THE EFFECT OF LACTIC CULTURES ON THE KEEPING QUALITY OF CREAM

by

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TABLE OF CONTENTS

INTRODUCTION

It is estimated that 75 per cent of the butter manufactured in this country is made from sour cream. Much of this cream is produced on farms where dairying is a sideline to some other type of farming. On the average farm it may require several days to accumulate a full can of cream. Due to the small quantity of cream produced the farmer sometime fails to give enough attention to cooling and other factors which prevent deterioration. Storage facilities on the farm and length of time held favor rapid spoilage.

The condition of the cream at the time of marketing has received wide spread attention, especially the last two or three years by the Federal Food and Drug Administration. The condemnation of adulterated cream containing extraneous material by federal inspectors has resulted in an extensive cream improvement campaign by the butter industry. Instructions on cooling tanks, more attention to sanitation, and more frequent delivery by the farmer have all aided in improving the quality. Reports issued by the State Dairy Commissioner indicate that cream stations are buying from 10 to 15 per cent second grade cream. In addition to this a considerable quantity of cream purchased as first grade cream becomes second grade before it reaches the creamery. It is

estimated that 25 per cent of the cream purchased in Kansas would not make 90 score butter.

The principle of adding starter to cream before churning to improve its flavor and keeping quality has been used by creameries with success for years. No mention has been found in the literature of any attempt to control the cream while it is being held on the farm by means of adding cultures of desirable bacteria. Work done by various investigators demonstrated that Streptococcus lactis can dominate the flora of ordinary milk products if the contamination of other types is not too extensive. It has also been found that S. lactis forms primarily lactic acid which may be more beneficial than harmful.

S. lactis has the ability to dominate the flora of cream and control to some extent the growth of other microorganisms. The lactic acid produced by S. lactis acts somewhat as a preservative for cream. Hence it seems logical to suppose that the principle of the addition of cultures to cream could be used to good advantage on the farm. The best temperature for the growth of S. lactis is about 70°F., this temperature could be maintained on the farm at less cost than a temperature low enough to prevent bacterial growth.

REVIEW OF LITERATURE

In a problem of cream improvement by using lactic cultures, several factors must be taken into consideration. There are four points of major importance. First, the ability of S. lactis to dominate the flora, and the effect of their growth on the cream. Second, the effect of S. lactis and the attendant rise in acidity on the various proteolytic organisms. Third, the effect of S. lactis growth and acidity on organisms causing such flavors as cheesy, strong, bitter, and malty. Fourth, the effect of acidity on mold and yeast development.

In milk or cream, if temperature conditions are satisfactory, the ever present S. lactis has an ideal medium for growth and development. There are many strains of this organism, which differ rather widely in their speed of growth and acid development. Work done by Frazier (7) shows that in the spontaneous souring of milk at ordinary temperatures, the streptococci usually predominate even in the early stages. As the acidity increases other types of bacteria are suppressed and when the fermentation is finally checked at a pH of 4.8 to 5.0, the flora consists almost entirely of the lactic streptococci; mostly of S. lactis type. If the S. lactis does not dominate the flora and yield lactic acid, slight amounts of acetic acid and other

by-products, Streptococcus kefir, Escherichia coli and Aerobacter aerogenese, the aroma forming organisms and many other milk bacteria tend to dominate, forming various end-products in addition to lactic acid, such as carbon dioxide, hydrogen, acetic, propionic, succinic, formic, butyric acids, acetons and ethanol.

Suzuki et al (25) and Hammer (9) report that S. lactis yields about 90 to 98 per cent lactic acid in breaking down lactose, the remaining percentage going to various end-products. These end-products are supposed to result from the respiratory process of the cells. According to Templeton and Sommer (26), E. coli and certain milk bacteria other than S. lactis produce a lactic acid yield of 39 to 80 per cent, the remainder being used in the manufacture of end-products, most of which are harmful to the flavor of cream, and hence may or may not be carried over into the butter.

In the normal lactic acid fermentation, Rogers and Whittier (19) have noted four rather definite stages. First, the period following production when there seems to be a lag in bacterial development. Second, if temperature is right, the period of rapid or logarithmic growth, until an acidity of .70 to .80 per cent lactic acid is formed. Third, the period of little or no increase in numbers. Fourth, fermentation ceases and cells begin to die. At this

point acid, enzymes, aldehydes and other by-products seem to combine to have an inhibiting effect on S. lactis.

There are many so-called milk bacteria which cause proteolysis, or protein decomposition. With the exception of cases of extreme contamination, the proteolysis usually does not occur until the normal fermentation has run its course. Proteolysis is undesirable in any case because of the variety of pungent odors produced. Belonowski (2) states that the rapid growth of S. lactis at first and later the growth of various bacilli aids greatly in keeping down casein decomposition by the ever present Escherichia-Aerobacter types. Work done by Hastings et al (12) indicate that acid development by S. lactis in cheese aids in controlling undue proteolysis.

Russell et al (21) have found that the normal lactic acid fermentation is soon stopped by the accumulation of by-products. Oospora lactis and other molds grow well on this medium, growing on the surface, utilizing the acids making the medium more alkaline. Putrefactive bacteria kept inactive due to acid now find conditions favorable for growth producing very undesirable by-products.

There are several acid and rennet-forming cocci and bacilli which cause proteolysis. Frazier (6) reports that the optimum temperature for most of these is at about blood

heat, but that they cause more actual decomposition at lower temperatures because at this high temperature high acid produced by other organisms will prevent their growth.

Spitzer and Epple (22) state that the growth of Bacillus panis Migula was inhibited by an acidity of .54 per cent lactic acid. Hence an increase in acidity will retard the proteolytic action of this organism. However, later work by Spitzer et al (23) when six other organisms were studied the opposite from the above was found. With these organisms an acidity of .50 to .60 per cent seemed to aid proteolysis and enzyme activity.

Much work has been done to determine whether the regular lactic acid bacteria are proteolytic. Frazier and Rupp (8) report that S. lactis, Lactobacillus bulgaricus, and Lactobacillus casei were able to decompose lactalbumen in milk serum to a slight extent. Work done by Peterson et al (18) on 22 strains of S. lactis shows that ammonia production was slight in all cases, in some cases it did not equal its consumption. A number of S. lactis cultures were studied by Anderegg et al (1) and Hammer et al (11) with and without calcium carbonate using 14-day periods of incubation. In general the rapidly coagulating cultures brought about little if any protein decomposition. It is the consensus of opinion of the various investigators that

S. lactis should not be listed as one of the actively proteolytic bacteria.

A number of off-flavors in cream due to bacterial action, such as cheese-like flavors, strong, bitter, and malty, are very destructive to the quality of cream. Not much is known about the causes of these flavors. The flavors are thought to be caused by by-products of various bacteria and mold.

Tracy and Ramsey (27) report that the regular acid formers retard the malty flavor development by organisms causing this defect and that Bacillus subtilis intensifies this off-flavor.

Burr (3) found that protein in butter was not the important factor in causing a cheese-like flavor but that the defect originated in the cream due to bacteria other than the regular lactic acid organisms. Henneberg (13) found that casein decomposers and to a degree the fat-splitting Bacillus cloacae produce putrid, cheesy and bitter flavors in butter. Orla-Jensen (16) showed that the cheese-sour flavor in butter reached its most intense form when lactobacilli in symbiosis with yeasts caused fat hydrolysis. According to Ruehle (20) cheesy flavors are not caused by Oospora lactis individually or when in association with S. lactis. Combs (4) has found that Oospora lactis and

Pseudomonas chrysogenum if allowed to appear in butter after 90 days storage.

