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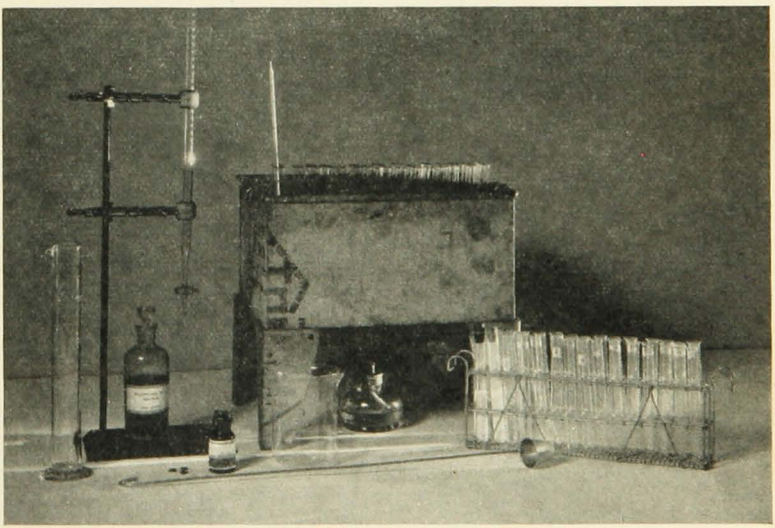

Apparatus Needed for the Methylene Blue Reduction Test

UNIVERSITY FARM, ST. PAUL

EXPERIMENTS WITH THE METHYLENE BLUE REDUCTION TEST FOR THE GRADING OF SWEET CREAM[1]

H. MACY

INTRODUCTION

The methylene blue reduction test has been widely used in this country and abroad for the grading of milk but not so much for cream. In sections where sweet cream is the major raw product of the creameries, there is a distinct need for a specific test that will supplement the systems of grading now in vogue, namely, taste, odor, or acidometry. It is a well-known fact that cream may be sweet but, nevertheless, of inferior quality from a bacteriological standpoint—thus indicating unsatisfactory sanitation conditions, insufficient cooling, or prolonged storage—as well as from the standpoint of undesirable flavors and odors other than those associated with the acid fermentation. The general movement toward improving the quality of cream used for buttermaking has emphasized the need for a rapid and simple test that will distinguish between inferior and superior lots of sweet cream. With this in mind, rather extensive studies of the usefulness of the so-called "reductase test" have been made at the University Farm and at representative Minnesota creameries.

METHODS

The tests were made according to the directions embodied in the Standard Methods of Milk Analysis except as indicated later (see Appendix). The standard tablets prepared by the National Aniline Company were used for the methylene blue solutions.

Preliminary Experiments

Preliminary studies were undertaken at the University Farm using cream obtained from milk produced by the University dairy herd.

A standard methylene blue solution was prepared according to the Standard Methods of Milk Analysis, namely: one tablet dissolved in 200 milliliters of water; originally, one milliliter of this solution was added to each ten milliliters of cream. This solution was found to be incapable of imparting sufficient color to cream to make an accurate

[1] The author gratefully acknowledges the helpful assistance of F. E. Nelson, S. T. Coulter, and E. O. Herreid in collecting the data, and the courtesy of those creameries and organizations which made the facilities of their plants available for these studies.

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reading possible, especially when the fat percentage was high. Consequently, trials were made using two milliliters of the standard solution, or one milliliter of a double- or triple-strength solution. The latter was found to yield the best results with average cream, altho the reduction time was somewhat prolonged. When the time required to reduce the color produced in cream by one milliliter of the standard solution was established as the unit, it was found that with two milliliters, the time was increased in the ratio 1:1.36; with one milliliter of double-strength solution, 1:1.37, and with one milliliter of triple-strength solution, 1:1.65. The range in relative reduction times is shown in the following:

Strength of solution	Number of milliliters	Distribution of samples according to ratio between standard and trial solutions				
		Ratio 1:1	Ratio 1:1 to 1:1.9	Ratio 1:2 to 1:2.9	Ratio 1:3 to 1:3.9	Ratio 1:4 to 1:5
		Number of samples				
Standard	2	19	11	4	2	0
Double	1	18	12	4	2	0
Triple	1	13	10	9	3	1

The prolongation of the reduction time was as evident in short as in long reduction periods. The deeper original color of the cream resulting from the use of triple-strength solutions made the readings more certain and more than compensated for any increase in time required for reduction. The triple-strength solution was adopted for the studies presented subsequently in this report.

Relationship of Reduction Time, Bacterial Content, and Acidity

The methylene blue test, (see Appendix) with the triple-strength solution, was applied to 159 samples of cream from the University supply and nearby creameries. The reduction time, percentage of butterfat and acidity, Breed microscopic count, standard plate counts at 37° C. (98.6° F.) after two days incubation, and plate counts at 20° C. (68° F.) after five days incubation were observed.

