

RESAZURIN REDUCTION TIME AFTER STORAGE AS A KEEPING QUALITY TEST FOR PASTEURIZED MARKET CREAM

By LORNA G. LIGHTBODY, M.Sc.*

SUMMARY

Reduction of resazurin to the white end-point at 37°C after KQ storage for 24 hr at 20°C has been used as a keeping quality test for pasteurized cream. A reduction time of not less than 1½ hr would seem to be a suitable standard. Resazurin reduction at 20°C after KQ storage gave less satisfactory results.

The resazurin reduction time at 37°C showed a significant correlation with the total bacterial count after KQ incubation. Most creams with a resazurin reduction time of less than 1½ hr after KQ incubation were classified as unsatisfactory on the results of initial bacteriological tests for total count, psychrophilic count and presence of coliform organisms. If a psychrophilic count was not performed, the results of total bacterial count and the coliform test did not show the unsatisfactory quality of some samples which had high total bacterial counts after KQ storage and which failed the keeping quality test.

The BCP grading test was performed on some samples. Results in this test were also significantly correlated with total bacterial count after KQ incubation. Creams classed as unsatisfactory on the results of total bacterial counts, psychrophilic counts or coliform counts were usually of Grade 3 or 4 in this test.

I. INTRODUCTION

Many workers have noted that direct tests on pasteurized milk or cream do not give reliable information of post-pasteurization contamination or of the keeping quality of the product. Provan and Rowlands (1943) found that neither the colony count nor the coliform test can be recommended for controlling the keeping quality of heat-treated milk, and they recommended the use of methylene blue or resazurin test after storage of the samples at 18°C for 18–24 hr. The keeping quality test using methylene blue reduction after storage was investigated under Queensland conditions by Smythe (1959), who recommended a methylene blue standard of 2 hr after incubation for 21–24 hr at 20°C. Berger and Anderson (1949) also showed that immediate tests on pasteurized milk for total count, coliform organisms or reduction time did not give reliable information concerning the conditions of production, and they favoured either a total count or reduction test after incubation at 17°C for 48 hr. Bertelsen, Mattsson, and Dufeu (1956) stated that a methylene blue test after storage at 17°C for 24 hr was useful in practice for determining the keeping quality of milk or cream but considered that

* Special Bacteriologist, Queensland Department of Primary Industries.

a coliform test on the incubated sample gave a more reliable indication than the reduction test. Olsen (1956) also favoured the use of a coliform test after incubation as a measure of keeping quality.

In earlier experiments in the Department's Dairy Research Laboratory (Lightbody and Smythe 1962), the methylene blue reduction test was found to be rather unsuitable for pasteurized cream, particularly high-fat creams, but much better results were obtained using resazurin as the redox indicator. The results of resazurin reduction tests after KQ storage showed a fairly close relationship with subsequent deterioration determined by taste and smell.

Further tests have now been made on routine samples of pasteurized cream to compare various tests for bacteriological quality, and to find a suitable standard for a resazurin reduction test. As psychrophilic contaminants may often be present in considerable numbers in pasteurized cream and may play a significant role in keeping quality, reduction tests were made at 20°C as well as at 37°C. A grading test in bromcresol purple (BCP) milk was proposed by Crossley (1948) to determine the suitability of market cream for commercial distribution. This test was also performed on some of the routine samples.

II. EXPERIMENTAL

The methods used for bacteriological analysis of the creams were the same as in previous work (Lightbody 1964).

Keeping quality tests were made by determining bacterial counts and resazurin reduction times after storage for 24 hr at 20°C. The reduction of resazurin was determined at both 37° and 20°C. The BCP grading test was carried out as suggested by Crossley (1948).

Sixty-eight samples of bottled pasteurized cream received at the laboratory for routine bacteriological analysis were examined for total bacterial count, coliform count, psychrophilic count, and resazurin reduction and bacterial counts after KQ storage. Tests for resazurin reduction time at 20°C after KQ storage and the BCP grading test were also made on 30 of these creams and the same tests were made on portions of 28 samples, pasteurized again in the laboratory. Another 174 samples of pasteurized cream were examined less extensively, but tests on these were made for total bacterial and coliform count initially, and total bacterial count and resazurin reduction time at 37° after KQ storage.