The dairy industry has been aware of the presence of mold and yeast in various dairy products, especially cheese and unsalted butter, and the effects of mold on these products have been studied quite extensively. Our information as to their effect on cream and the resulting butter is rather limited. Russell and Hastings (21) state that the molds play an important part in lowering the acidity of cream, making it a desirable medium for the proteolytic bacteria which can not grow well at a high acidity. Stoltz (24) states that acidity in cream will decrease as mold growth increases. White and Hood (28) found that the pH of the medium seemed to have no effect on mold growth. Yeast grew well at a pH of 3.4 and lower and as high as 6.0. Macy and Anderson (14) have shown that the degree of acidity did not have a very marked effect on the extent of growth of O. lactis. Combs and Eckles (5) found that molds growing on sweet cream caused an off-flavor in the cream and also in the butter. Molds grown on sour cream caused no off-flavor in the cream but made butter of poor keeping quality. Parfitt (17) states that stirring cream would increase mold growth because, in the process of stirring the mycelia are broken up, air is incorporated, new surfaces are exposed and

better conditions for growth are afforded.

Yeasts are usually considered along with molds mainly because like molds they can always be isolated from milk and cream, and they grow in about the same medium. Hammer and Cordes (10) have shown that the organisms are not true yeasts but are torulae which cause a yeasty odor and flavor. The two most important types are Torula cremoris and Torula sphaerica, sometimes called white milk yeast. Because of their acid tolerance they grow well where many organisms die. They produce carbon dioxide and ethyl alcohol from lactose. Nelson (15) has found that the action of the white yeast is slow and does not have much influence on changes brought about in dairy products other than breaking down of lactose and causing yeasty flavors and odors.

EXPERIMENTAL PROCEDURE

The purpose of this experiment is to determine the practicability of adding lactic acid starters to cream on the farm as a cream improvement measure. In as far as possible this experiment was carried out under average farm conditions.

After testing the cream for butter fat and acid, it was placed in clean sterilized containers. All lots of cream other than the controls were then inoculated with the desired amount of lactic culture and set at the desired temperature for further observation. The temperature was recorded and regulated twice daily.

Each morning for the next seven days, the surface of the cream was examined for mold growth. The cream was then stirred and the flavors and odors noted. After an acid test was made, a fresh lot of cream was added to each container and stirred. After the seventh day no additional cream was added but observations were made for three more days. The cream was then graded, neutralized, pasteurized, ripened and churned. The samples were churned in a small Dazey churn and the butter was worked by hand. The butter samples were placed in paraffined cartons for scoring. One set of samples was held at 45°F. and scored after 24 hours and at two week

intervals for twelve weeks. Another set of samples was held at 0°F. and was scored after being in storage four months.

Cultures used. Thirteen different cultures were used in this study. Some were cultured in our own laboratory, while others were regular commercial cultures. They included the following:

1. Hansens' Lactic Butter Culture.
2. Ericcsons' Butter Culture.
3. Pure S. lactis Culture.
4. National Dairy Products No. 122 Butter Culture
5. National Dairy Products No. K 500 Butter Culture
6. National Dairy Products No. BM2 (lacto bacillus culture used at 71 and 98°F.)
7. National Dairy Products No. H521.
8. S. lactis Slow Litmus Reducer.
9. S. lactis Fast Litmus Reducer.
10. S. lactis Fast Curdling No. 1.
11. S. lactis Slow Curdling No. 1.
12. S. lactis Slow Curdling No. 2.
13. S. lactis Fast Curdling No. 2.

Temperatures used. Cream was stored at temperatures of 60, 66, 68, 70, 72, 75, 80 and 98°F. in an attempt to approach storage conditions on the average farm. These temperatures were obtained by placing the cream container in a tank surrounded by water, or by placing it in a room at the desired temperature and in some cases a thermostatically controlled incubator was used.

Cream used. The quality of cream used in the experiment varied considerably. It consisted of:

1. Cream separated from grade A milk.
2. Cream separated from grade B milk.

3. Cream from ungraded milk separated with separator, bowl of which was washed every other day.
4. Cream from ungraded milk separated with separator, bowl of which was washed once weekly.
5. Sweet cream from a patron of the college creamery.

Procedure in selecting pure S. lactis cultures. A sample of grade A raw milk was allowed to sour naturally at a temperature of 75°F. This milk was then plated on regular whey agar using hundred thousand, million and ten million dilutions. The plates were incubated at 80°F. which is near the optimum temperature for S. lactis. Material from eighteen well isolated, characteristic, ellipsoid, subsurface colonies were transferred to individual tubes of litmus milk. S. lactis cultures were identified by - reduction of the litmus from the bottom of the tube, formation of a pink band at surface of the milk and fairly rapid coagulation of the milk. A pure S. lactis culture designated as No. 3 was selected for use in the experiment without further study.

All pure cultures of S. lactis do not show the same speed of reaction in litmus milk. The eighteen selected pure cultures of S. lactis were studied to note the difference in fast and slow curdling and fast and slow reduction of litmus milk. A standard loop full of inoculum was transferred from each culture to a separate tube of litmus milk which was held at 70°F. An indication of the speed of coagulation and litmus reduction was obtained by observing the

time required to complete the reaction. A fairly accurate index to the speed of reaction of the cultures was secured by repeating the operation three times.

Procedure in carrying mother cultures. Regular 150 cc. water blank bottles were used as milk containers and 9 cc. cream pipettes were used for transferring the cultures. The bottles and pipettes were washed in hot water and washing powder and rinsed in clear boiling water. The pipettes were dry air sterilized at 350°F. for three hours. One hundred cubic centimeters of good quality milk was placed in each bottle and boiled vigorously for 15 minutes. After cooling naturally to 158°F., it was cooled with tap water to 70°F., allowed to stand for 5 minutes and inoculated. An inoculation of 8 to 11 drops of regular culture would coagulate the milk in from 15 to 18 hours. The slow curdling cultures required 20 to 22 drops of inoculum to curdle the milk in 15 to 18 hours. The cultures were incubated at 70°F. and iced as soon as a fairly firm curd was formed.

Checking quality of cream. The acidity of the cream was determined by titrating a 9 gram sample with n/10 sodium hydroxide using phenolphthalein as an indicator. Flavor and odor were noted by tasting and smelling the cream. Mold growth on the surface of the cream was determined by the aid of a hand lens.

Yeast and mold count. To determine more accurately the extent of yeast and mold growth in the cream, regular plate counts were made using acidified whey agar. One cubic centimeter of one per cent lactic acid was added to each plate to lower the pH to approximately 3.6. This would prevent bacterial growth and make the yeast count more accurate. One hundred, thousand and ten thousand dilutions were made on each sample of cream. The plates were counted after a five day incubation period at 70°F.

The effect of acidity on the growth of mold. A good quality sweet cream containing .12 per cent lactic acid was sterilized at 15 pounds steam pressure for 25 minutes in an autoclave and cooled to about 70°F. Some of the cream was used as a control and a heavy inoculation of pure culture of Oospora lactis was added to the remainder of the cream. Fifty cubic centimeter portions of the contaminated cream were pipetted into sterile jars. A series of jars of the contaminated cream was acidified to range from 0.12 to 1.17 per cent titratable acidity with a range of 6.0 to 3.2 pH as determined by the Quinhydrone Electrode method. Duplicate samples of the contaminated and acidified cream were poured into petri plates, incubated at 70°F. and observed daily for 8 days. Jars containing about 10 cc. of the contaminated and acidified cream were placed in a refrigerator at 45°F.

for daily observation.

RESULTS OF THE EXPERIMENT

Comparison of various cultures. Preliminary trials were for the purpose of determining the merits of various cultures. Several commercial butter cultures, lactobacillus cultures, and pure cultures of S. lactis isolated in our laboratory were used in cream. These produced butter which scored 89 to 91, 88 or less, and 89 to 90, respectively. The difference in the score of butter was not significant when commercial butter cultures and pure S. lactis were used. The scores of the two samples of butter made from cream inoculated with lactobacillus cultures were two points lower than the butter made from the control cream.