The relation between the reduction time and the bacterial counts by the three methods are graphically illustrated in Figures 1, 2, and 3. It will be noted that the reduction time is inversely proportional to the number of bacteria as determined by the plate or microscopic methods. The agreement with data for milk is quite evident. The few instances where samples stray from the straight line relationship are not unexpected. The location of the samples containing starter (represented by "S") in the three graphs is interesting and illustrates the difficulty of obtaining satisfactory counts of such products with the ordinary media and incubation temperatures. It indicates as well the activity of the starter organisms as reducing agents.

Table 1

Relation Between Bacterial Counts and Reduction Time

Reduction time, minutes	Logarithmic average of		
	Direct microscopic count	20° C. (68° F.) count	37° C. (98.6° F.) count
Less than 25.....	8.282	7.985	7.826
25 to 105.....	7.528	7.518	7.274
120 to 300.....	6.428	5.969	5.858
Over 300	5.280	4.646	4.319

Note: For those not familiar with logarithms, the data above may be interpreted by the following:

4.000 represents	10,000 per milliliter
5.000 represents	100,000 per milliliter
6.000 represents	1,000,000 per milliliter
7.000 represents	10,000,000 per milliliter
8.000 represents	100,000,000 per milliliter

Table 1 presenting the averages of the logarithms of the bacterial counts further indicates that there is a direct correlation between the reduction time and the bacterial content of the cream. The data also show that the microscopic method gave the highest average count, and the standard 37° C. (98:6° F.) incubation, the lowest. The bacterial counts, that could be made, ranged from 500 to 520,000,000 per milliliter at 37° C. (98.6° F.), and 1,000 to 670,000,000 at 20° C. (68° F.). Microscopic counts of less than 120,000 could not be determined satisfactorily, and the highest count that could be made accurately was 1,400,000,000 per milliliter. The relationships existing between the counts obtained by the three methods are illustrated in more detail by the following analysis of the data:

The counts on plates incubated at 20° C. (68° F.) for five days were:

(a) for all normal samples—1.720 times higher,
(b) where counts were 1,000,000 or less—1.919 times higher,
(c) where counts were 1,100,000 to 50,000,000—1.755 times higher,
(d) where counts were over 50,000,000—1.398 times higher,
(e) where samples contained starter—1.553 times higher,

than the counts on plates at 37° C. (98.6° F.) for two days.

The microscopic counts were:

(a) for all normal samples—5.124 times higher,
(b) where counts were 1,000,000 or less—8.397 times higher,
(c) where counts were 1,100,000 to 50,000,000—4.373 times higher,
(d) where counts were over 50,000,000—3.037 times higher,
(e) where samples contained starter—37.121 times higher,

than the counts on plates at 37° C. (98.6° F.) for two days.

These data will be useful in interpreting the results given subsequently.

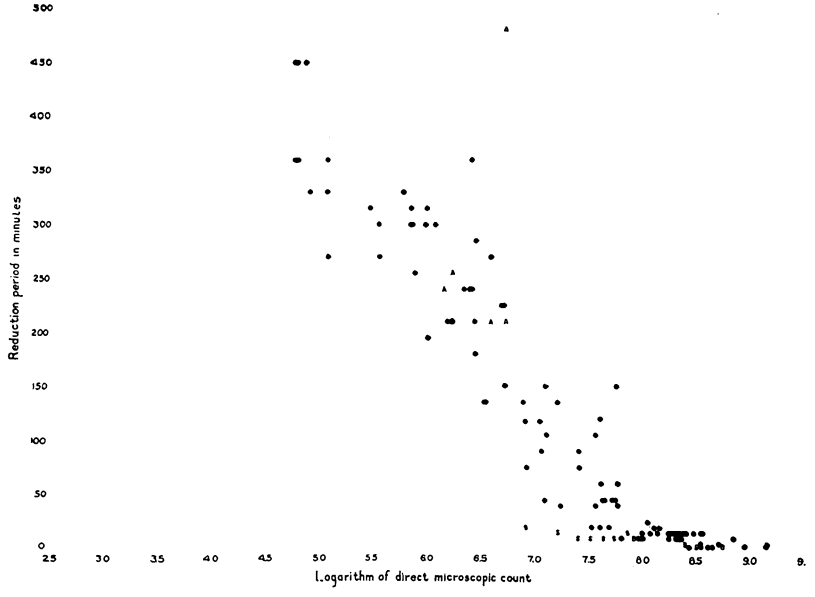

Fig. 1. Relation between the methylene blue reduction time of cream and the bacterial content as determined by the microscopic method.

