THE EFFECT OF SMALL AMOUNTS OF CHLORINE ON THE REDUCTION TIME OF MILK

By H. BARKWORTH

South-Eastern Agricultural College, Wye, Kent

Introduction

It would be expected that the addition of chlorine to milk, as hypochlorite or in some other form, would increase the reduction time. Further consideration will show that a large number of variables are involved—the amount of chlorine, the age of the milk sample at the time of addition, the form in which chlorine is added, the age of the sample when tested, the period which has elapsed since the addition of chlorine, the number and kinds of bacteria present, and the temperature. It would be difficult to assess the relative importance of each and all of the factors in a single experiment, and the object of the experiments described in this paper was to ascertain the minimum amount of chlorine which significantly increases the reduction time of the sample.

Wilson (1938) tested fifteen samples, of which four had reduction times of less than 20 min. Part of each sample was stored in the refrigerator. Six different chlorine compounds were used in concentrations ranging from 25 to 400 parts per million of available chlorine. Chlorine water was the most and Milton the least effective as judged by the increase in reduction time (R.T.). Cold storage reduced the effect of the chlorine, and the increase in R.T. was also less in the case of the four poor-quality samples (R.T. less than 20 min.) An increase of about 30 min. was given for the better class samples by 25 p.p.m. av. Cl, and of about 10 min. for the other four samples. Chlorine could be tasted in all cases when 100 p.p.m. av. Cl was present and Milton could be detected at 50 p.p.m.

Anderson (1938) also made trials. An inspection of his results suggested that occasionally as little as 5 p.p.m. av. Cl might affect the reduction time, and 50–100 p.p.m. av. Cl could be detected by taste in milk and cream. Wright & Anderson (1938), however, state that even 200 p.p.m. av. Cl half an hour after adding, cannot be detected by odour. They also suggest that amounts of less than 50 p.p.m. av. Cl have no preservative effect (apparently measured as keeping quality). Wilson (1938) found that the average increase in keeping quality for eleven samples was 1 hr. 27 min. when chlorine in the form of Milton was added to give a concentration of 25 p.p.m. av. Cl.

In an attempt to measure the interaction of storage temperature, age on adding chlorine and concentration of chlorine, Barkworth (1940) prepared twenty-five subsamples from each sample. Chlorine was added at 0, 5 and 10 p.p.m. either at 10.0 a.m. or at 5.0 p.m., using samples of morning milk.

Samples were stored at 40, 60 and 70° F. till 5.0 p.m., and from then on, till time of testing at 10.0 a.m. next day, either at 60° F. or in the refrigerator. Plate counts and reduction tests were made on arrival and on each subsample at 5.0 p.m. and at 10.0 a.m. next day. Owing to the war only four samples were so treated. The results suggested that even 5 p.p.m. av. Cl might affect the reduction time of the sample.

EXPERIMENTS

The experiments here reported were of a simpler design and were intended to test only the significance of the increase in R.T. on adding a known amount of chlorine. As in the previous experiments (Barkworth, 1940), Milton was used as the source of chlorine, because it appeared to have the minimum effect on R.T. Samples were taken at a nearby creamery and reached the laboratory by 12 noon. After testing for plate count, 100 ml. was poured into another sample bottle and to this portion was added the requisite amount of Milton. The remainder of the 8 oz. samples served as the untreated portion. Plate counts were made on both portions at 10.0 a.m. next day, at which time reduction tests were also started. Milton contains about 1 % available chlorine, but the actual strength was frequently checked by titration, and from this was calculated the amount of Milton which would have to be added to 100 ml. milk to give the desired concentration of chlorine in parts per million. To obtain 10 p.p.m. av. Cl in 100 ml. of milk, about 0·5 ml. of a 1:10 dilution had to be added. These experiments were carried out between November 1941 and January 1942 and when stored 'at air temperature', the samples being left in the open porch of the laboratory, the temperature next morning was 50–56° F.

RESULTS

Reduction time

Series 1. Two batches of twelve samples each were treated with about 180 p.p.m. av. Cl, resulting in increased R.T. in twenty-two cases, ranging from ½ to 5 hr. increase. The samples were stored at 60° F. and twenty-one of the untreated samples gave R.T. of 1½ hr. or less. The increases are shown in Table 1.

Series 2. Chlorine was reduced to 10 p.p.m. and samples were again stored at 60° F., but later storage was amended to 'air temperature' in order to increase the R.T. of the untreated portions. Table 1 shows the increase in R.T. recorded in this series.

Series 3. Chlorine was added to give 10 p.p.m. av. Cl, but both the untreated and the treated portions were tested in duplicate for reduction time. The increases obtained in this series were not as great as those in series 2, but were nevertheless statistically significant.

The mean increase was taken as the difference between the average R.T. of the two tests on the treated portions and the average R.T. of the two tests

on the untreated portions. A t test was made on these differences. Even if the observed increases are compared against the total samples the result is significant.

