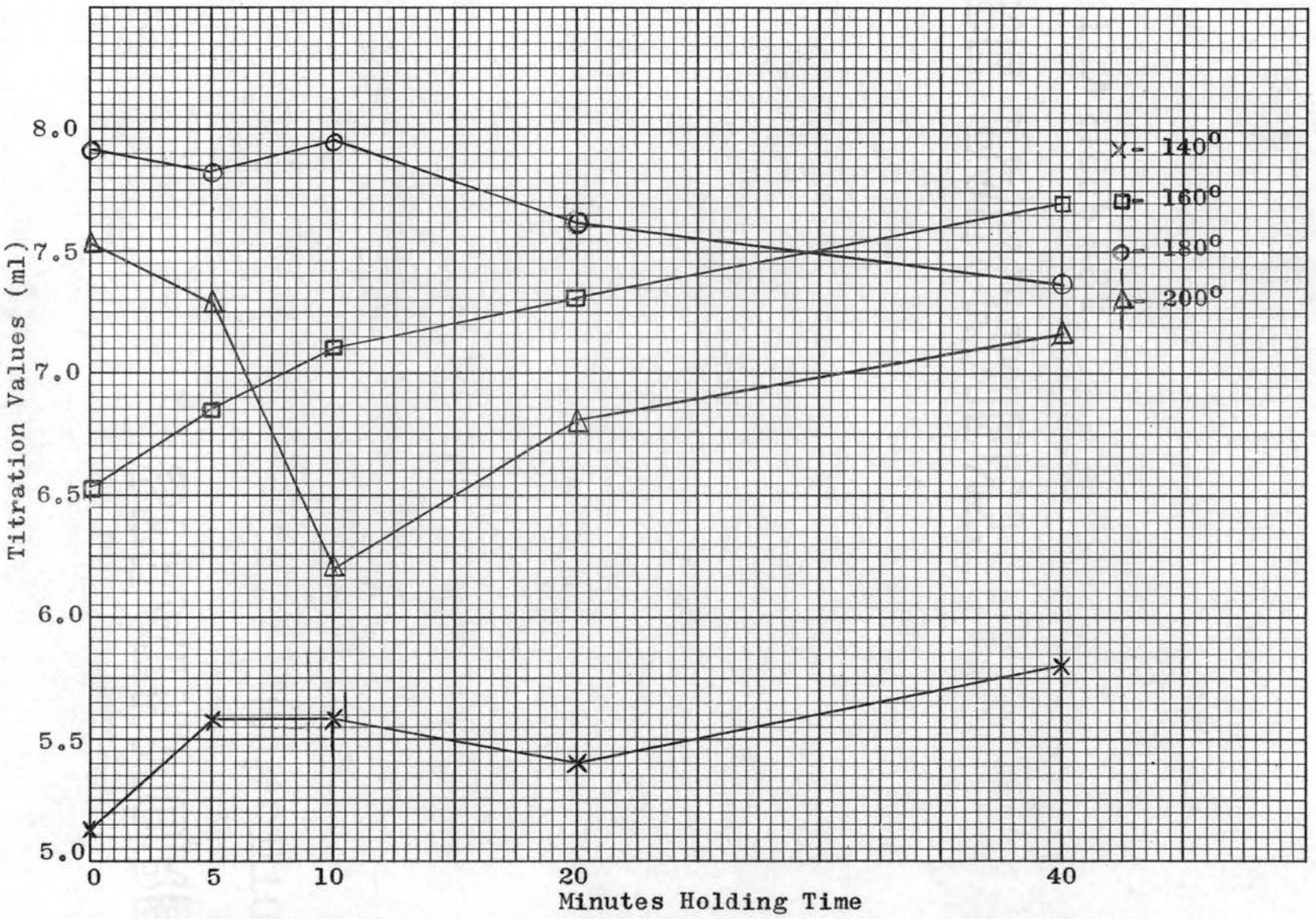

Figure 2. Rates of acid production by lactic cultures in skimmilk subjected to various heat exposures.

10 minutes than in the lot heated to 200°F with zero holding time. The sharp decrease in the rate of acid production was most likely due to production of toxic compounds in the heated milk and the subsequent increase was most likely due to the expulsion or destruction of the toxic compounds. These results appear to support the theory (14) that the toxic materials produced by certain heat exposures are partially destroyed or expelled by longer exposures at the same temperature.

Skimmilk Plus Whole Milk. Many dairy processing plants use a mixture of skimmilk and whole milk for cultured buttermilk. Two trials were conducted to determine what effects various heat treatments of the skimmilk and whole milk mixture would have on the rates of acid production by lactic cultures. Five cultures were used in each trial involving heat treatments of 160°F, 180°F, and 200°F for 30 minutes and 250°F for 15 minutes. A summary of the results are presented in Table IV.

The mean rate of acid production was highest in the milk heated at 250°F followed by that in the milk heated at 200°F, 160°F, and 180°F respectively. All differences among the mean titration values were highly significant ($P < .01$). Although the rate of acid production was highest in the lots heated at 200°F and at 250°F, the intense cooked flavor, the brown coloration, and the weak body resulting from such high heat treatments would be very detrimental to the quality of the finished buttermilk.