Note: In Figures 1, 2, 3, and 4, the dots represent normal samples of cream, "A" indicates addition of an acid, "B" the addition of a base, and "S" the addition of a starter culture.

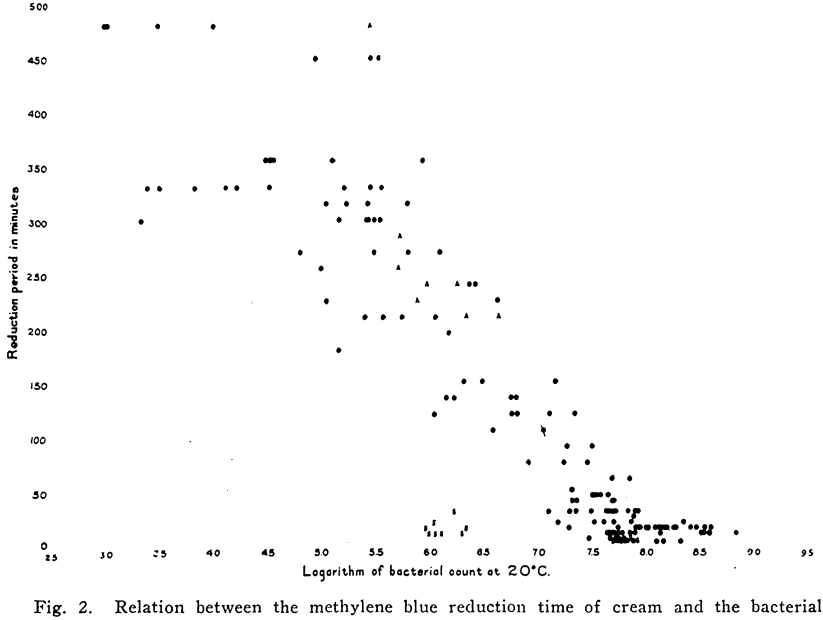

Fig. 2. Relation between the methylene blue reduction time of cream and the bacterial content as determined by the plate method with incubation at 20° C. (68° F.) for five days.

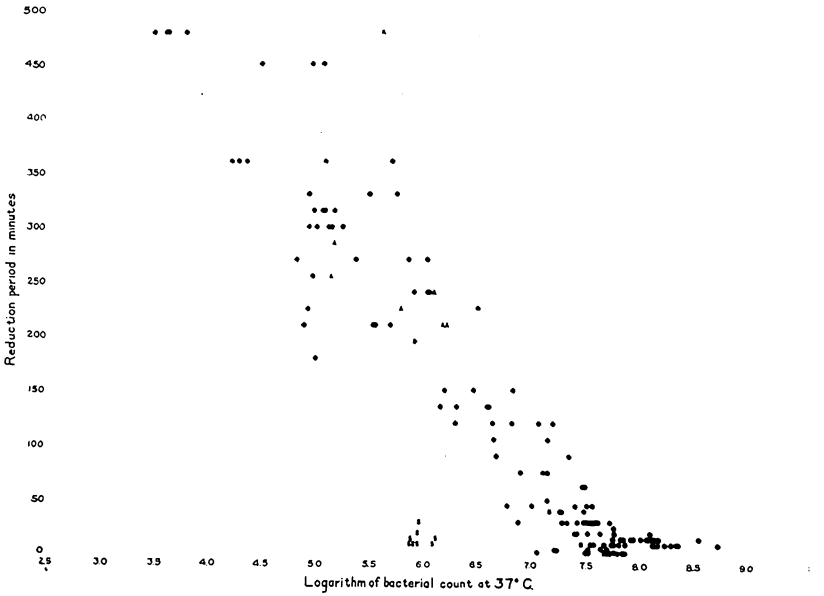

Fig. 3. Relation between the methylene blue reduction time of cream and the bacterial content as determined by the plate method with incubation at 37° C. (98.6° F.) for 48 hours.