Cream storage temperatures used. Storage temperatures of 60, 66, 68, 70, 72, 75, 80 and 98°F. were used because they are representative of those employed on many dairy farms. The butter made from inoculated and noninoculated cream held at 75°F. and above had an average score of 88. Cultured and noncultured cream held below 75°F. produced butter with an average score of 90.2 and 89.7, respectively. These results indicate that the storage temperature of the cream had more effect on the butter score than the addition of lactic cultures.

Grades of cream used. Cream produced under different conditions contains various amounts of contaminating organisms. Trials were made to determine the effect of the addition of cultures to cream of good and poor quality. Five batches of sweet cream produced under various conditions were inoculated with ten per cent culture and held with uninoculated controls at 70°F. The results are shown in Table I.

Table I. The Effect of Adding Lactic Cultures to Cream of Varied Quality on the Score of the Butter.

Grades of Cream	Score of Butter from inoculated cream	Score of Butter from non-inoculated cream
Cream from grade A milk	90	89.5
Cream from grade B milk	88	89
Cream from ungraded milk, separator washed once daily.	90	90.5
Cream from ungraded milk, separator washed once weekly	88	89
Sweet cream from patron of college creamery	87	88
Average score of all samples	88.6	89.2

The data in Table I. shows that the butter made from inoculated cream had an average score of 88.6 compared with 89.2 for the uninoculated control cream.

The effect of adding culture on the rate of acid development. Three batches of grade B sweet cream inoculated with ten per cent culture and three batches of noninoculated

grade B cream were held at 70°F. to determine the effect of cultures on the rate of acid development. The titratable acidity of the cream was determined daily, and the results are shown in Table II. Curves showing the daily development of acid are plotted in Figures I and II, and in Figure III. The curves show a comparison of the average rate of acid development in the three inoculated and the three non-inoculated control batches.

Table II indicates that the acidity of the inoculated cream at the end of the first day ranged from 0.50 to 0.60 per cent, increasing slightly on the second day followed by a decrease of 0.06 to 0.10 per cent on the third and fourth days. The acidity then steadily increased throughout the ten day period. The noninoculated cream tested 0.40 per cent acid at the end of the first day, followed by a steady increase until the tenth day. Figure III indicates that the rate of acid development in the cultured cream was greater the first 24 hours, however, the final acidity was about the same in the cultured and the noncultured cream.

Table II. The Effect of Adding Cultures to Cream held at 70°F. on Acid Development, Flavor, Grade of Cream, Butter Score, and Keeping Quality.

Age of cream days	With Culture Acid	Without Culture Acid	With Culture Acid	Without Culture Acid	With Culture Acid	Without Culture Acid
1	.11	.11	.11	.11	.11	.11
2	.55	.39	.54	.40	.58	.40

Table II - Continued

Age of cream days	With Culture Acid	Without Culture Acid	With Culture Acid	Without Culture Acid	With Culture Acid	Without Culture Acid
3	.56	.46	.55	.49	.59	.48
4	.54	.51	.52	.55	.50	.45
5	.52	.53	.53	.60	.49	.50
6	.52	.56	.55	.67	.51	.55
7	.54	.59	.63	.71	.55	.56
8	.60	.70	.77	.75	.56	.58
9	.65	.86	.84	.88	.59	.58
10	.68	.91	.90	.93	.66	.62
Cream Flavor	putrid stale	cheesy stale	slight musty stale	yeasty stale putrid	slight bitter stale	stale yeasty
Cream Grade	3	3	2	3	2	3
Butter Score Fresh	87	87	89	91	88	90
*Butter Score	85	85	88.5	90	88	89
**Butter Score	86	87	90	90	87	91

* Butter score after holding 8 weeks at 45°F.
**Butter score after hoding 4 months at 0°F.

Figure I shows that batches of the same cream uniformly inoculated and held at the same temperature do not always yield uniform ultimate acidities. This suggests that different organisms may dominate the flora in spite of the heavy inoculum.

In Figure IV curves are plotted comparing the daily average acidity of 12 batches of inoculated cream with that of 12 uninoculated controls. The 12 batches of cream varied in quality. They were held at temperatures ranging from 60

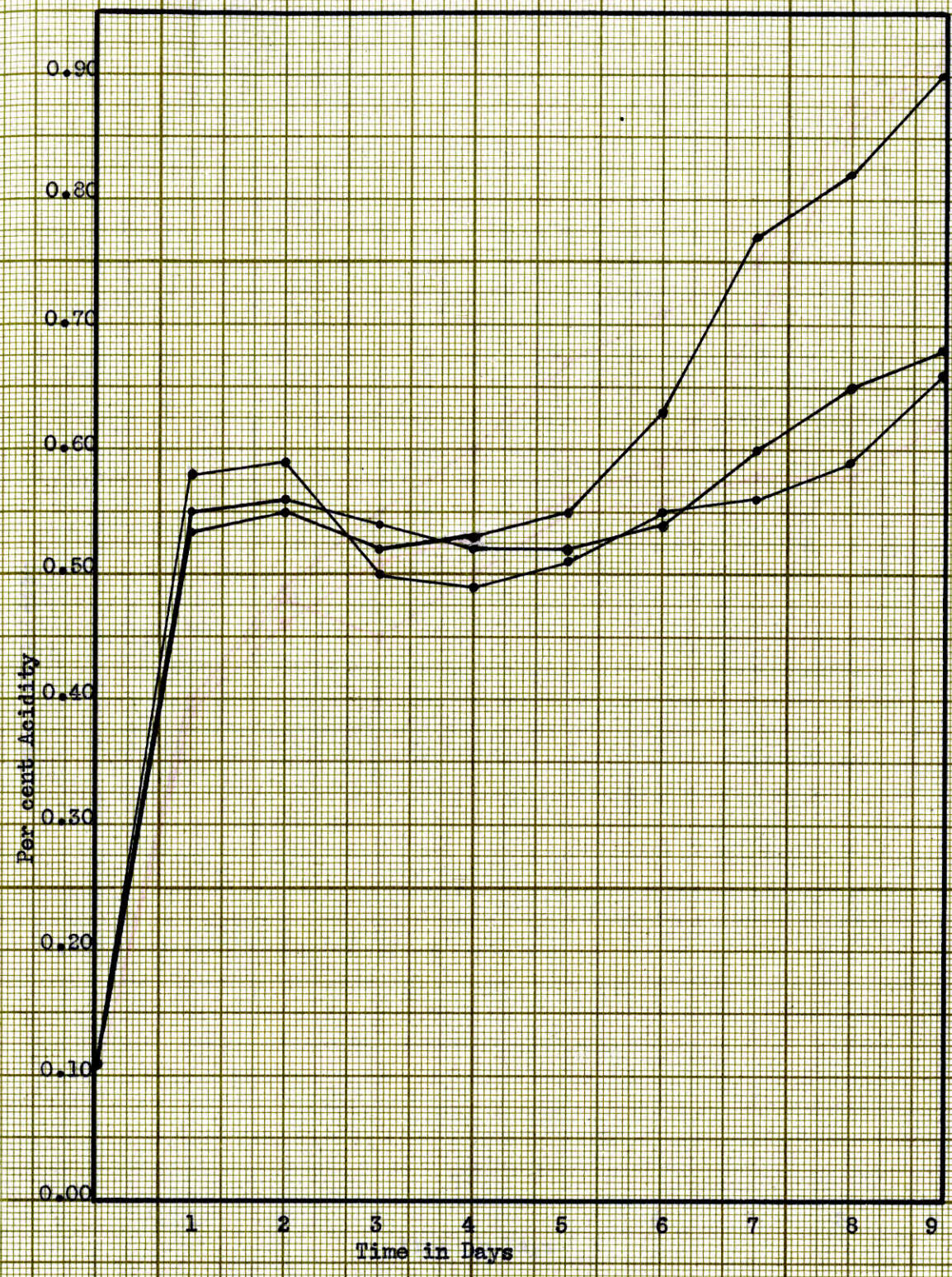

FIGURE I. Curves Showing Daily Acid Content of Three Batches of Cream Inoculated with 10 per cent Culture and Held at 70°F. Data from Table II.