III. RESULTS

A summary of the results of tests on 68 routine cream samples is shown in Table 1. Samples of cream with different fat percentages have been considered together, as previous results showed that the fat content did not influence the bacteriological results during keeping quality incubation.

To compare the results of various tests for keeping quality, correlation coefficients were calculated, and their level of significance determined by the test.

TABLE 1
RESULTS OF BACTERIOLOGICAL TESTS ON 96 SAMPLES OF PASTEURIZED CREAM

	Commercially Pasteurized	Laboratory Pasteurized	Total
Total bacterial count/ml—			
< 10,000	22	17	39
10,000–100,000	39	10	49
100,000 and over	7	1	8
Coliforms/ml—			
< 1	21	28	49
> 1	47	—	47
Psychrophiles/ml—			
< 10	18	28	46
10–< 1,000	29	—	29
1,000 and over	21	—	21
Total bacterial count/ml after KQ incubation—			
< 10 ⁶	2	15	17
10 ⁶ –10 ⁷	15	6	21
10 ⁷ –10 ⁸	26	6	32
> 10 ⁸	25	1	26
Resazurin reduction time at 37° after KQ incubation (hr)			
$\frac{1}{2}$	24	11	35
1	11	6	17
1 $\frac{1}{2}$	16	3	19
> 1 $\frac{1}{2}$	17	8	25
Resazurin reduction time at 20° after KQ incubation (hr)			
$\frac{1}{2}$ or 1	18	12	30
1 $\frac{1}{2}$ or 2	5	4	9
> 2	7	12	19
BCP test—grade			
1	3	16	19
2	4	11	15
3	8	1	9
4	15	—	15

Results are shown in Table 2. There was a highly significant correlation between the total bacterial count after KQ incubation and the resazurin reduction time at 37°. When the resazurin reduction test was conducted at 20° the correlation coefficient was lower. The correlation between the BCP test and the total bacterial count after KQ incubation was also highly significant but the correlation coefficients between the BCP test and resazurin reduction times at 20° and 37° were lower.

TABLE 2
RELATIONSHIPS BETWEEN KEEPING QUALITY TESTS

Test	No. of Samples	Correlation Coefficient	Level of Significance (%)
TC/KQ v. res. redn. at 37° ..	174	-0.66	0.1
	58	-0.46	0.1
TC/KQ v. res. redn. at 20° ..	58	-0.38	1
TC/KQ v. BCP test ..	58	0.66	0.1
BCP test v. res. redn. at 37° ..	58	0.29	5
BCP test v. res. redn. at 20° ..	58	0.34	1

The samples of routine cream were classified on the results of initial bacteriological tests, using as standards for satisfactory quality total bacterial count less than 50,000 per ml, coliform organisms less than 1 per ml, and psychrophilic organisms less than 10 per ml, less than 100 per ml, or less than 1,000 per ml. These results were compared with the results of resazurin reduction tests after KQ incubation taking standards in this test at $\frac{1}{2}$ hr, 1 hr and $1\frac{1}{2}$ hr. The results of chi-square tests are shown in Table 3. The highest chi-square values were obtained when a resazurin standard of $1\frac{1}{2}$ hr was taken, irrespective of the level which might be considered desirable for psychrophilic organisms. The total samples included the results on 28 samples of laboratory-pasteurized cream, so the results of the other 68 samples were also analysed separately. However, the overall results of the two groups were similar.