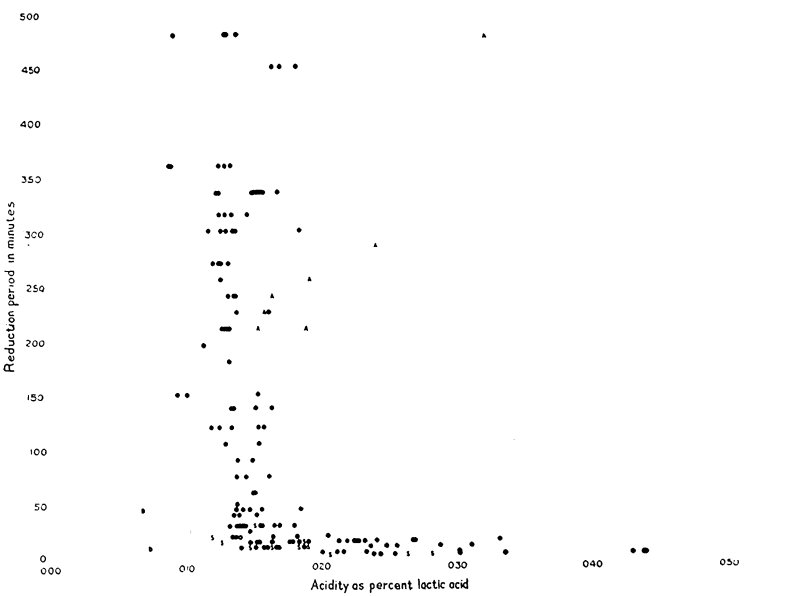

Fig. 4. Relation between the methylene blue reduction time and the acidity of cream.

The relation between the acidity of the cream and the reduction time is shown in Figure 4. Here it will be noted that with two exceptions, in both of which acid had been added, all samples with acidities over 0.21 per cent reduced in 15 minutes or less. The position of the samples that had been neutralized emphasized the lack of direct relationship between titrable acidity and reduction time. The reduction time of samples with acidities of 0.20 per cent or less varied between 8 minutes and 8 hours, with the majority requiring 30 minutes or more. If the data are analyzed for "sweet" cream (acidity of 0.20 per cent or less), it is obvious that there is no significant correlation between such natural acidity of cream and the reduction time. This fact is further borne out by the following comparison:

Reduction time	Acidity of samples in each group	
minutes	average per cent	range in per cent
Less than 25	0.227	0.135—0.440
25 to 105	0.149	0.134—0.186
120 to 300	0.137	0.096—0.189
Over 300	0.145	0.092—0.208

The acidity of certain samples was increased by the addition of one per cent lactic or tartaric acid, but there was no significant change in reduction time when the results were compared with check samples. The same held true when acidities were reduced by the addition of N/1 NaOH. On the other hand, the inoculation of sweet cream with small quantities of butter culture led to a marked decrease in reduction time. Samples that had required several hours to reduce lost their color in 2 to 30 minutes even tho the acidity was increased only slightly. This demonstrates the marked reducing capacity of starter organisms.

The data indicated that the acidity of the cream samples was correlated with the bacterial content as shown below:

	Logarithms of		
Acidity, per cent	Direct microscopic count	20° C. (68° F.) count	37° C. (98.6° F.) count
Less than 0.150	av. 6.6245 min. 4.778 max. 8.130	av. 6.1874 min. 3.030 max. 7.920	av. 6.0199 min. 3.477 max. 7.755
0.150 to 0.199	av. 7.3570 min. 4.778 max. 8.365	av. 6.8105 min. 3.380 max. 8.342	av. 6.5701 min. 2.698 max. 8.130
0.200 to 0.249	av. 8.0811 min. 4.778 max. 8.591	av. 7.8992 min. 5.462 max. 8.826	av. 7.6619 min. 4.954 max. 8.716
0.250 or more	av. 8.6631 min. 8.298 max. 9.146	av. 8.1915 min. 7.477 max. 8.600	av. 7.9524 min. 7.505 max. 8.540

These results also demonstrate that cream that is "sweet," in the ordinarily accepted sense, may nevertheless contain large numbers of bacteria.

The effect of the butterfat on the reduction time is shown in the results given below. The creams in each group were separated from the same whole milk and the bacterial counts were essentially the same in each group.

Butterfat per cent	Reduction time minutes	Butterfat per cent	Reduction time minutes
Sample A		Sample D	
14.7	135	14.7	Held at 20° C. for 10 hours. 15
18.5	135	18.5	
25.5	120	25.5	
51.7	120		
Sample B		Sample E	
11.2	480	14.7	Held at 60° C. for 78 hours. 30
14.8	480	18.5	
16.9	480	25.5	
48.2	480		
Sample C		Sample F	
14.7	Held at 37° C. for 4 hours. 15	17.5	360
18.5	10	39.1	360
25.5	10	57.0	360

Further studies with cream standardized by skimmilk gave the results illustrated below:

Butterfat per cent	Reduction time minutes	Butterfat per cent	Reduction time minutes
Sample A		Sample B	
18.3	360	17.2	315
29.3	210	26.4	315
38.5	180	35.5	315

There seems to be some tendency for the samples with higher fat percentages to reduce more rapidly than the thinner creams, but this is not a constant relationship. The differences are too slight to have any great significance.

Observations on the Use of Methylene Blue Reduction Test in Creameries

The application of the methylene blue reduction test under practical conditions was made in three typical Minnesota creameries.

