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# Bacteriological tests as indices for the development of off-flavours in cream

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SUMMARY

Bacteriological tests alone are not capable of predicting the keeping quality of cream, since taints can develop which are due to non-bacterial action. When spoilage is due to bacterial growth, the water agar test was found to be more accurate than the other tests that were examined.

## INTRODUCTION

Although recognizing the occurrence of anomalous results, a working party of the Public Health Laboratory Service recommended the methylene blue reduction test as being suitable for the bacteriological grading of fresh cream at an advisory level (Report, 1958). The working party also reported that this test gave reasonably good correlation with the keeping quality of cream held at atmospheric temperature. Other workers have been unable to recommend methylene blue reduction as a useful index for keeping quality (Crossley, 1948; Lightbody & Smythe, 1962; Cox, 1970). Preliminary incubation has been used in an attempt to obtain more concordant results, but this modification is not entirely successful (Druce & Thomas, 1968). Tekinsen & Rothwell (1974) concluded that the predominant bacterial species present in fresh cream were probably thermoduric and that counts of psychrotrophic organisms made after incubation at 5° C. for 10 days indicated the degree of contamination. When compared with mesophilic organisms, psychrotrophs are weak dye-reducers (Thomas, Druce, Davis & Bear, 1966), and their presence could be the cause of anomalous results. This fact was recognized by a second Public Health Laboratory Service working party (Report, 1971). The proteolytic count of the water agar test has been shown to be a more sensitive index of bacterial contamination than any of the tests in current use (Taylor, 1971, 1975), and this test has been examined in conjunction with other commonly used tests to predict the keeping quality of cream.

## METHODS

Cartons or jars of 11 brands of double cream, either pasteurized or ultra-high temperature treated, were obtained from retail sources or directly from the dairy by members of the staff or Ayr Burgh Sanitary officers and delivered to the Institute with a history of the sample. The samples (231) were examined

immediately and the remainder of the sample stored at 5° C. and examined for taste, smell and appearance on Mondays, Wednesdays and Fridays. A further 18 samples were subdivided into 4–5 oz. quantities and stored at 5° and 3·5° C. These samples were tasted daily for taints by one person who examined all the samples. The bacterial contents of the creams stored at 5° and 3·5° C. were examined on Mondays, Wednesdays and Fridays.

The age of the samples varied from freshly separated to the last day recommended for the sale of that cream. The creams were all made from tanker milk. Heat-treated cream was sold under 8 of the brand names, the remaining 3 brands separated cream from pasteurized milk.

Bacteriological examinations were made for colony and lipolytic count and presumptive coliform test after incubation at 30° C. for 72 hr. and a confirmatory coliform count in violet red-bile agar (VRB) after incubation at 30° C. for 24 hr. using British Standard methods (British Standard 4285: 1968; Supplement No. 1 (1970) to B.S. 4285: 1968). The water agar test (Taylor, 1971, 1975) was carried out at 30° C. and also, when appropriate, at 5° C. in conjunction with a colony count incubated at 5° C. for 7 days. Other tests used were the methylene blue and phosphatase tests (Statutory Instrument 1571: 1963).

## RESULTS AND DISCUSSION

The phosphatase test showed that, irrespective of age and origin, all the samples of cream examined in this work had received adequate heat treatment.

Spoilage which could definitely be attributed to either bacterial or non-bacterial action was most clearly shown with creams of good initial bacteriological quality. The relation between the composition of the bacterial flora, as shown by the various tests, and the keeping quality is illustrated by the changes that occurred when creams *A* and *B* were held at 5° and 3·5° C. (Fig. 1). These two creams would normally be considered of excellent quality, having a colony count of $10^3$ colonies/g. or less, no presumptive coliforms in 1/10 ml., a coliform count in violet red-bile (VRB) agar and lipolytic count of less than 50/g. (not shown in Fig. 1) and a methylene blue reduction time of more than 7½ hr. However, a small number of proteolytic organisms was found in cream *B*, correctly predicting that this cream would show spoilage before cream *A*. A sour taste was the off-flavour developed in both these creams. The initial lipolytic count could not be related to the development of taints in these or any of the other creams that were examined.