TABLE 3
RELATIONSHIP BETWEEN INITIAL BACTERIOLOGICAL RESULTS AND RESAZURIN REDUCTION TIME AFTER KQ INCUBATION—CHI-SQUARE VALUES

Standard Considered Satisfactory for Psychrophilic Count	Standard Taken as Satisfactory for Resazurin (68 Routine Samples)			Reduction Time After KQ Storage (hr) (Total 96 Samples)		
	$\frac{1}{2}$	1	$1\frac{1}{2}$	$\frac{1}{2}$	1	$1\frac{1}{2}$
< 10 per ml	0.08 n.s.	0.63 n.s.	4.17*	1.65 n.s.	0.67 n.s.	5.11*
< 100 per ml	0.60 n.s.	3.70 n.s.	7.32**	2.63 n.s.	2.95 n.s.	7.59**
< 1000 per ml	2.78 n.s.	6.97**	8.55**	5.36*	5.57*	8.83**

n.s. Not significant. * Significant at 5 per cent. level. ** Significant at 1 per cent. level.

The distribution of the 68 samples when the standard for psychrophilic count was taken as 1000 per ml is shown in Table 4. If the standard for resazurin reduction time was taken as $1\frac{1}{2}$ hr, 8 samples which failed to conform to the standards for initial bacteriological quality still passed the keeping quality test. Of these 8 samples, 6 were failed because of coliform counts of between 1 and 15 per ml, and the other 2 for high psychrophilic counts. With a standard of $1\frac{1}{2}$ hr resazurin reduction time, 8 samples failed this KQ test although on initial bacteriological results they were classed as satisfactory. Five of these had psychrophilic counts greater than 10 per ml and total bacterial counts after

KQ incubation greater than 10 million per ml. The other 3 creams in this category had relatively low bacterial counts. These results suggest that a resazurin reduction time of $1\frac{1}{2}$ hr after KQ incubation would not be an unreasonable standard for a keeping quality test. Even with this lenient standard of not more than 1000 per ml for psychrophilic count, many creams with unsatisfactory initial quality would pass the KQ test if the standard for resazurin reduction time was taken as $\frac{1}{2}$ hr or 1 hr.

TABLE 4

RELATIONSHIP BETWEEN INITIAL BACTERIOLOGICAL TESTS AND RESAZURIN REDUCTION TIME AFTER KQ INCUBATION FOR 68 SAMPLES

Initial Quality	Resazurin Reduction Time (hr)					
	$\frac{1}{2}$	$>\frac{1}{2}$	$\frac{1}{2}$ or 1	>1	$\frac{1}{2}$, 1 or $1\frac{1}{2}$	$>1\frac{1}{2}$
Satisfactory	3	15	4	14	8	10
Unsatisfactory	21	29	31	19	42	8

The relationships between initial bacteriological quality, and the BCP grading test and total bacterial counts after KQ incubation, are shown in Table 5. The creams were graded as being satisfactory in initial tests if the total bacterial count was less than 50,000 per ml, coliform organisms were absent in 1 ml and the psychrophilic count less than 10 per ml.

TABLE 5

COMPARISON OF RESULTS IN INITIAL BACTERIOLOGICAL TESTS WITH BCP GRADE AND TOTAL BACTERIAL COUNT AFTER KQ INCUBATION

	Initial Bacteriological Tests	
	Satisfactory	Unsatisfactory
BCP grade—		
1	18	1
2	9	6
3	2	7
4	1	14
TC/KQ-log—		
<6	14	1
>6-7 ..	9	4
>7-8 ..	7	9
>8	0	14

There was a significantly different distribution of creams with satisfactory and unsatisfactory initial bacteriological quality within the grades in the BCP test (chi-square = 14.02, $P < 0.001$) and also between the classes of total bacterial counts after KQ incubation (chi-square = 25.23, $P < 0.001$). However,

if psychrophilic counts were omitted from the initial bacterial tests on the cream, these differences became less significant. Nine creams which were classed as unsatisfactory because of high psychrophilic counts would then be classed as satisfactory. Seven of these were Grade 3 or 4 in the BCP test, and 8 had total bacterial counts after KQ incubation greater than 10^7 . The contamination in these creams had a considerable influence on keeping quality, although no coliform organisms were present in 1 ml.