The first observations were made at the Minnesota State Experimental Creamery at Albert Lea (Creamery A) and included bacterial counts by the microscopic method and reduction tests on samples of sweet cream during the four seasons of the year, including the months of August and November, 1932, and January, March, and April, 1933. An additional set of observations was made at another creamery (Creamery B) in September, 1932. Samples were taken in the weigh-room as cream was delivered by the patrons. One man was able to make reduction tests and prepare smears for 130 lots of cream a day.

The readings on reduction time were made in the usual way. The data were assembled under grades according to the usually accepted classification, namely, good, fair, unsatisfactory, very unsatisfactory.

At Creamery A, 614 samples of cream were taken in August, 258 in November, 394 in January, and 456 in March-April. At Creamery B, 304 samples were taken. This made a total of 2,026 samples.

The distribution of samples according to bacterial count was as follows:

Microscopic count per milliliter	Per cent of samples
61,000,000 or more	13.6
31,000,000–60,000,000	12.7
15,600,000–30,000,000	15.5
6,600,000–15,000,000	19.7
3,000,000– 6,000,000	12.0
600,000– 2,400,000	17.4
120,000– 480,000	7.8
Less than 120,000	1.3

The reduction periods for the 2,026 samples were as follows:

Grade	Reduction time in minutes	Per cent of samples
Good	Less than 25	27.6
Fair	25 to 105	26.7
Unsatisfactory	120 to 300	27.3
Very unsatisfactory	More than 300	18.4

Table 2 indicates the relationship between reduction time and bacterial content. A direct correlation exists between the number of bacteria and the time required for the reduction of methylene blue. If the ordinary ratio between microscopic and plate counts, or the ratio determined

in the preliminary experiments, is taken into consideration, it will be seen that the various grades set up by standard methods for milk fit in reasonably well with these results obtained on cream.

Table 2

Distribution of Samples of Sweet Cream According to the Methylene Blue Reduction Time, and The Number of Bacteria as Determined by the Microscopic Method (2,026 samples)

Grade according to standard methods	Reduction time, minutes	Distribution of samples according to microscopic count, per cent							
		61,000,000 or more	31,000,000 to 60,000,000	15,600,000 to 30,000,000	6,600,000 to 15,000,000	3,000,000 to 6,000,000	600,000 to 2,400,000	120,000 to 480,000	Less than 120,000
Very unsatisfactory	Less than 25	48.8	35.2	15.5	0.5	0.0	0.0	0.0	0.0
Unsatisfactory	25-105	0.4	11.3	40.6	42.2	5.2	0.3	0.0	0.0
Fair	120-300	0.0	0.0	1.4	29.8	34.0	28.9	5.9	0.0
Good	More than 300	0.0	0.0	0.0	0.9	7.2	51.2	33.5	7.2

The average reduction time for samples with different bacterial counts is shown in Table 3. There is a marked consistency in results during the year at the two creameries. The seasonal effect is noticeable, indicating the relationship of temperature to the numbers of bacteria. However, the general tendency is the same throughout.

Table 3

Reduction Time for Cream Samples in Relation to Bacterial Content (2,026 samples)

Number of bacteria by microscopic method	Average reduction time					
	Creamery A August	Creamery B September	Creamery A November	Creamery A January	Creamery A March-April	All samples
per milliliter	minutes	minutes	minutes	minutes	minutes	minutes
61,000,000 or more.....	6	9	11	9	7	7
31,000,000–60,000,000.....	16	19	29	24	18	19
15,600,000–30,000,000.....	32	34	63	46	39	38
6,600,000–15,000,000.....	89	83	153	117	119	107
3,000,000– 6,000,000.....	154	174	268	216	214	205
600,000– 2,400,000.....	238	293	408	347	328	334
120,000– 480,000.....	305	373	480	440	432	413
Less than 120,000.....	...	490	555	520	452	498

Subsequent visits were made at another creamery (Creamery C) during August, 1933, and a further test at Creamery B at the same time. In these two cases, bacterial counts were not made, but 975 samples were checked for reduction time.

The results of all trials at the three creameries are summarized in Table 4. The remarkable uniformity of results during August is noticeable. In September, the weather shows its effect, resulting in fewer poor samples. This is further accentuated in November when the nights are cold and cooling is more certain. In January, the atmospheric temperature is much lower, and dairymen in attempting to keep cream from freezing expose it to higher temperatures, thus giving results less satisfactory than in November. The climatic conditions have changed somewhat in March and April, with warmer nights but less danger of freezing, and the results are about the same as in January. The effect of the lack of cooling in August is evident.