TABLE IV

RATES OF ACID PRODUCTION BY LACTIC CULTURES IN A MIXTURE OF SKIMMILK AND WHOLE MILK SUBJECTED TO VARIOUS HEAT EXPOSURES

10 observations with 10 cultures in 2 trials				
	Mean Titration Values[1] ml	Differences between means	Significance LSR[2]	Significance P< .01
180°F for 30 min.	5.35	250°-180° = 2.22	.56	≠
160°F for 30 min.	5.88	250°-160° = 1.69	.55	≠
200°F for 30 min.	6.55	250°-200° = 1.02	.53	≠
250°F for 15 min.	7.57	200°-180° = 1.20	.55	≠
		200°-160° = .67	.53	≠
		160°-180° = .53	.53	≠

[1]Ranked from low to high
[2]Least Significant Range = difference required for significance

A heat treatment of 160°F for 30 minutes would result in a finished product having a weaker body than that of milk heated at 180°F for 30 minutes; however, the amount of undesirably cooked flavor would be less in the milk receiving the 160°F treatment. From these results it can be concluded that a pasteurization temperature and exposure in the range of 160°F to 180°F for 30 minutes should be used to produce high quality cultured buttermilk.

Reconstituted Non-fat Milk. A trial involving 15 cultures was conducted to show the effects of heat treatments on reconstituted non-fat milk. The rates of acid production were compared in lots of the milk heated at 160°F, 180°F, and 210°F for 30 minutes and at 250°F for 15 minutes. A summary of the results is given in Table V.

The results show that the rate of acid production in the milk heated at 160°F was significantly lower ($P < .01$) than that in the milk heated at any of the other temperatures. These results are in almost direct contrast to those obtained with fresh skimmilk. This discrepancy appears to be due to the fact that the non-fat dry milk was subjected to considerable heat treatment incident to the drying process and the additional heat given the reconstituted non-fat milk was sufficient to produce the compounds which were toxic to lactic cultures at exposures as low as 160°F for 30 minutes. The additional heat exposure of 210°F for 30 minutes was apparently sufficient to expell or destroy at least part of the toxic compounds.

TABLE V

RATES OF ACID PRODUCTION BY LACTIC CULTURES IN RECONSTITUTED NON-FAT MILK SUBJECTED TO VARIOUS HEAT EXPOSURES

1 trial with 15 cultures						
	Mean Titration Values[1] ml	Differences between means	Significance			
			LSR[2]	P < .05	LSR[2]	P < .01
160°F for 30 min.	6.22	210°-160° = 0.84	.28	≠	.37	≠
250°F for 15 min.	6.86	210°-250° = 0.20	.27	-	.36	-
180°F for 30 min.	6.91	210°-180° = 0.15	.26	-	.34	-
210°F for 30 min.	7.06	180°-160° = 0.69	.27	≠	.36	≠
		180°-250° = 0.05	.26	-	.34	-
		250°-160° = 0.64	.26	≠	.34	≠

[1]Ranked from low to high
[2]LSR = Least significance range = difference required for significance

Variations in Responses of Cultures. In the analysis to test for interaction between cultures and heat treatments, there was some interaction observed ($P < .10$). Also, it was observed that certain cultures seemed to produce acid much more slowly in milk pasteurized at 140°F to 143°F than in milk from the same lots pasteurized at 160°F. Conversely, a few cultures seemed to be rather resistant to the bactericidal property in milk pasteurized at 143°F. Among the 20 different cultures used in the various heat treatment trials, six cultures were selected for further study. Group 1 included three cultures that produced acid rather slowly in milk pasteurized at 140°F to 143°F, but showed rapid acid production in milk pasteurized at 160°F. Group 2 included three cultures that produced acid rapidly in milk pasteurized at 140°F to 143°F and at 160°F. Three trials were conducted in which portions of lots of skimmilk were pasteurized at 143°F in a vat or in the laboratory and portions of the same lots were pasteurized in flowing steam (210°F). Exposure times were 30 minutes. These lots were then used in an activity test which simulated the setting of milk for cheese. Briefly, the test involved inoculating measured 9 ml quantities of the milk contained in rubber stoppered test tubes with 0.5 ml of lactic culture, tempering to 90°F, incubating at this temperature for 4 hours and then titrating with 0.1N NaOH, using phenolphthalein as the indicator. The results were expressed as the ml of 0.1N NaOH required to neutralize the entire contents of each tube.

With Group 1, the mean titration values were 5.63 ml and 6.92 ml, for the lots pasteurized at 143°F and at 210°F, respectively, while the corresponding values for Group 2 were 7.06 ml and 6.40 ml, respectively. The difference in the mean titration values for Group 1 were highly significant ($P < .01$). The difference in the rates of acid production in Group 2 was also significant ($P < .05$). The results show that the two groups had contrasting responses to the heat treatments. If the 6 cultures had been tested for activity in milk which had been pasteurized at 210°F only, those in Group 1 would have been selected as being the most active, but it is obvious that their rates of acid production in cheese milk would be rather low, whereas those in Group 2 would perform very satisfactorily. It was of interest to note that the cultures in Group 1 were obtained from cultured buttermilk while those in Group 2 were characterized as cheese cultures.

While the above data were obtained on cultures that represented the extremes in responses to heat treatments, they emphasize the importance of testing the activity of cheese cultures in milk pasteurized at temperatures used for pasteurizing cheese milk.