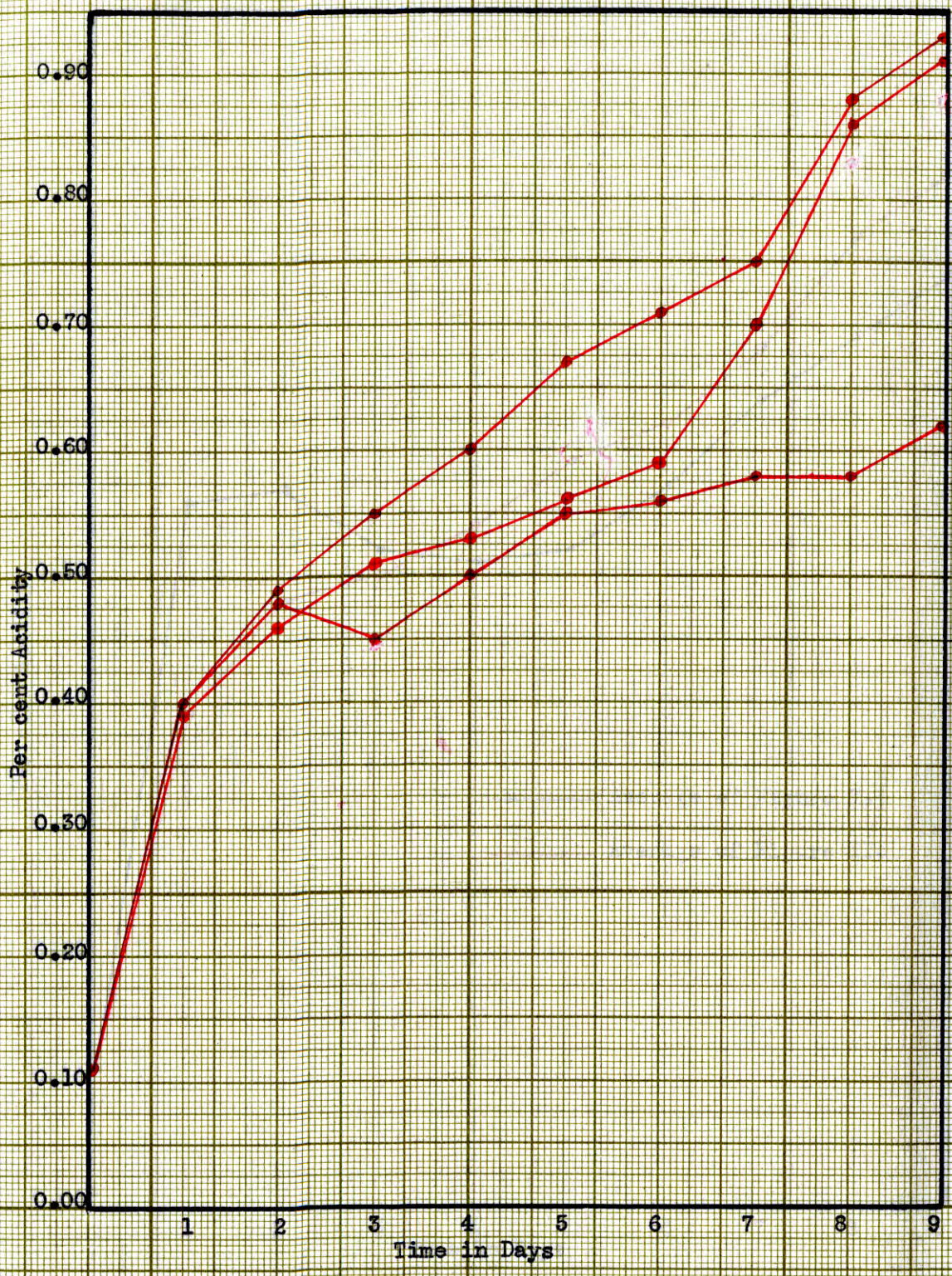

FIGURE II. Curves Showing Daily Acid Content of Three Batches of Non-inoculated Cream Held at 70°F. Data from Table II.

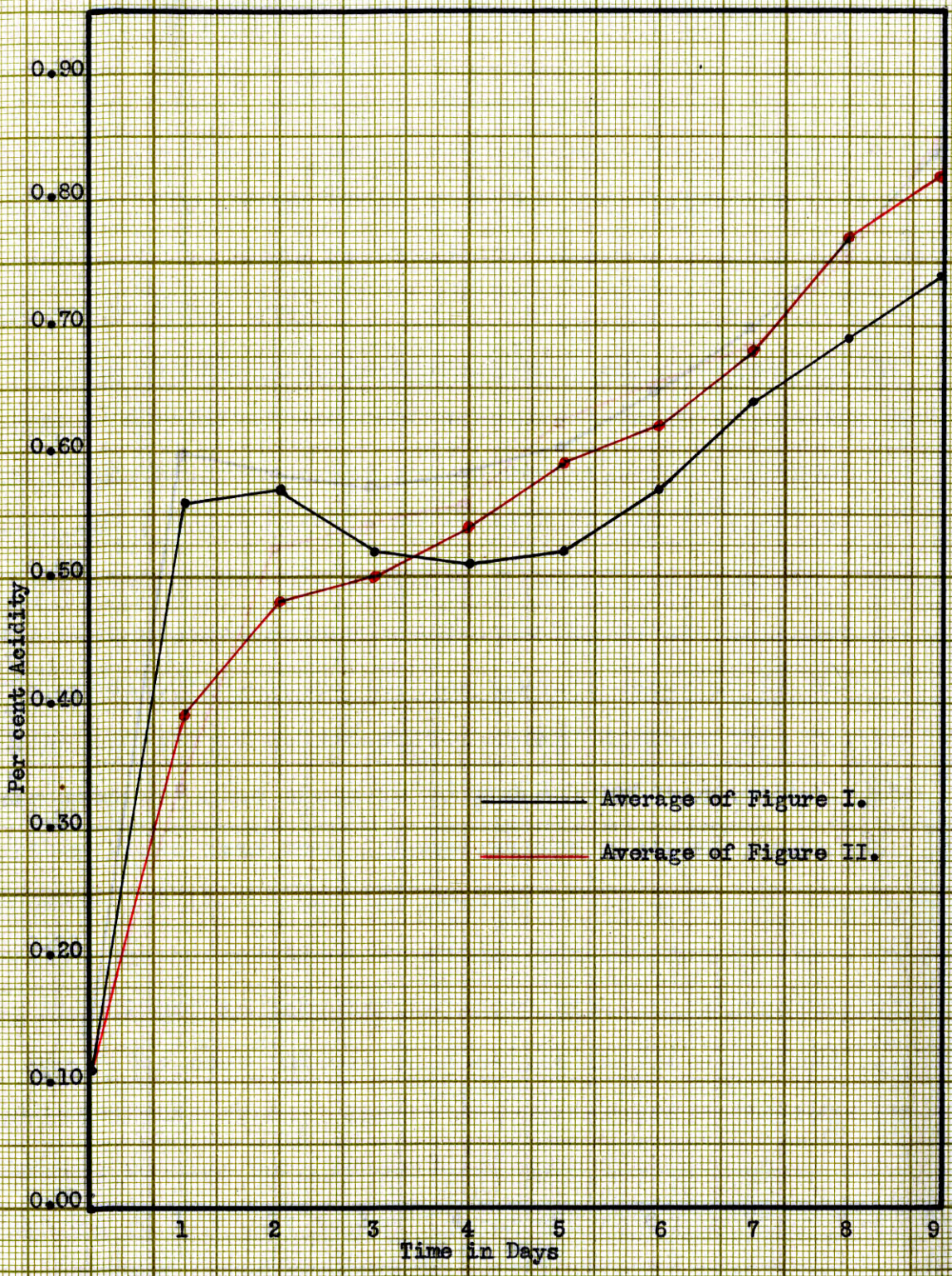

FIGURE III. Curves Plotted Comparing Average Daily Acid Content of Three Batches of Inoculated Cream with Three Controls. Data from Table II.