It has been shown that the standard deviation (S.D.) of the R.T. is 9·48 min. when the result is the mean of two tubes (Barkworth, Irwin & Mattick, 1942). We can apply this figure to our table of differences as follows:

$$\text{S.D.} = 9{\cdot}48 \text{ min.} = 0{\cdot}158 \text{ hr.}$$

S.E. of diff. between two means of $8 = 0{\cdot}158\sqrt{\frac{2}{8}}$.

Table 1. *Increase in reduction time due to adding chlorine*

No. of samples	Av. Cl p.p.m.	Increase in R.T. over untreated portion: ½	1	1½	2	2½	3	3½	4	4½	5	No. of samples affected	Storage conditions
						Series 1							
12	188	3	2	1	—	3	—	—	—	—	1	10	a.m., p.m. and mixed milk stored at 60° F.
12	177	2	3	2	—	3	1	—	—	—	1	12	
24												22	
						Series 2							
12	10	4	—	1	—	3	1	—	—	—	—	9	a.m., p.m. and mixed milk stored at 60° F.
36	10	3	1	—	—	—	—	—	—	—	—	4	All a.m. milk stored at 60° F.
36	10	7	2	1	—	—	—	—	—	—	—	10	All a.m. milk stored at air temp. 50° F.
84												23	

This method has been used for both the affected samples and also for the total samples, but the result remains significant in all cases.

The R.T. of the untreated portions of the affected samples varied from 1 to 2 hr.

Series 4. Available chlorine was reduced to 5 p.p.m., and in case differences should be small it was decided to observe the tubes every 15 min. The results have been analysed on the basis of both half-hourly and quarter-hourly observations (see Table 2). As might be expected, some differences which were noticeable on a 15 min. basis were eliminated when observations were assumed to have been made only every half-hour, which is of course the standard interval. The differences were more variable than in series 3, and in two cases the chlorinated portions had a shorter R.T. Tests of significance were made as in series 3, and in all cases the value of t was significant. The R.T. of the untreated portion of the affected samples (½ hr. observations) varied from 1½ to 7½ hr.

Plate count

It has been noted that increase in R.T. was obtained at all levels of reduction time, and in Table 3 the plate count on arrival has been correlated with the increase in R.T.

It would seem that when, as in series 1, a large amount of chlorine is added, then samples of lower plate count are more affected. This is in agreement with Wilson (1938). When minimal amounts of chlorine are present (5 and 10 p.p.m.)

Table 2. *Effect of adding chlorine, reduction time in duplicate on treated and untreated portions (series 3)*

Av. Cl p.p.m.	No. of samples	No. affected	R.T. observed every	Mean increase in R.T. hr.	S.E. hr.	t	Mean observed differences in affected samples: Max.	Min.
10	24	8	½ hr.	(*a*) 0·50	0·047	10·6	0·75	0·25
				(*b*) 0·50	0·079	6·3		
				(*c*) 0·17	0·051	3·2		
				(*d*) 0·17	0·046	3·7		
5	33	15	½ hr.	(*a*) 0·45	0·123	3·7	1·00	−0·50
				(*b*) 0·45	0·058	7·8		
				(*c*) 0·20	0·066	3·0		
				(*d*) 0·20	0·038	5·3		
		20	¼ hr.	(*a*) 0·33	0·103	3·2	1·25	−0·625
				(*b*) 0·33*	0·050	6·6		
				(*c*) 0·19	0·067	2·9		
				(*d*) 0·19*	0·038	5·2		

(*a*) On no. of affected samples and observed differences.
(*b*) On no. of affected samples and assumed S.D. 9·48 min.
(*c*) On total no. of samples and observed differences.
(*d*) On total no. of samples and assumed S.D. 9·48 min.

* S.D. 9·48; no adjustment for 15 min. inspections.

Table 3. *Effect of plate count on arrival on increase in reduction time due to adding chlorine*

No. of samples	Plate count (log): Mean	Plate count (log): S.D.	Av. choline p.p.m.	r	
22	5·22	0·68	177 and 188	−0·6823	Significant
23	4·94	0·64	10	−0·2768	—
23	5·02	0·45	5 and 10 (tested in duplicate)	0·1582	—
68	5·01	0·90	—	−0·1210	—

this relationship no longer holds good, and increases of ½–1 hr. may occur at any level of R.T. This suggests that in these circumstances kind of bacteria is more important than number.

SUMMARY

Chlorine at the rate of 5 and 10 parts per million of available chlorine in the form of Milton significantly increased the reduction time of milk samples.

Observed increases varied from −0·50 to +1·25 hr.

At this level of chlorination the increase in R.T. is not correlated with the initial plate count and also occurs at all levels of R.T.

REFERENCES

ANDERSON, E. B. (1938). Unpublished data.
BARKWORTH, H. (1940). Min. Agric., meeting Advisory Bacteriologists.
BARKWORTH, H., IRWIN, J. O. & MATTICK, A. T. R. (1942). *J. Dairy Res.* (in the Press).
WILSON, G. S. (1938). Unpublished data.
WRIGHT, R. C. & ANDERSON, E. B. (1938). *Analyst*, **63**, 745, 252.

(*MS. received for publication* 2. III. 42.—Ed.)