Effects of Milk Fractions

Many stimulants have been used to improve the rate of acid production by lactic cultures. Most of them, however, are materials foreign to milk and adding them to milk might be considered adulteration. In the research reported herein, the rates of acid production by lactic cultures in various fractions of milk were compared to those in skimmilk. All milk and milk fractions except the raw portions were pasteurized at 210°F for 30 minutes.

Skimmilk, whole milk and buttermilk. Two analyses were made to test for interaction between cultures and treatments (milk fractions). The first test involved 2 replicate trials with 7 cultures. In these trials the rate of acid production in skimmilk was compared to those in whole milk and in buttermilk. The buttermilk was used alone and in mixtures with skimmilk in proportions of 3-1, 1-1, 1-3, and 1-7 (75%, 50%, 25% and 12.5% buttermilk). The results are presented in Table VI.

The results of this analysis show that there was no significant interaction between cultures and treatments ($P > .05$). The effects of treatments were highly significant ($P < .01$).

In the second analysis 2 replicate trials were conducted using 8 cultures. In these trials the rate of acid production in skimmilk was compared to those in whole milk, buttermilk and mixtures of buttermilk with skimmilk

TABLE VI

TEST FOR INTERACTION BETWEEN CULTURES AND MILK FRACTIONS
(Skimmilk, whole milk, and 12.5% to 100% buttermilk)

Source	df	S.S.	M.S.	F*
Total	97	89.34	.92	
Trials	1	28.71	28.71	
Cultures	6	6.00	1.00	2.17
Treatments	6	26.80	4.46	9.69
Treatment x Culture	36	5.60	.15	.32
Error	48	22.23	.46	

*F value required for treatments alone = 3.2 at the 1% level.
*F value required for treatment x culture interaction = 1.66 at the 5% level.

TABLE VII

TEST FOR INTERACTION BETWEEN CULTURES AND MILK FRACTIONS
(Skimmilk, whole milk, and 2.5% to 40% buttermilk)

Source	df	S.D.	M.S.	F*
Total	175	259.05	1.48	
Trials	1	0.9	0.9	
Cultures	7	10.7	1.52	0.93
Treatments	10	90.5	9.05	5.52
Treatment x Culture	70	13.5	0.19	0.12
Error	87	143.45	1.64	

*F value required for treatments alone = 2.54 at the 1% level.
*F value required for treatment x culture interaction = 1.43 at the 5% level.

(buttermilk concentrations of 40%, 20%, 10%, 5% and 2.5% were used). The results of this analysis are presented in Table VII.

These results were in close agreement with those obtained in the first analysis in that there was no significant interaction between cultures and treatment ($P > .05$) and the effects of the treatments were highly significant ($P < .01$).

Since the above results showed that there was no significant interaction between cultures and milk fractions, three additional trials were conducted. These trials involved different lots of milk and 18 observations were made with 12 cultures. The mean rates of acid production in whole milk and buttermilk were compared to that in skimmilk. The buttermilk was used alone and in mixtures with skimmilk (50%, 25% and 12.5% buttermilk were used). A summary of the results obtained is given in Table VIII.

The highest rates of acid production occurred in the mixtures containing 25 and 50% buttermilk. The rate of acid production in whole milk was slightly higher than that in skimmilk, but this difference was not significant ($P > .05$). Significantly higher rates of acid production occurred in all concentrations of buttermilk than in the skimmilk ($P < .01$).

Based on the results obtained in the above trials two more trials, involving 23 observations with 16 cultures using lower concentrations of buttermilk, were conducted.

TABLE VIII

RATES OF ACID PRODUCTION BY LACTIC CULTURES
IN SKIMMILK, WHOLE MILK, AND BUTTERMILK
(12.5% to 100% Buttermilk)

18 observations with 12 cultures in 3 trials				
	Mean Titration Values ml	d'[1]	Significance[2] P <.05	P <.01
Skimmilk	4.02			
Whole Milk	4.67	0.65	-	-
100% Buttermilk	5.58	1.56	/	/
50% Buttermilk	6.22	2.20	/	/
25% Buttermilk	6.21	2.19	/	/
12.5% Buttermilk	5.92	1.90	/	/

[1] d' = mean titration value minus mean titration value for skimmilk.
[2] d' values of 0.71 and 0.90 were required for significance at the 5% and 1% levels, respectively.

Buttermilk was mixed with skimmilk to obtain concentrations of 40%, 20%, 10%, 5% and 2.5% buttermilk. The rate of acid production in whole milk was also determined. A summary of the results is presented in Table IX.

The rates in the whole milk and in all concentrations of buttermilk were significantly higher than that in the skimmilk ($P < .01$). The highest rate occurred in the mixture of 40% buttermilk and 60% skimmilk. The rates of acid production in the remaining mixtures decreased as the buttermilk concentration decreased, except that there was no difference in the mean titration values obtained in the mixtures containing 2.5% and 5% buttermilk. The mean rate of acid production in the whole milk was slightly higher than those in the latter mixtures.