When the methylene blue reduction time was related to the stage of bacterial growth, it was found that the reduction time was not less than 7½ hr. until after the growth had entered the logarithmic phase (Fig. 1). However, various anomalies were shown when the reduction time was related to keeping quality. After incubation at 5° C. for 7 days, cream *A* still had a methylene blue reduction time of greater than 7½ hr. but developed a taint 3–4 days later. A proteolytic count had been detected in this cream after incubation for only 4 days. A similar anomaly was shown when cream *B* was incubated at 3·5° C. Thus, a long methylene blue

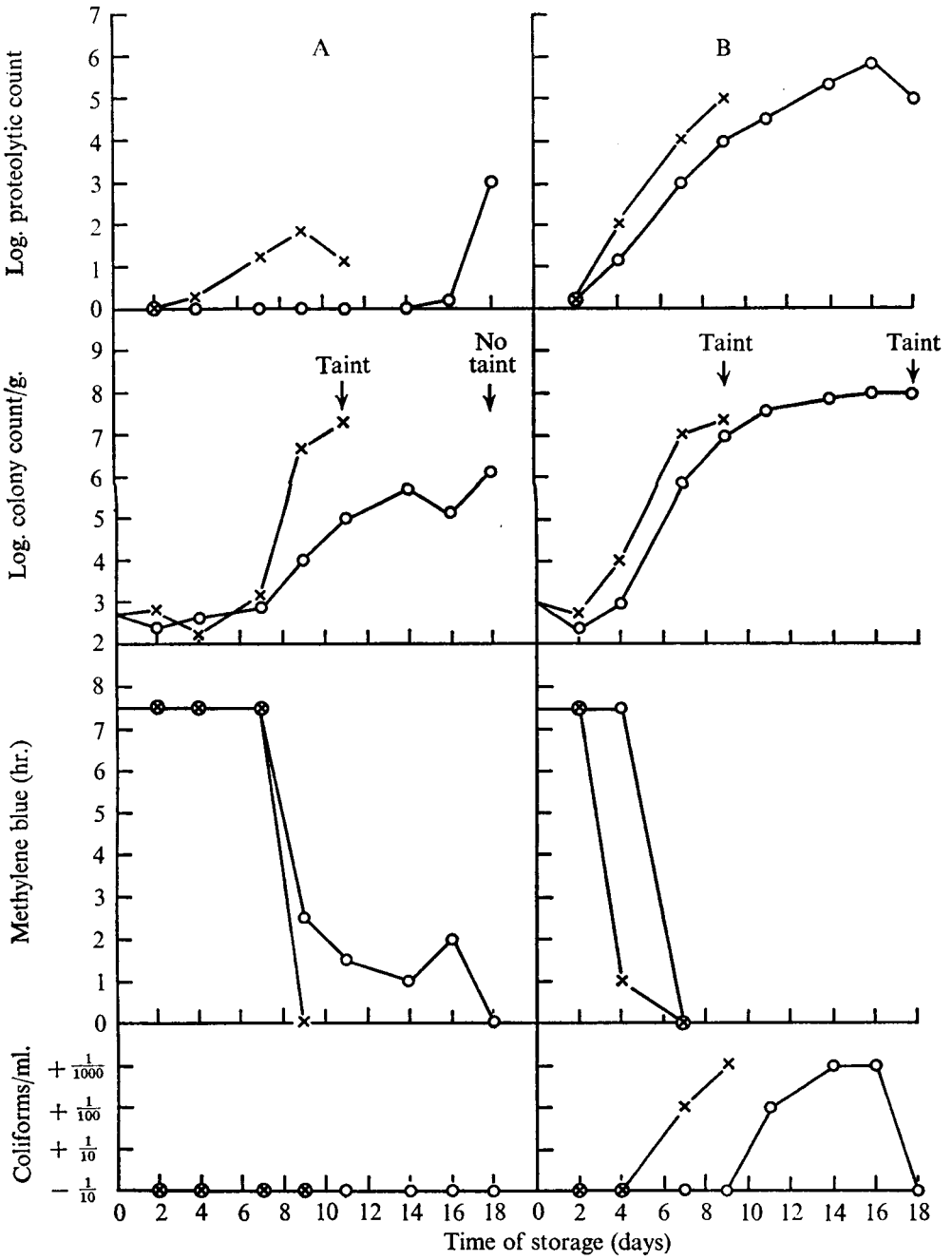


Fig. 1. The changes in bacterial flora and keeping quality of two creams (*A*, *B*) during storage at 5° C. (×——×) and 3·5° C. (○——○).

Table 1. *The percentage incidence of different values of colony count, presumptive coliforms, coliform count on violet red-bile agar (VRB), methylene blue reduction time (MB) and proteolytic count in the water agar test (WA), in the initial samples of cream that developed off-flavour after storage at 5° C. for different lengths of time*