IV. DISCUSSION

Following experiments on laboratory-pasteurized creams (Lightbody and Smythe 1962), it was suggested that the resazurin reduction time to a bright pink end-point (Disk 1) following KQ incubation might provide a suitable keeping quality test for pasteurized creams. When commercially pasteurized cream samples were examined, reduction times were so short that it was necessary to take the reduction time to white to obtain differentiation between creams. In the first series of tests only 33 per cent. of commercially pasteurized cream samples had resazurin reduction times after KQ storage greater than $1\frac{1}{2}$ hr. However, the bacteriological quality of routine cream samples has shown improvement during the past 12 months, and in the last three months 50 per cent. of all samples and 61 per cent. of the high-fat cream samples passed a keeping quality test with a standard of not less than $1\frac{1}{2}$ hr resazurin reduction time after KQ storage.

The time and temperature used for KQ storage of cream were the same as used in this laboratory for the keeping quality test for pasteurized milk. This incubation seemed to provide suitable conditions for a KQ test. A high bacterial count after KQ storage was found to be indicative of post-pasteurization contamination (Lightbody 1964) because of the relatively slow growth of thermoduric organisms. For this reason the total bacterial count after KQ incubation provided a much better indication of keeping quality than the initial bacterial count of the pasteurized product. The resazurin reduction test after KQ storage gave results which were correlated significantly with the total count after KQ storage, and thus would seem to provide the most practical means of determining quality after KQ incubation. Bertelsen, Mattsson, and Dufeu (1956) and Olsen (1956) considered that a coliform test after incubation was superior to a dye-reduction test for assessing keeping quality. However, pasteurized cream may contain other contaminant organisms when coliform organisms are absent or present only in very low numbers. These contaminants may exert a considerable influence on the keeping quality of the cream, particularly if they are psychrophilic. For this reason a reduction test to indicate total bacterial growth during KQ storage is preferred to a test for coliform organisms after incubation of the sample.

A few samples with a low resazurin reduction time but with a relatively low total bacterial count after KQ storage were found. Such occasional unusual results may be due to considerable bacterial growth in the cream before pasteurization (Lightbody 1964).

The BCP grading test (Crossley 1948) was carried out on some samples. Although results obtained in this test agreed well with total bacterial counts and reduction times after KQ storage, the test did not seem to offer any advantages over the reduction test. It was not always easy to read the tubes in this test, so different operators did not always agree on the grade of the sample.

V. ACKNOWLEDGEMENT

The author wishes to thank Mr. D. A. Beere for technical assistance.

REFERENCES

- BERGER, G., and ANDERSON, L. (1949).—Methods for testing the keeping quality of milk with special reference to after infection. Proc. 12th Int. Dairy Congr. 3 (3):366-71.
- BERTELSEN, E., MATTSSON, N., and DUFEU, J. (1956).—The applicability of bacteriological tests in determining the keeping quality of market milk and cream. Proc. 14th Int. Dairy Congr. 3(1):316-28.
- CROSSLEY, E. L. (1948).—Studies on the bacteriological flora and keeping quality of pasteurized liquid cream. *J. Dairy Res.* 15:261-76.
- LIGHTBODY, Lorna G., and SMYTHE, V. R. (1962).—Effect of heat treatment on bacteriological quality and phosphatase activity of market cream. *Qd J. Agric. Sci.* 19:77-92.
- LIGHTBODY, Lorna G. (1964).—Effect of low-temperature storage on the bacteriological quality of pasteurized market cream. *Qd J. Agric. Sci.* 21:311-21.
- OLSEN, S. J. (1956).—Determination of the keeping qualities of milk. Proc. 14th Int. Dairy Congr. 3(2):339-54.
- PROVAN, A. L., and ROWLANDS, A. (1943).—Dye reduction tests for heat treated milk. *Dairy Ind.* 8:693-9.
- SMYTHE, V. R. (1959).—Keeping quality test for pasteurized milk in warm climates. Proc. 15th Int. Dairy Congr. 3:1312-8.