In order to determine if the apparent stimulatory effects of whole milk and of buttermilk were due to a lessening of the inhibitory effect due to heat treatment of the milk, a trial involving 9 cultures was conducted, using both heated and raw products. Skimmilk was combined with buttermilk to obtain concentrations of 75%, 50%, 25% and 12.5% buttermilk. The heated and raw products were treated exactly the same, except for the heat treatment. A summary of the results is presented in Table X.

The results with the heated products, in general, confirm those in the previous trials in that the rates of acid production in the whole milk, buttermilk and the mixtures of buttermilk and skimmilk were significantly

TABLE IX

RATES OF ACID PRODUCTION BY LACTIC CULTURES IN SKIMMILK, WHOLE MILK, AND BUTTERMILK (2.5% to 40% buttermilk)

23 observations with 16 cultures in 2 trials

	Mean Titration Values ml	d'[1]	Significance[2] P < .01
Skimmilk	5.08		
Whole milk	6.05	.97	≠
40% Buttermilk	6.85	1.77	≠
20% Buttermilk	6.47	1.39	≠
10% Buttermilk	6.31	1.23	≠
5% Buttermilk	6.02	.94	≠
2.5% Buttermilk	6.02	.94	≠

[1]d' = mean titration value minus mean titration value for skimmilk.

[2]d' value of at least 0.68 was required for significance at the 1% level.

TABLE X

RATES OF ACID PRODUCTION BY LACTIC CULTURES IN RAW AND IN HEATED SKIMMILK, WHOLE MILK AND BUTTERMILK

	Average of 9 cultures						
	HEATED			RAW			
	Mean Titration Value ml	d'[1]	Significance[2] P <.01	Mean Titration Value ml	d'[1]	Significance[3] P <.05	P <.01
Skimmilk	3.29			4.37			
Whole milk	4.02	.73	≠	4.98	.61	≠	-
100% Buttermilk	5.74	2.45	≠	6.72	2.35	≠	≠
75% Buttermilk	6.89	3.60	≠	6.98	2.61	≠	≠
50% Buttermilk	7.29	4.00	≠	7.01	2.64	≠	≠
25% Buttermilk	7.28	3.99	≠	6.34	1.97	≠	≠
12.5% Buttermilk	6.71	3.42	≠	5.90	1.53	≠	≠

[1]d' = mean titration value minus mean titration value for skimmilk
[2]d' value of at least 0.43 was required
[3]d' values of 0.55 and 0.70 were required for significance at the 5% and 1% levels, respectively.

higher than that in the skimmilk ($P < .01$). The results obtained with the raw products show that the rates of acid production in buttermilk, and in mixtures of buttermilk and skimmilk, were significantly higher than that in skimmilk ($P < .01$). The difference between rate of acid production in the raw whole milk and in the raw skimmilk was not as great as that obtained with the heated products ($P < .05$ vs. $P < .01$). A possible explanation for this is that the organisms normally present in raw milk produced acid during the incubation which could account, in part, for the higher acidity in the raw products as compared to that in the pasteurized products. These results indicate that, since higher rates of acid production were obtained in both raw and heated products containing butterfat or buttermilk, the fat globule membrane appears to contain materials that stimulate acid production by lactic cultures.

Reconstituted Non-Fat Milk and Buttermilk. Since reconstituted non-fat milk is used to some extent in propagating lactic cultures, it was deemed advisable to determine if buttermilk could be used to improve the nutritional qualities of the milk. Two trials involving 19 cultures were conducted to compare the rates of acid production in reconstituted non-fat milk with those in buttermilk and mixtures of buttermilk and reconstituted non-fat milk. These mixtures contained the following concentrations of buttermilk: 80%, 60%, 50%, 40%, 30%, 20% and 10%. The non-fat dry milk used for the reconstituted

product was selected as the best of 7 lots tested for acid production by lactic cultures and was considered satisfactory for propagating the cultures. A summary of the results is presented in Table XI.

These results indicate that the highest rate of acid production occurred with the 100% buttermilk and that as the amount of buttermilk in the mixtures decreased, the rates of acid also decreased. The differences between the rates of acid production in the buttermilk and the mixtures of buttermilk with reconstituted non-fat milk and that in the reconstituted non-fat milk were all significant at the 1% level, except for that in the mixture containing 10% buttermilk which was significant at the 5% level. The cultures appeared to require higher concentrations of the buttermilk in reconstituted non-fat milk than in fresh skimmilk.

Source of the Stimulatory Material. Since the results of previous trials indicated that the stimulatory factor (or factors) was associated with the fat globule membrane, an attempt was made to pin-point the source. Two trials were conducted in which spray dried buttermilk and spray dried non-fat milk were reconstituted and the casein precipitated from each with rennet extract and filtered. The filtration presumably removed all the casein and some of the other proteins which had been denatured in the drying process. The resulting buttermilk whey was milky in appearance, due to the presence of the fat globule membrane material, while the skimmilk whey was practically clear.

TABLE XI

RATES OF ACID PRODUCTION BY LACTIC CULTURES IN RECONSTITUTED NON-FAT MILK AND IN BUTTERMILK

19 observations with 19 cultures in 2 trials				
	Mean Titration Values ml	d'[1]	Significance[2] P < .05	P < .01
Recon. non-fat milk	4.41			
100% Buttermilk	5.89	1.48	≠	≠
80% Buttermilk	5.84	1.43	≠	≠
60% Buttermilk	5.49	1.08	≠	≠
50% Buttermilk	5.24	.83	≠	≠
40% Buttermilk	5.17	.76	≠	≠
30% Buttermilk	5.00	.59	≠	≠
20% Buttermilk	4.79	.38	≠	≠
10% Buttermilk	4.53	.12	≠	-

[1] d' = mean titration value minus mean titration value for skimmilk.