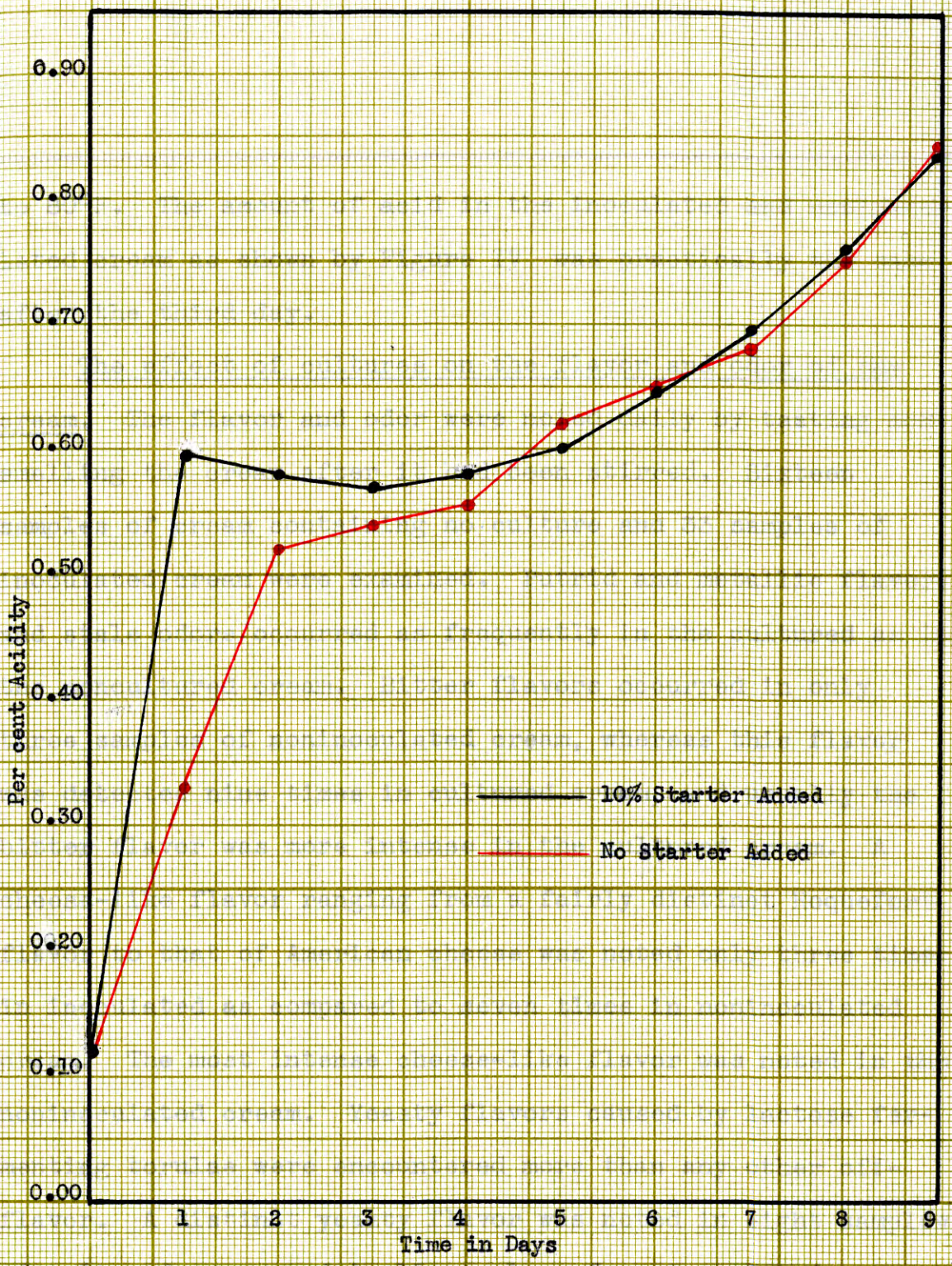

FIGURE IV. Curves Plotted Comparing Average Daily Acid Content of 12 Lots of Inoculated Cream and the 12 Controls. The Lots of Cream Plotted include Cream of Varied Quality and Held at Different Temperatures.

to 80°F. The amount of acid in the inoculated and noninoculated cream as shown by Figure IV was practically the same after the third day.

The effect of cultures on the flavor and odor of the cream. The flavor and odor were noted daily by tasting and smelling the cream after it had been stirred. Sixteen samples of cream containing no culture and 33 samples of inoculated cream were examined. Putrid and metallic flavors and stale odors occurred as frequently in the cultured as in the noncultured cream. Bitter flavors occurred in only three samples of noninoculated cream, whereas this flavor was detected nine times in cultured cream, and usually the bitter flavor was more intense in the cultured cream. A cheese-like flavor ranging from a fairly distinct Roquefort flavor to that of American cheese was noted only three times in inoculated as compared to seven times in noninoculated cream. The most intense cheese-like flavor was noted in the noninoculated cream. Yeasty flavors caused by lactose fermenting torulae were encountered more than any other off-flavor. A distinct yeasty flavor was noted in eight samples of cultured cream and in 15 samples of noninoculated cream. At temperatures of 75, 80 and 98°F. the flavor occurred in both the inoculated cream and the noninoculated control. Only one batch of yeasty flavored cream became definitely gassy; it was noninoculated and was held at 70°F. and had a

yeast count of 200,000 per cc. as determined by the plate method.

Effect of cultures on grade of cream. Each batch of cream was graded at the end of the ten day holding period. Figure V shows a comparison of the grades of 24 inoculated batches of cream and 24 uninoculated controls. Twelve of the inoculated samples were found to grade the same as the controls. Apparently the culture did not have any marked effect on the grade of the cream.

Effect of cultures on the score and keeping quality of the butter. The butter was held at 45°F. and scored when it was fresh and at two week intervals for eight weeks; another set of samples was scored after holding for four months at 0°F.

Figure VI is a diagram showing the difference in score of butter made from inoculated and noninoculated cream. In 11 cases the score of the butter made from inoculated cream was more than that made from the noninoculated controls. Whereas, in eight trials the score of the butter made from the inoculated cream was lower, and in six instances the scores were identical.

In Figures VII and VIII are shown the difference in the scores of butter after holding at 45°F. and 0°F. for two and four months respectively.