Table 4

Distribution of Samples of Sweet Cream According to Grade at Various Seasons of the Year (3,001 samples)

Grade according to standard methods	Distribution of samples according to grade							
	Creamery A Aug.	Creamery B Aug.	Creamery C Aug.	Creamery B Sept.	Creamery A Nov.	Creamery A Jan.	Creamery A Mar.-Apr.	All samples
	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
Very unsatisfactory..	43.0	50.3	52.4	25.7	4.7	23.9	24.5	35.4
Unsatisfactory	30.6	28.4	23.8	33.5	14.7	24.3	25.4	26.5
Fair	24.3	18.2	20.4	25.3	34.1	26.4	29.6	24.7
Good	2.1	3.1	3.4	15.5	46.5	25.4	20.4	13.5

These observations are substantiated by the data presented in Table 5, in which the calculated average reduction times for the different grades are given. The same general uniformity of results in August, at the three creameries, and the seasonal changes agree closely with the results shown in Table 4.

Table 5

Average Reduction Time for Each Grade of Cream Classified According to Standard Methods (3,001 samples)

Grade according to standard methods	Average reduction time							
	Creamery A Aug.	Creamery B Aug.	Creamery C Aug.	Creamery B Sept.	Creamery A Nov.	Creamery A Jan.	Creamery A Mar.-Apr.	All samples
	minutes	minutes	minutes	minutes	minutes	minutes	minutes	minutes
Very unsatisfactory ...	10	10	10	13	15	11	13	11
Unsatisfactory	53	55	58	54	56	52	54	55
Fair	197	190	192	215	213	200	202	201
Good	365	346	367	405	460	431	410	424

At creamery B in August, 1933, visits were made to various dairymen to observe the conditions under which cream was produced and handled. The results of some of these visits are given below:

Patron	Cream grade according to reduction time	Cooling device	Cooling medium	Temperature of water	Care of separator	General sanitation	Remarks
14	Good	IN-WT	IW	43° F.	2x	VG	
17	Good	WT	IW	42° F.	2x	VG	
153	Good	WT	IW	43° F.	2x	VG	
85	Fair	WT	IW	46° F.	1-2x	VG	
98	Fair	CT	IW	49° F.	2x	VG	
60	Fair	CT	IW	49° F.	2x	F	MM
92	Fair	WT-WP	W	51° F.	2x	G	
238	Fair	WT	W	50° F.	2x	VG	
39	Fair	WT	W	56° F.	2x	VG	MM
19	Bad	ST	W	54° F.	2x	G	
171	Very bad	WT	W	56° F.	1x	F	
4	Very bad	WT	IW	46° F.	2x	VG	
127	Very bad	WT	W	57° F.	1x	F	
64	Very bad	WT	W	61° F.	1x	P	

IN = Insulated
WT = Wooden tank
CT = Concrete tank
WP = Well pit
ST = Stock tank
W = Water
IW = Ice water

1x = Separator washed once daily
2x = Separator washed twice daily
VG = Very good
G = Good
F = Fair
P = Poor
MM = Milking machine

This brief report of the correlation, between farm conditions and the grade of cream according to reduction time, is suggestive. The influence of temperature, methods of cooling, and sanitation are quite striking and present possibilities as to avenues of approach in the general program for cream improvement.

It was also observed during the course of these studies that certain patrons consistently produced excellent or poor cream. This may be taken to mean that their practices were good or bad. To show these tendencies, the following results from Creamery A are given:

Patron	Reduction time in minutes for samples taken during			
	August	November	January	March-April
23.....	300-240-360-360-240-60	600-720	540-540-330	540-540-540-510
122.....	390-360-420-390-300	600-660	540-420-450	510-540-390-420
160.....	150-360-240-360	360-480	480-480-450	540-540-420-390
72.....	10-5-5-40-5-75	30-60	20-30-45	45-15-10-45
119.....	2-2-10-2-5-2	5-60-240	5-5-5	5-5-5-10
77.....	5-5-20-45-5-5	270-570	360-330-195	5-5-90
89.....	10-15-10-5-30-5	150-240	240-240-180	45-45-15-30

Inspections of these farms revealed the fact that Patrons 23, 122, and 160 were operating their dairies under satisfactory sanitary conditions and cooled the cream adequately during all seasons of the year, while the others were either careless about their methods of production or cooled the cream insufficiently. The better results obtained by Patrons 77 and 89 during the winter months are to be attributed primarily to lower temperatures prevailing at that time, rather than to any radical changes in sanitation.

The results obtained from these experiments in the field make it clear that the methylene blue reduction test is a practical and reasonably accurate method for determining the quality of sweet cream received at the creameries in this state. Further trials are advisable in a larger number of creameries, especially in connection with a program involving farm inspection and improvements in sanitation, cooling, and delivery.