[2] d' values of 0.12 and 0.37 were required for significance at the 5% and 1% levels, respectively.

Seventeen observations with 13 cultures were made to compare the rates of acid production in reconstituted non-fat milk and in the milk with buttermilk and with skimmilk wheys added. The buttermilk and the skimmilk wheys were mixed with reconstituted non-fat milk in concentrations of 10%, 5%, and 2%. The results of these trials are presented in Table XII.

The results show that skimmilk whey had no significant effect on the rate of acid production by cultures ($P > .05$). The highest rate of acid production occurred in the mixture containing 10% buttermilk whey and then the rates decreased as the amount of buttermilk whey decreased. The rates of acid production were significantly higher in all concentrations of the buttermilk whey than in the reconsituted non-fat milk. These differences were significant at the 1% level for the rates in mixtures containing the 10% and the 5% level for the rate in the mixture containing 2% buttermilk whey. These results indicate that the stimulatory property of buttermilk is associated with or present in the fat globule membrane and not with the casein.

To further prove that the stimulatory property of buttermilk was associated with or present in the fat globule membrane, a trial was conducted using reconstituted non-fat milk and washed cream buttermilk. Practically all milk solids other than the fat globules were removed by the washing process. The fat was removed by churning

TABLE XII

RATES OF ACID PRODUCTION BY LACTIC CULTURES IN RECONSTITUTED NON-FAT MILK AND WHEYS FROM RECONSTITUTED NON-FAT MILK AND FROM BUTTERMILK

17 observations with 13 cultures in 2 trials

	Mean Titration Value ml	d'[1]	Significance[2] P < .05	P < .01
Recon. non-fat milk	5.84			
10% Buttermilk whey	6.38	0.54	≠	≠
5% Buttermilk whey	6.29	0.45	≠	≠
2% Buttermilk whey	6.13	0.29	≠	-
10% Skimmilk whey	5.91	0.07	-	-
5% Skimmilk whey	5.73	-0.11	-	-
2% Skimmilk whey	5.78	-0.06	-	-

[1] d' = mean titration value minus mean titration value for skimmilk.

[2] d' values of 0.21 and 0.27 were required for significance at the 5% and 1% levels, respectively.

leaving only the fat globule membrane in the buttermilk. The washed cream buttermilk was mixed with reconstituted non-fat milk to obtain concentrations of 10%, 5%, 2% and 1% washed cream buttermilk. Eight cultures were used in this trial. A summary of the results is given in Table XIII.

These results indicate that the rate of acid production by lactic cultures was increased by the presence of the fat globule membrane material. The rates of acid production in the milk with 5% and with 2% washed cream buttermilk were significantly higher than that in the reconstituted non-fat milk ($P < .05$). The rates of acid production in the milk with 10% and with 1% washed cream buttermilk were higher than that in the reconstituted non-fat milk, but this difference was not significant ($P > .05$). These results indicate that the stimulatory factor is located or partially located in the fat globule membrane. It was noted that the stimulatory effect of the washed cream buttermilk was not as great as those obtained with the normal buttermilk or buttermilk whey. This indicates that part of the stimulatory factor may have been removed during the washing process. It has been shown in past work (16) that part of the proteins adsorbed to the fat globule membrane were removed during the washing process.

Comparison of Buttermilk With Pancreas Extract. After having proved that buttermilk was stimulatory to lactic cultures, a trial was conducted to compare the stimulatory effects of buttermilk and of pancreas extract (a proven

TABLE XIII

RATES OF ACID PRODUCTION BY LACTIC CULTURES IN RECONSTITUTED NON-FAT MILK AND WASHED CREAM BUTTERMILK

8 observations with 8 cultures			
	Mean Titration Value ml	d'[1]	Significance[2] P <.05
Recon. non-fat milk	5.31		
10% Washed cream buttermilk	5.48	0.17	-
5% Washed cream buttermilk	5.65	0.34	≠
2% Washed cream buttermilk	5.66	0.35	≠
1% Washed cream buttermilk	5.54	0.23	-

[1] d' = mean titration value minus mean titration value for skimmilk.
[2] d' value of 0.29 was required.

starter stimulant). The rates of acid production by lactic cultures in skimmilk, buttermilk, and skimmilk containing 0.1% pancreas extract were compared. Fresh buttermilk was used alone and as a 1-1 mixture with skimmilk (50% concentration). Two cultures were used in this trial. The media were prepared and heated in 100 ml quantities, cooled, inoculated (about 1%), dispensed in measured 9 ml quantities into sterile, rubber-stoppered test tubes and then incubated. The media were inoculated before dispensing to insure uniform inoculation in all tubes of each medium. Ten tubes of each medium were prepared for each culture. Acid production was determined initially and at 8, 9, 10, 11, 12, 14, 16, and 18 hours of incubation at 72°F. The results are presented in Figure 3.