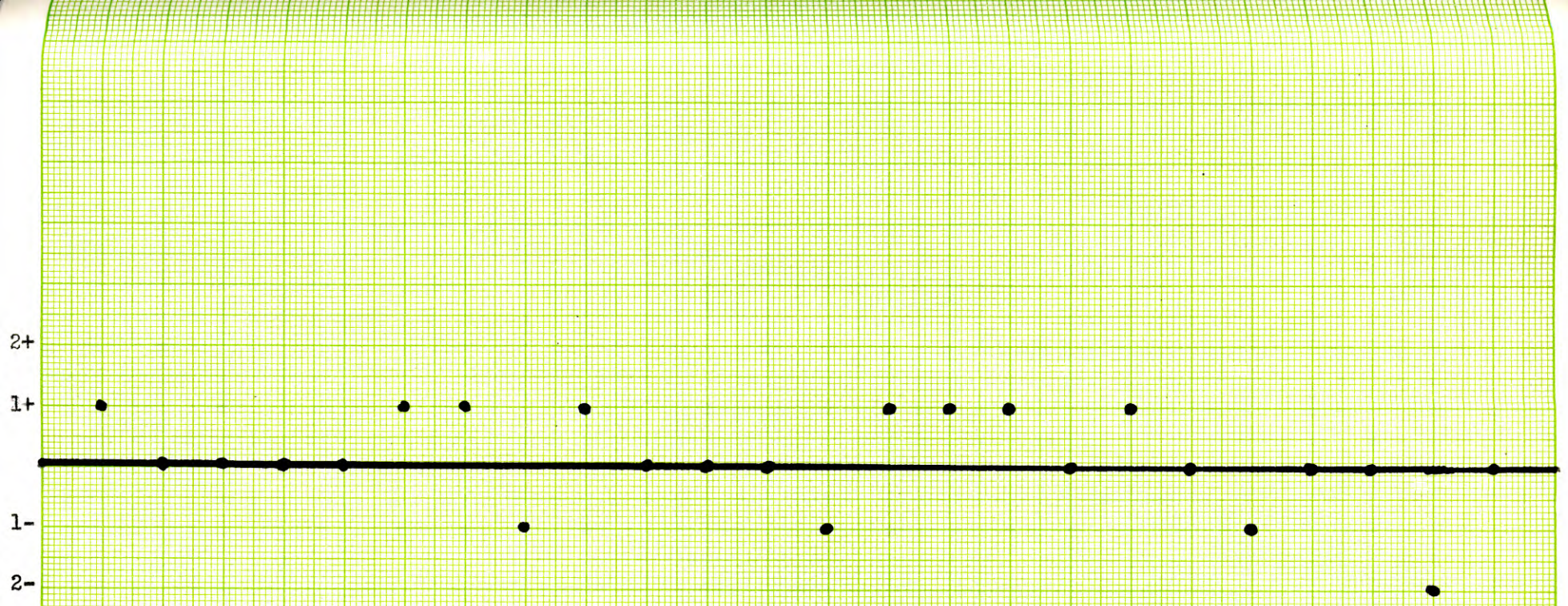

Figure V. Showing The Effect of Lactic Cultures On The Grade of Cream

The heavy line represents the grade of the control cream. The dots on the line indicate grade of inoculated cream same as the control. Dots above the line indicate grade of inoculated cream better than control. Dots below the line indicate grade of inoculated cream less than control.

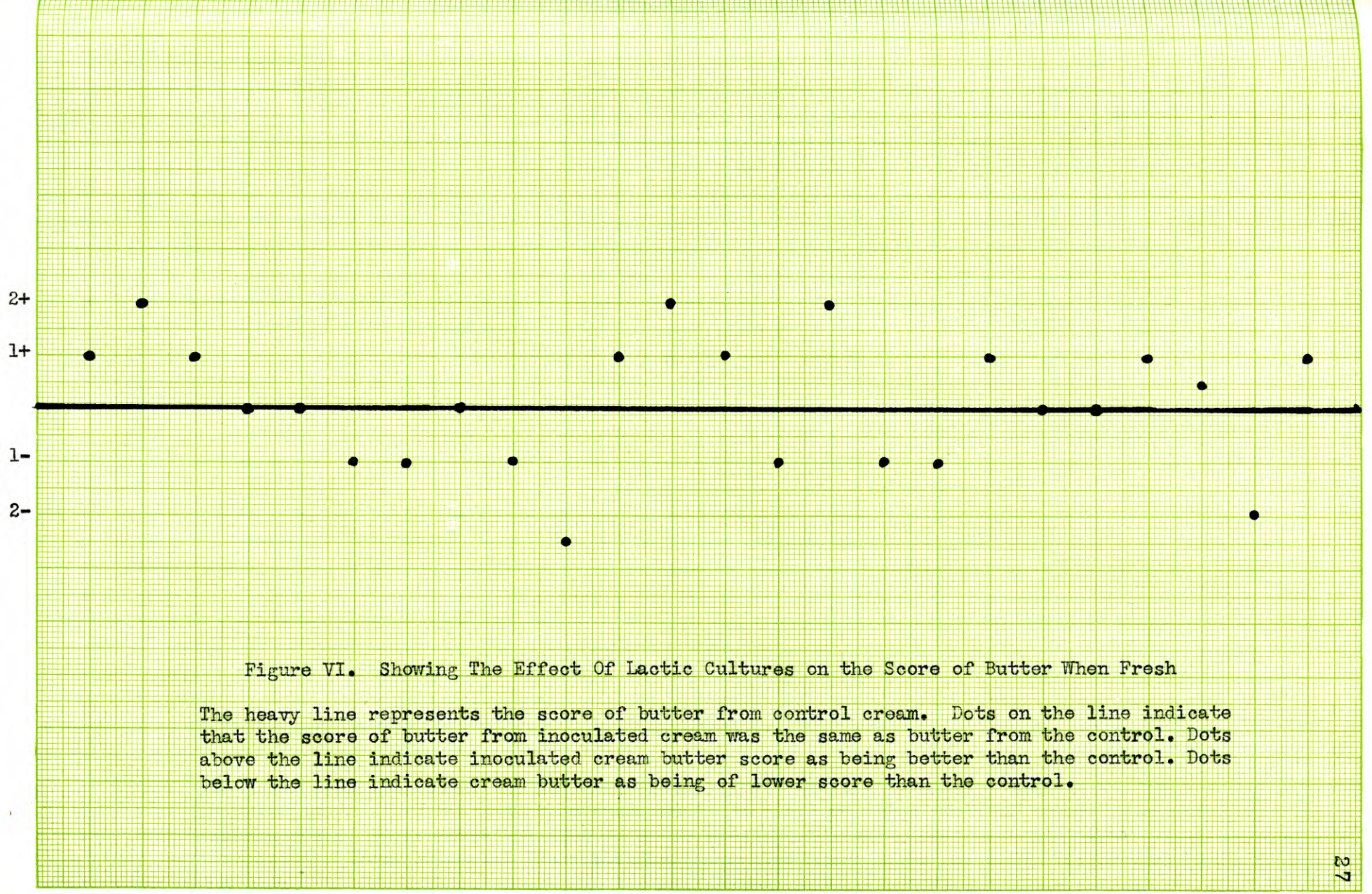

Figure VI. Showing The Effect Of Lactic Cultures on the Score of Butter When Fresh

The heavy line represents the score of butter from control cream. Dots on the line indicate that the score of butter from inoculated cream was the same as butter from the control. Dots above the line indicate inoculated cream butter score as being better than the control. Dots below the line indicate cream butter as being of lower score than the control.