Observations on Churnings of Butter made from Cream of Different Grades as Indicated by the Methylene Blue Reduction Test

At Creameries B and C during August, 1933, samples of cream were selected to represent the good and poor grades of cream. Sufficient cream was taken to constitute a churning in a Dazey churn. The cream was pasteurized at 65.5° C. (150° F.) for 30 minutes. At Creamery B it was cooled to 4.5° C. (40° F.) and held for 12-14 hours before churning. At Creamery C the cream was cooled to 9-10° C. (48-50° F.) and churned after two hours. Further trials were made at Creamery A, using raw cream. The butter was stored at 2° C. (35.6° F.) for periods ranging from two to six weeks.

The results are presented in Tables 6 and 7.

At Creamery C the scores on unsalted butter were somewhat better as a rule at the end of four weeks storage in the case of butter from the better cream, but this was not true after six weeks storage. In the latter instance neither group of samples was especially good.

The results at Creamery B with unsalted butter were somewhat more favorable toward the better cream, but the results were not altogether consistent.

With salted butter, the samples made from the better cream at both creameries showed a tendency toward better keeping quality. There were some exceptions to this, however.

The raw cream butters, made at Creamery A, were much alike in quality after storage. If there was any advantage at all, it was in favor of the cream with the higher reduction time.

These preliminary observations on the usefulness of the methylene blue reduction test for selecting cream for making superior butter are not extensive enough to justify any general conclusions. There are so many factors involved in the deterioration of butter that one may not expect results which are altogether consistent. Further work should be done in this connection, however, to determine whether or not cream of low bacterial content is more desirable for buttermaking, or whether the reduction test is simply useful as a measurement of the sanitation, cooling procedure, and frequency of delivery.

Table 6

Scores on Butter Made From Cream of Different Grades as Determined by Methylene Blue Reduction Time

Creamery	Date	Average reduction time in minutes	Unsalted butter			Salted butter	
			Score after storage at 2° C. (35.6° F.) for			Score after storage at 2° C. (35.6° F.) for	
			2 weeks	4 weeks	6 weeks	4 weeks	6 weeks
C	8-14-33	15	93	87	86	91	92
C	8-14-33	285	93	92	88	92	92
C	8-15-33	13	92½	92	88	92	91
C	8-15-33	400	93	93	86	91	92½
C	8-16-33	15	91	90	88	88	90
C	8-16-33	320	93	93	85	91	93
C	8-17-33	14	93	93	91½	90	92½
C	8-17-33	280	93	92	89	91	92½
C	8-18-33	12	93	90	90	92	92½
C	8-18-33	300	93	93	92	91	92½
B	8-11-33	5	91	91	91	91	91
B	8-11-33	14	91	92	91	90	90
B	8-11-33	360	93	92	92	91	92
B	8-14-33	5	93	92	93	92	91
B	8-14-33	5	92	92	91	92	90
B	8-14-33	100	92½	93	93	92	92
B	8-14-33	270	93	90	90½	92	93
B	8-17-33	8	92	93	92	92	91
B	8-17-33	14	92	93	88½	91	91
B	8-17-33	160	93	92	90	92	92½
B	8-17-33	310	92	93	93	92	92
B	8-19-33	5	92	92	92	92	92
B	8-19-33	6	92	92	92½	92	91
B	8-19-33	225	93	93	93	92	92
B	8-19-33	350	93	93	93	93	92

Table 7

Scores on Butter Made From Raw Cream of Different Grades as Determined by Methylene Blue Reduction Time (Creamery A)

Sample	Date	Average reduction time in minutes	Type of butter	Score after storage at 2° C. for			
				2 weeks	4 weeks	6 weeks	
A	3-29-33	11	Unsalted	91	88	86	Putrid
B	3-29-33	453	Unsalted	91	89	86	Fat hydrolysis
A&B	3-29-33	...	Unsalted	90	88	85	Putrid Cheesy
C	3-31-33	83	Unsalted	91	89	86	Cheesy Putrid
D	3-31-33	283	Unsalted	92	89½	87	Unclean
C&D	3-31-33	...	Unsalted	91½	88½	86	Cheesy Putrid
A	3-29-33	11	Salted	92	90	89	Old Unclean
B	3-29-33	453	Salted	92	91	88	Tallowy Old
A&B	3-29-33	...	Salted	92	91	90	Old Stale
C	3-31-33	83	Salted	92	91	89	Unclean
D	3-31-33	283	Salted	93	91	90	Old Stale
C&D	3-31-33	...	Salted	92	91	89	Unclean

Note: Samples A&B and C&D represent mixtures of good and bad cream.

SUMMARY

1. Experiments on the use of the methylene blue reduction test in the grading of sweet cream are reported.

2. A triple-strength solution of methylene blue was more satisfactory for cream than the solution recommended for milk in the Standard Methods of Milk Analysis.