This graph indicates that the rate of acid production in the buttermilk was higher than that in the skimmilk, but not as high as that in the skimmilk containing 0.1% pancreas extract; however, the pancreas extract was foreign to milk and the buttermilk was not. The buttermilk appeared to support a slightly higher rate of acid production than the 50% buttermilk and skimmilk mixture. This graph also illustrates the importance of determining the acid production near the middle or late logarithmic phase of growth when comparing rates of acid production by cultures, because, as the incubation period increased beyond the logarithmic phase, acidities of all cultures approached common level.

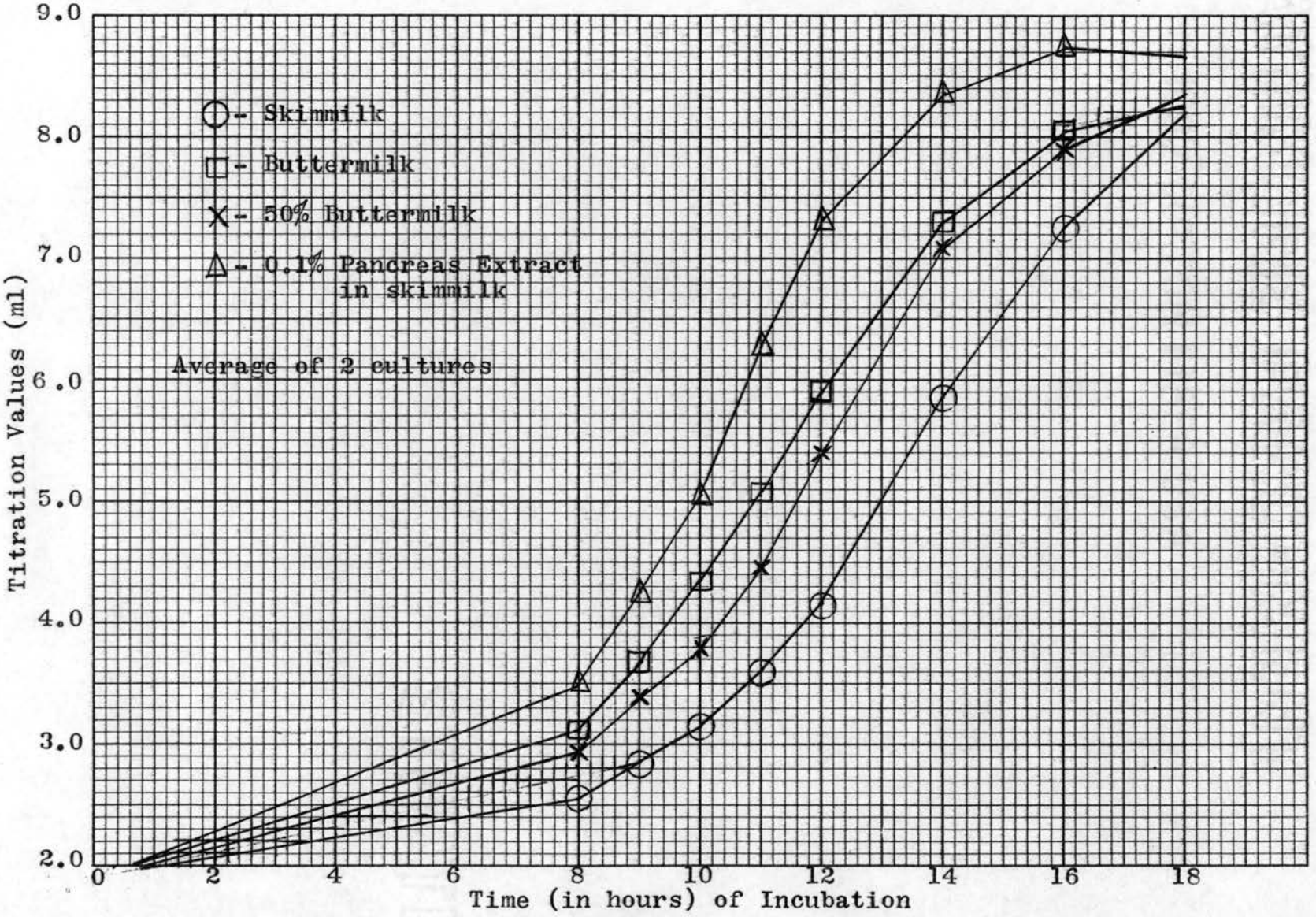

Figure 3. Rates of acid production by lactic cultures in skimmilk, buttermilk and skimmilk containing pancreas extract.

Separator Slime. Since buttermilk was found to be stimulatory to the rate of acid production by lactic cultures, it was deemed necessary to determine also the effects of separator slime. Three trials were conducted in which the rate of acid production in skimmilk containing 5%, 2%, 1%, 0.5%, and 0.25% separator slime were compared to that in skimmilk. Twenty-six observations were made with 14 cultures. A summary of these results is presented in Table XIV.

TABLE XIV

RATES OF ACID PRODUCTION BY LACTIC CULTURES IN SKIMMILK AND SEPARATOR SLIME

26 observations with 14 cultures in 3 trials

	Mean Titration Values ml	d'[1]	Significance[2] P <.01
Skimmilk	4.13		
5% Separator Slime	5.84	1.71	/
2% Separator Slime	5.26	1.13	/
1% Separator Slime	5.06	.93	/
0.5% Separator Slime	4.96	.83	/
0.25% Separator Slime	4.90	.77	/

[1] d' = mean titration value minus mean titration value for skimmilk.
[2] d' value of 0.47 was required.