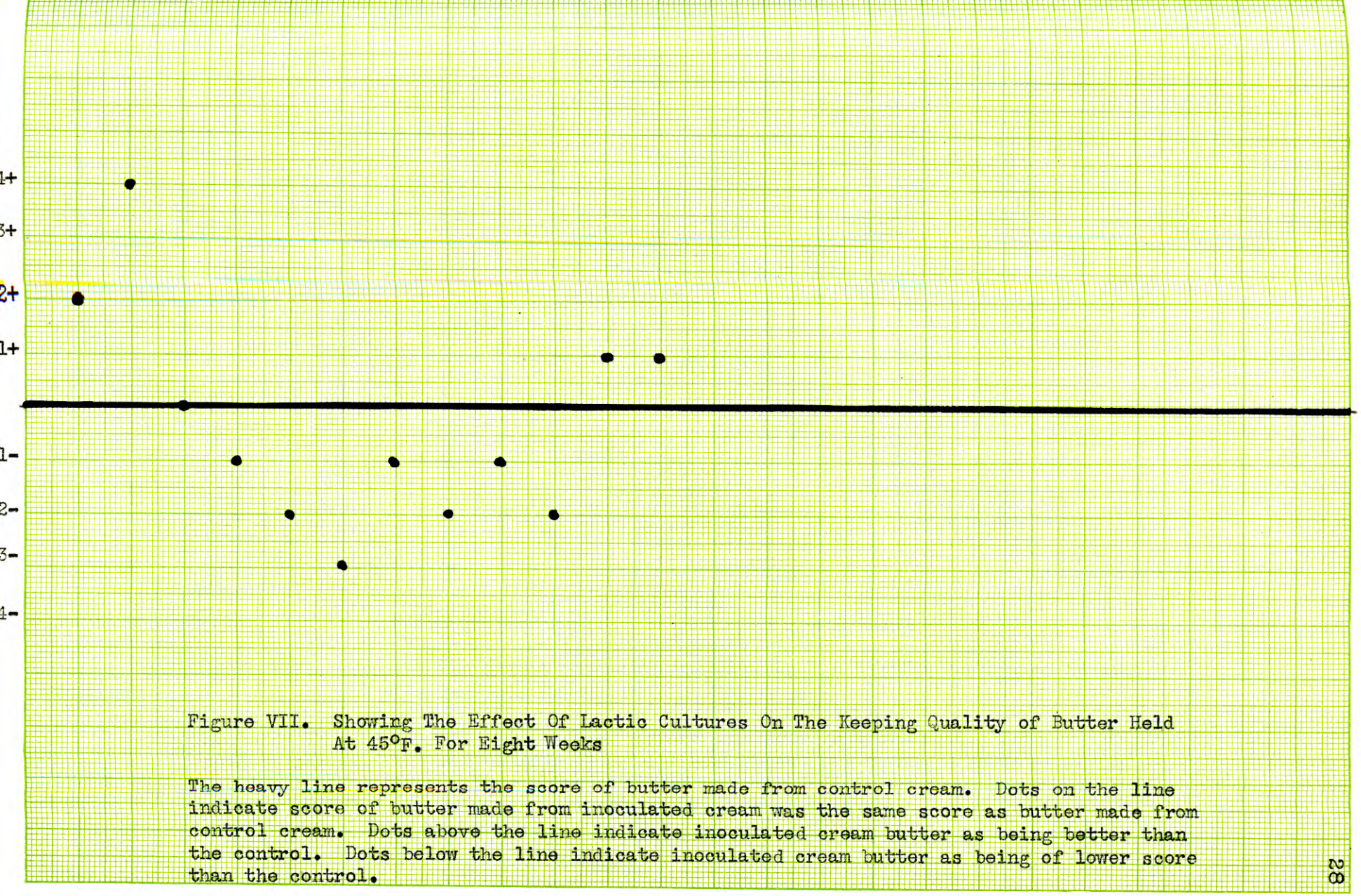

Figure VII. Showing The Effect Of Lactic Cultures On The Keeping Quality of Butter Held At 45°F. For Eight Weeks

The heavy line represents the score of butter made from control cream. Dots on the line indicate score of butter made from inoculated cream was the same score as butter made from control cream. Dots above the line indicate inoculated cream butter as being better than the control. Dots below the line indicate inoculated cream butter as being of lower score than the control.

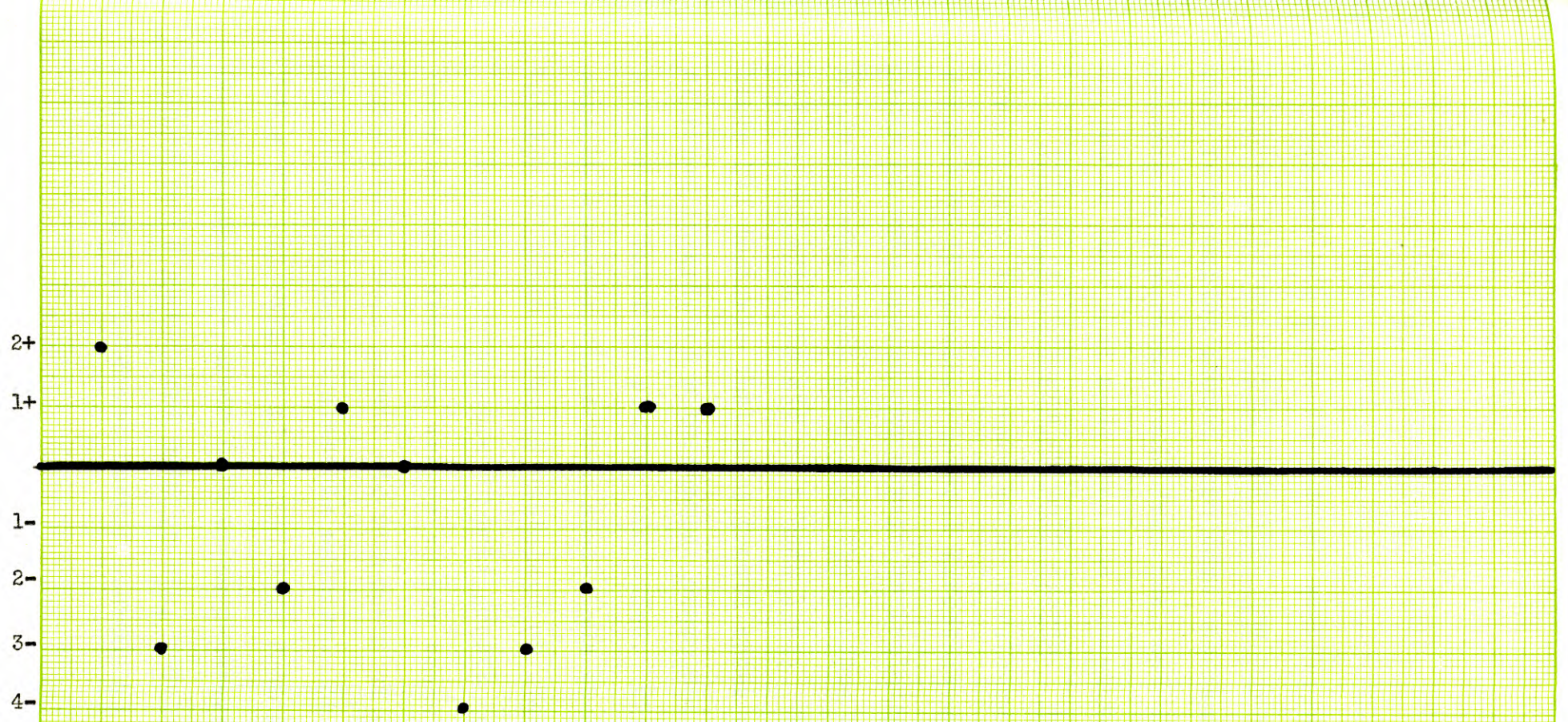

Figure VIII. Showing The Effect Of Lactic Cultures On The Keeping Quality of Butter Held At 0°F. For Four Months.

The heavy line represents the score of butter made from control cream. Dots on the line indicate the score of butter made from inoculated cream was the same score as butter made from the control cream. Dots above the line indicate inoculated cream butter score as being better than the control. Dots below the line indicate inoculated cream butter score as being lower than the control.

Figure VII shows that seven samples of butter made from inoculated cream scored less than the control butter, whereas only four samples scored more than the control. The keeping quality of the butter made from the control cream was slightly better than that made from the inoculated cream.

Effect of cultures on mold growth. Oospora lactis, the common white mold of cream makes a velvety growth on the surface of the cream. Several investigators report that the oidia or mold spores can always be isolated from any sample of raw cream. Mold growth on the surface of the cream was determined with the aid of a hand lens. The results in Table III indicate that mold growth appeared on 19 samples of cream inoculated with starter organisms and only on nine samples to which no starter was added. This can not be considered a marked difference since less than one third of the batches of cream observed contained no starter.