3. The time required for the reduction of methylene blue was inversely proportional to the bacterial content of the cream.

4. The reduction time of samples of cream showing less than 0.21 per cent acidity showed no direct relationship to the acidity of the cream.

5. The reduction time was not significantly influenced by the per cent of fat in the cream.

6. The methylene blue reduction test was found to be a satisfactory index of the bacterial content of sweet cream and expedient for use in creameries for the grading of cream.

7. The sanitary conditions and methods of cooling on dairy farms were reflected in the reduction time.

8. Butter, unsalted and salted, made from the better quality of cream possessed somewhat better keeping quality than the butter from inferior cream. The differences, however, were not marked.

9. The methylene blue reduction test should serve as a very convenient aid in a program directed toward the improvement in the quality of sweet cream.

APPENDIX

Methylene Blue Reduction Test for Sweet Cream[2]

This test, which has been called the "reductase test," has been found to be useful for the grading of cream having acidities less than 0.21 per cent. It is not adaptable to sour cream. The time required for the disappearance of the blue color imparted to the cream by a methylene blue solution is a measure of the bacterial content of the cream. Where the color disappears rapidly, it signifies that the number of bacteria in the cream is high; if it requires several hours for such decolorization, it indicates that the bacterial content is proportionately lower. Inasmuch as the bacterial content is influenced by the sanitation on the farm or in the factory, the rapidity and degree of cooling and storage, as well as the age of the cream, the determination of the number of bacteria by such a simple test should give helpful information concerning the history of the cream and its possible influence on the quality of products made from it.

Apparatus Needed[3]

1. Pipette or metal dipper having a capacity of ten (10) milliliters (10 cubic centimeters).
2. Burette, graduated to one (1) milliliter, and apparatus stand provided with burette clamps; or, pipette graduated to one (1) milliliter.
3. Test tubes, thick-walled, ⅝″x6″ or ⅝″x5″. (It is often convenient to grind an area ½″ square on the upper portion of the tube by means of an emery wheel so that each tube may be marked easily with a lead pencil. Such markings may be erased when the tubes are washed.)
4. Cork or rubber stoppers for each tube.
5. Water bath. (The size depends upon the number of tests made.)
6. Wire racks for test tubes. Single or double row type is advisable.
7. Alcohol lamp, gas burner, or electric hot plate.
8. Methylene blue tablets. Standard tablets are manufactured by the National Aniline and Chemical Company, Inc., New York City, and are available at creamery supply houses.
9. Thermometer. (Centigrade or Fahrenheit.)
10. Graduated cylinder.

[2] See "Standard Methods of Milk Analysis," published by the American Public Health Association, 50 West 50th St., New York City, for a complete description of the methylene blue reduction test for milk.

[3] See cover page.

Collection of Sample

Collect a ten (10) milliliter (10 cubic centimeter) sample of cream in weigh-room, directly from patron's can if possible, by means of a metal dipper or a pipette. Place sample in a clean, boiled or sterilized test tube. Number properly. Place boiled or sterilized stopper in tube. Set tubed sample in rack in ice water bath until all samples are collected or until methylene blue solution is added. Rinse dipper or pipette thoroly in cold water and then in boiling water before taking the next sample.

Addition of Methylene Blue

As soon as possible after the samples are taken, add one milliliter of the methylene blue solution to each sample of cream. Mix thoroly. (It is usually convenient to collect a number of samples before adding the solution[4] in order to simplify and to expedite the reading of results. However, the samples must be kept cold during the interval before making the color test.)

Warm the cream samples to 37° C. (98.6° F.) immediately after adding the solution. (Record time when that temperature is reached. This is the starting point for observations on the time required for the disappearance of the blue color.) Keep tubes in water bath or incubator at that temperature until the samples are decolorized. (The alcohol lamp, gas burner or electric hot plate may be helpful in maintaining a constant temperature of the water bath.) The samples should be kept away from light during the period of observation.

Readings

Observe the tubes at intervals of ten minutes or less during the first hour. After that readings may be made each half hour.

Record the time required for the blue color to disappear from each sample. This is called the "reduction time."

Grading the Cream

The samples may be graded, on the basis of reduction time, according to the following classification:

GOOD: not decolorized in 5½ hours.

FAIR: decolorized in less than 5½ hours, but not less than 2 hours.

POOR: decolorized in less than 2 hours, but not less than 20 minutes.

VERY POOR: decolorized in 20 minutes or less.

[4] Methylene blue solution: Dissolve three (3) methylene blue tablets in fifty (50) milliliters of boiling water. Then add sufficient cold water to bring the total volume to two hundred (200) milliliters. Note: Three tablets are necessary for cream, while one is sufficient for milk.)