These results indicate that the rates of acid production in all mixtures of separator slime and skimmilk were significantly higher than that in skimmilk alone (P <.01).

The highest rate of acid production occurred in the skimmilk containing 5% separator slime and then the rates decreased with each decrease in separator slime content. The fact that separator slime proved to be stimulatory could account in part for the higher rates of acid production obtained with whole milk.

SUMMARY AND CONCLUSIONS

Determinations were made of the effects of various heat treatments of skimmilk, whole milk, reconstituted non-fat milk, and certain mixtures of these on the rates of acid production by lactic cultures. The heat treatments used involved temperatures ranging from 140°F to 250°F with exposure times of 0 to as long as 40 minutes. An analysis was made to test for interaction between the cultures and the heat treatments.

The effects of various milk fractions on the rate of acid production by lactic cultures were studied. Individual lots of fresh whole milk were separated and the cream churned. The rates of acid production in the whole milk, skimmilk, buttermilk and in certain mixtures of these media were determined, as well as in skimmilk with separator slime added. The comparative rates of acid production in raw and in heated fractions were also determined. The stimulatory effect of buttermilk was further demonstrated by determining the rates of acid production in this product, in reconstituted non-fat milk, and in mixtures of these two media.

After it was established that buttermilk contained a factor (or factors) which stimulated acid production by lactic cultures, attempts were made to pin-point the source

of the factor (or factors). The influence on the rate of acid production of whey prepared from dried buttermilk, which contained the fat globule membrane material, was compared with that of whey from non-fat dry milk. The wheys were used in mixtures with reconstituted non-fat milk. Buttermilk prepared from washed cream was also used to further test for the presence of the stimulatory factor in the fat globule membrane material.

The heat treatment milk received influenced the rate of acid production by lactic cultures. In skimmilk the rate of acid production was highest in the lots of milk pasteurized at 160°F for 30 minutes, followed in order by that in the sterilized milk (250°F for 15 minutes), 143°F, 180°F and 210°F for 30 minutes, respectively. From these results it appeared that sterilized skimmilk would be best for propagation of lactic cultures and that milk for bulk cultures for cheese making should be pasteurized at 160°F for 30 minutes. There is the possibility that bacteriophage surviving in milk pasteurized at 160°F could cause trouble in mother cultures.

In mixtures of 2 parts of skimmilk to 1 part of whole milk, such as is often used for making cultured buttermilk, the most rapid acid production occurred in the sterilized milk, followed in order by that in the lots pasteurized at 200°F, 160°F, and 180°F for 30 minutes, respectively. Since temperatures higher than 180°F impart pronounced cooked flavors and cause browning of the

milk, they are not practical for use on milk intended for making buttermilk. It appeared that milk for buttermilk should be pasteurized at 160°F to 180°F for 30 minutes.

In reconstituted non-fat milk the highest rate of acid production occurred in the milk heated at 210°F for 30 minutes followed in order by that in the lots heated at 180°F for 30 minutes, 250°F for 15 minutes, and at 160°F for 30 minutes. These results were in direct contrast to those obtained with fresh skimmilk, presumably due to the heat treatment to which the non-fat dry milk had been exposed during the drying process. Because of the effects of heat treatments and of the milk used on the rates of acid production, these factors must be considered in tests for activity of lactic starter cultures.

Most cultures seemed to be rather sensitive to the bactericidal property present in milk pasteurized at 143°F for 30 minutes, while certain other cultures produced acid rapidly in milk exposed to this treatment. From the results obtained with the heat treatments of various types of milk it appeared that, in the evaluation of cultures, the milk and pasteurization temperature used should be similar to those employed for cheese making.

The rates of acid production by lactic cultures were higher in whole milk, buttermilk, and in mixtures of skimmilk with buttermilk and with separator slime than in skimmilk from the same source. Buttermilk was the most stimulatory fraction tested. The optimum mixture of

skimmilk with buttermilk for the highest rate of acid production was generally in the range of 25% to 50% buttermilk. The butterfat globule membrane material was found to contain a factor (or factors) which was stimulatory to lactic cultures. The stimulatory effect of the butterfat globule membrane material was not as great as that of pancreas extract.

LITERATURE CITED

1. Anderson, A. W. and Elliker, P. R. The Nutritional Requirements of Lactic Streptococci Isolated from Starter Cultures. I. Growth in a Synthetic Medium. J. Dairy Sci., 36:161. 1953.

2. Anderson, A. W. and Elliker, P. R. The Nutritional Requirements of Lactic Streptococci Isolated from Starter Cultures. II. A Stimulatory Factor Required for Rapid Growth of Some Strains in Reconstituted NFMS. J. Dairy Sci., 36:608. 1953.

3. Anderson, A. W., Parker, R. B. and Elliker, P. R. The Nutritional Requirements of Lactic Streptococci Isolated from Starter Cultures. III. Variations in Growth Promoting Properties of Fresh Whole Milk. J. Dairy Sci., 38:1083. 1955.