The first appearance of mold growth on the cream was found to vary from the second to the tenth day of the holding period. Mold growth did not occur on any cream with less than 0.46 per cent titratable acidity. As indicated in Table III, 11 samples of butter made from moldy cream scored 90 or above. All cream held at temperatures of 75°F. or higher had a pronounced mold growth, resulting in third grade cream and produced butter scoring 88 or less. All

cream held at 75°F. or above not only showed heavy mold growth after a few days, but also showed pronounced off-flavors resulting from the growth of other micro-organisms at the high temperature of storage. Obviously, the low score of butter made from such cream should not be attributed to moldiness alone.

Effect of acidity on growth of Oospora lactis. In order to determine the effect of acidity on mold growth in absence of bacterial action, sterile cream was inoculated with a pure culture of O. lactis. Fifty cubic centimeter portions of the inoculated cream were transferred to sterile jars. Acidities of these samples were adjusted to values ranging from 0.12 to 1.17 per cent and with pH values of 6.0 to 3.2 as determined by the quinhydrone electrode. Duplicate samples of these were poured into Petri plates, incubated at 70°F. and observed daily for eight days.

The data in Table IV indicates that mold growth did not occur on cream testing 0.35 per cent acid or less, and with pH of 5.0 or more. Extensive mold growth was observed on all cream testing 0.41 per cent acid or more and a pH of 4.8 or less. Inasmuch as the cream was sterile until inoculated with pure culture of mold, the results indicate that mold growth is dependent on acid content of cream rather than bacterial association. When replicate samples of the same acidified and inoculated cream were incubated at 45°F., mold

Table III. Acid Development and Mold Growth in Inoculated and Noninoculated Cream Held at Various Storage Temperatures and their Effect on Final Score of Butter.

Sample No.	Temp. of Cream	First Appearance of Mold Growth on Cream			Final Flavors of Cream	Cream Grade	Butter Score Fresh
		Per cent Acid	Day	Flavor of Cream			
39	70	.46	6		yeasty	2	90
32-C*	70	.46	8	sl. stale	sl. yeasty	1	91
37-C	70	.47	7		yeasty	2	89
31-C	70	.48	8	sl. stale	sl. stale	1	89
38-C	70	.49	7		sl. stale Stale	1	90
13-C	70	.49	5		sl. bitter stale	2	88
9-C	70	.52	6	yeasty slight	putrid sl. musty	3	87
28-C	68	.52	6	metallic stale	metallic cheesy	2	90
10	70	.53	5	yeasty	yeasty stale	3	87
11-C	70	.53	5		sl. yeasty cheesy	2	89
17-C	75	.53	5	sl. stale	sl. yeasty	3	88
30	68	.53	6	sl.yeasty	yeasty yeasty	2	91
43-C	71	.53	5		Sl. bitter musty	2	90
29-C	68	.54	6	metallic	metallic musty	3	88
27-C	68	.54	6	sl. musty	strong stale	2	91
14	70	.55	6	stale	yeasty	3	89
33-C	70	.55	10	cheesy	sl. yeasty cheesy	1	90
18	75	.56	5	yeasty	yeasty yeasty	3	88
6	80	.57	5	yeasty	putrid sl. yeasty	3	87
23-C	68	.57	7	sl.yeasty cheesy	sl. cheesy cheesy	2	90
35-C	70	.58	6	yeasty	yeasty	3	87
				sl.yeasty	yeasty		
12	70	.60	5	stale	putrid	3	92
21	68	.62	8	sl. stale	sl. yeasty yeasty	1	90
25	68	.62	8	metallic sharp	metallic acid, stale	3	87
36-C	70	.66	7	acid	yeasty cheesy	3	87
5-C	80	.76	5	stale sharp	bitter bitter	3	87
40-C	98	1.10	2	acid sharp	yeasty bitter	3	88
41-C	98	1.18	2	acid	yeasty	3	88

* Containing lactic cultures

Table IV. Effect of per cent of Lactic Acid on Mold Growth in the Absence of Bacterial Action in Cream for 8 Days at 70°F.

cc. of 10 per cent Acid added to 50 cc. Portions of Cream	Per cent acid in cream	The pH value of cream	Day Mold Growth Appeared
Control, sterile Cream			
0	.12	6.0	no mold
3.2	.60	4.2	" "
Cream Inoculated with O. lactis			
0	.12	6.0	no mold
2.5*	.23	5.8	" "
5.0*	.26	6.4	" "
7.5*	.35	4.9	" "
1.0	.31	5.1	" "
1.25	.35	4.9	" "
1.50	.41	4.8	3
3.25	.63	4.1	2
3.50	.72	4.0	2
3.75	.77	3.87	2
4.00	.78	3.82	2
4.25	.83	3.79	2
4.50	.86	3.70	2
4.75	.89	3.68	2
5.00	.95	3.47	3
5.25	.98	3.39	3
5.50	1.03	3.34	2
5.75	1.07	3.32	2
6.00	1.12	3.27	2
6.25	1.15	3.25	2
6.50	1.17	3.24	2

* One per cent Acid

growth was not evident after 25 days. There was, however, a marked stale odor in those samples which had been inoculated with mold which was not evident in the sterile controls.

Effect of cultures on yeast count in cream. Yeasty flavors occurred less frequently in inoculated cream than in uninoculated cream. To determine the effect of addition of lactic cultures to cream on yeast growth, counts were made on six batches of inoculated cream and one control held at 70°F. for the regular ten day period.

Table V. The Effect of Various Lactic Cultures on Acidity and Yeast Count of Cream.

Cultures Used	Final Acidity	Yeast Count per cc.
Control (no culture)	.85	200,000
Slow Curdling (S. lactis)	.70	30,000
" " "	.75	25,000
Fast Curdling "	.74	15,000
" " "	.72	5,000
Number 122 (butter culture)	1.67	0*
" " " "	.96	0

* No yeast colonies on a 1:1,000 dilution.

The results in Table V show that the cultured cream had an average yeast count of 12,500, as compared to 200,000 for the noncultured control. Although the significance of the results of this single experiment is limited, the results do concur directly with the repeated observation made throughout this work, namely, that there was a definite tendency for inoculated cream to show yeasty flavors less often than the noninoculated cream.

SUMMARY

The practice of adding cultures to cream at the time of production failed to show any significant improvement in the quality of the finished butter, as indicated by results obtained in 49 experimental churnings. Addition of cultures accelerated acid development during the first 24 hours and apparently checked the growth of yeast and of bacteria which produce cheese-like flavors. The addition of cultures, apparently did not prevent growth of organisms responsible for the bitter flavor in cream, and may have accentuated it. Mold growth was not accelerated by the addition of cultures to cream. This would be expected since the acidity was about the same after the first two days in the inoculated and noninoculated cream. When noninoculated cream is held at temperatures of 70°F. or less, the flora seems to be sufficiently controlled by the lactic acid producing organisms which get into cream during production, to prevent undue deterioration in a reasonable holding period. The addition of cultures to cream for the purpose of improving its quality seems to have some possibilities. However, it should not be recommended as a general practice until further work can be done to demonstrate its value under farm and commercial conditions.

CONCLUSIONS

1. The rate of development of acid during the first 24 hours was greater in the cream to which culture had been added than in the noninoculated cream. The final acidity was about the same in cultured and noncultured cream.

2. Putrid and metallic flavors and stale odors occurred in as many samples of cultured cream as in noncultured cream.

3. Bitter flavors occurred more often in cultured samples.

4. Yeasty and cheese-like flavors occurred more in the noninoculated cream than in the inoculated cream.

5. The rate of mold growth was not materially affected by the inoculation of the cream with lactic acid producing cultures of bacteria.

6. Mold growth did not appear on any cream having a titratable acidity of less than 0.46 per cent.

7. Mold growth is apparently more dependent on the acid content of the cream than on bacterial association.

8. There is evidence to indicate that the inoculation of cream with lactic acid producing organisms tends to curtail the development of yeast.

9. There was no marked difference in the score and keeping quality of the butter made from inoculated and non-inoculated cream.

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* Original not seen.