4. Auclair, J. E. The Inhibition of Micro-organisms by Raw Milk. III. Distribution and Properties of Two Inhibitory Substances Lactenin-1 and Lactenin-2. J. Dairy Res., 21:323. 1954.

5. Auclair, J. E. and Hirsch, A. The Inhibition of Micro-organisms by Raw Milk. I. The Occurrence of Inhibitory and Stimulatory Phenomena. Methods of Estimation. J. Dairy Res., 20:45. 1953.

6. Babel, F. J. Dairy Cultures--Factors Influencing Growth and Acid Production. Purdue Agri. Exp. Sta., Bul. 573. 1952.

7. Dill, C. W., Roberts, W. M. and Aurand, L. W. Production of Sulfur Compounds in Skimmilk Heated by Direct Steam Injection. J. Dairy Sci., 45:1332. 1962.

8. Elliot, K. A. C. Milk Peroxidase. Its Preparation, Properties, and Action With H_2O_2 on Metabolites. With a Method for Determining Small Amounts of H_2O_2 in Complex Mixtures. Biochem. J., 26:10. 1932.

9. Foster, E. M. The Effect of Heat on Milk as a Culture Medium for Lactic Acid Bacteria. J. Dairy Sci., 35:988. 1952.

10. Garvie, Ellen I. and Mabbitt, L. A. Acid Production in Milk by Starter Cultures - The Effect of Peptone and Other Stimulatory Substances. J. Dairy Res., 23:305. 1956.

11. Gillies, A. J. Role of Fat Globules in the Inhibition of Certain Strains of Streptococcus cremoris in Pasteurized Milk. Qd. J. Agri. Sci., 18:123. 1961.

12. Gould, I. A. Some Chemical Changes Produced in Milk by High Temperature Heat Treatment. Milk Plant Mo., 35:70. 1946.

13. Green, V. W. and Jezeski, J. J. Studies on Starter Metabolism. II. Influence of Heating Milk on the Subsequent Response of Starter Cultures. J. Dairy Sci., 40:1054. 1957.

14. Green, V. W. and Jezeski, J. J. Studies on Starter Metabolism. III. Studies on Cysteine-Induced Stimulation and Inhibition of Starter Cultures in Milk. J. Dairy Sci., 40:1062. 1957.

15. Hammer, B. W. and Babel, F. J. Bacteriology of Butter Cultures: A Review. J. Dairy Sci., 26:83. 1943.

16. Hare, J. H., Schwartz, D. P. and Weese, S. J. The Amino Acid Composition of the Fat-Globule Membrane Protein of Milk. J. Dairy Sci., 35:615. 1952.

17. Herald, C. T. and Brunner, J. R. The Fat-Globule Membrane of Normal Cow's Milk. I. The Isolation and Characteristics of Two Membrane-Protein Fractions. J. Dairy Sci., 40:948. 1957.

18. Herald, C. T., Brunner, J. R. and Bass, S. T. A Spectrographic Analysis of Two Protein Fractions of the Fat-Globule Membrane of Normal Cow's Milk. J. Dairy Sci., 40:446. 1957.

19. Johns, C. K. and Berzins, I. Observations on the Determination of Antibiotics in Milk. J. Milk and Food Tech., 19:14. 1956.

20. Jones, F. S. and Little, R. B. The Bactericidal Property of Cow's Milk. J. Exp. Med., 45:319. 1927.

21. Lightbody, Lorna G. The Use of Buttermilk for the Growth of Butter Cultures. Aust. J. Dairy Tech., 16:25. 1961.

22. Mac Leod, P. and Gordon, D. F. Peptides as Sources of Essential Amino Acids for Lactic Streptococci. J. Dairy Sci., 44:237. 1961.

23. Morrison, R. D. Personal Communication. March 23, 1963.

24. Niven, C. F. Nutrition of Streptococcus lactis. J. Bacteriol., 47:343. 1944.

25. Ransom, M. Good Buttermilk. Milk Plant Mo., 42:39. 1953.

26. Sandine, W. E., Speck, M. L. and Aurand, L. W. Identification of Constituent Amino Acids in a Peptide Stimulatory for Lactic Acid Bacteria. J. Dairy Sci., 39:1532. 1956.

27. Speck, M. L. Symposium on Lactic Starter Cultures. IV. Starter Culture Growth and Action in Milk. J. Dairy Sci., 45:1281. 1962.

28. Steel, R. G. D. and Torrie, J. H. Principles and Procedures of Statistics. New York, Toronto, and London:McGraw and Hill, 1960.

29. Stadhouders, J. and Veringa, H. A. Some Experiments Related to the Inhibitory Action of Milk Peroxidase on Lactic Streptococci. Neth. Milk Dairy J., 16:96. 1962.

30. Wilson, A. T. and Rosenblum, H. The Antistreptococcal Property of Milk. I. Some Characteristics of the Activity of Lactenin in vitro. The Effect of Lactenin on Hemolytic Streptococci of Several Serological Groups. J. Exp. Med., 95:25. 1952.

31. Wilson, A. T. and Rosenblum, H. The Antistreptococcal Property of Milk. II. The Effects of Anaerobiosis, Reducing Agents, Thiamine, and Other Chemicals on Lactenin Action. J. Exp. Med., 95:39. 1952.

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