| | | Flavour acceptable at (days) | | | | |
|---|---|---|---|---|---|---|
| | | ≥ 14 | 10–< 14 | 7–< 10 | 3–< 7 | < 3 |
| Number of samples | ... | 46 | 12 | 26 | 28 | 42 |
| Colony count | | | | | | |
| | $< 10^3$ | 37 (17)* | 42 (5) | 19 (5) | 3 (11) | 10 (4) |
| | $10^3$–$< 10^4$ | 52 (24) | 50 (6) | 38 (10) | 14 (4) | 30 (13) |
| | $10^4$–$< 10^5$ | 9 (4) | — | 35 (9) | 29 (8) | 12 (5) |
| | $10^5$–$< 10^6$ | 2 (1) | 8 (1) | 4 (1) | 29 (8) | 7 (3) |
| | $\geqslant 10^6$ | — | — | 4 (1) | 25 (7) | 41 (17) |
| Presumptive coliforms | | | | | | |
| | − 1/10 | 100 (46) | 100 (12) | 61 (16) | 39 (11) | 52 (22) |
| | + 1/10 | — | — | 23 (6) | 3 (1) | 10 (4) |
| | + 1/100 | — | — | 8 (2) | 29 (8) | 10 (4) |
| | + 1/1000 | — | — | 8 (2) | 29 (8) | 28 (12) |
| VRB count | | | | | | |
| | ⩽ 50 | 100 (46) | 100 (12) | 77 (20) | 57 (16) | 57 (24) |
| | 51–$< 10^3$ | — | — | 19 (5) | 21 (6) | 10 (4) |
| | $10^3$–$< 10^4$ | — | — | 4 (1) | 14 (4) | 7 (3) |
| | $10^4$–$< 10^5$ | — | — | — | 4 (1) | 10 (4) |
| | $\geqslant 10^5$ | — | — | — | 4 (1) | 16 (7) |
| MB (hours) | | | | | | |
| | $\geqslant 7\frac{1}{2}$ | 72 (33) | 84 (10) | 31 (8) | — | 40 (17) |
| | $4\frac{1}{2}$–7 | 17 (8) | 8 (1) | 19 (5) | 3 (1) | 5 (2) |
| | $\frac{1}{2}$–4 | 7 (3) | — | 46 (12) | 43 (12) | 10 (4) |
| | 0 | 4 (2) | 8 (1) | 4 (1) | 54 (15) | 45 (19) |
| WA | | | | | | |
| | < 5 | 87 (40) | 75 (9) | 50 (13) | 4 (1) | 43 (18) |
| | 5–50 | 7 (3) | 17 (2) | $11\frac{1}{2}$ (3) | — | — |
| | 51–$< 10^3$ | 2 (1) | — | 27 (7) | 64 (18) | 14 (6) |
| | $10^3$–$< 10^4$ | 2 (1) | 8 (1) | $11\frac{1}{2}$ (3) | 18 (5) | 7 (3) |
| | $10^4$–$< 10^5$ | 2 (1) | — | — | 14 (4) | 14 (6) |
| | $\geqslant 10^5$ | — | — | — | — | 22 (9) |

* Figures in parentheses are numbers of samples.

reduction time did not necessarily indicate a long keeping quality. A short reduction time could give an equally anomalous result. When cream A was incubated at 3·5° C., the methylene blue reduction time was greater than $7\frac{1}{2}$ hr. after incubation for 7 days but had fallen to $2\frac{1}{2}$ hr. after 9 days. This result indicated quite correctly that the bacterial growth had entered the logarithmic phase. The cream would normally be considered to have failed the methylene blue reduction test. However, proteolytic colonies were not detected before the 16th day of incubation and the cream was still palatable on the 18th day at the end of the experimental period. The proteolytic count at 30° C. indicated the presence of these organisms at 5° C. (Taylor, 1971). The slope of the growth curve suggests that this cream would have remained palatable for at least a further 7–10 days.

These anomalous results may possibly be explained by the fact that methylene blue reduction is being examined in a medium with a high fat content. The dye-reducing powers of an organism are normally tested in skim milk. The high fat content of cream will greatly affect the results since the reduced form of the dye is preferentially adsorbed by fat. This permits the reduction of this dye at a higher oxidation-reduction potential than would normally occur (Wilson, 1935).

The absence of coliforms in a cream does not necessarily indicate that coliforms were not present before examination. Presumptive coliforms were absent from 1/10 ml. and the confirmatory coliform count in VRB agar was less than 50/g. during the 18 days that cream *A* was stored at both 5° and 3·5° C. In cream *B*, coliforms developed at both these temperatures (Fig. 1). However, by the time this cream had developed a taint at 3·5° C., coliforms were no longer detectable.

In general, it was found that taints due to bacterial action developed in cream stored at 5° C. when the colony count was not less than about $10^7$ colonies/g. for 1–2 days. In creams that had been held at 3·5° C., taints developed when a count of about $10^8$ colonies/g. had been maintained for 4–6 days at least. This finding does not agree with the results of Tekinsen & Rothwell (1974) who found a considerable variation in the colony count at 30° C. at spoilage. The lack of agreement might be due to the difference in the length of incubation for the counts, 3 days in the present work as compared with 5 days used by Tekinsen & Rothwell. Another possible cause for lack of agreement was the finding in the present work that considerable bacterial lysis and regrowth could occur. A slight effect of this type is shown by the growth curve for cream *A*, stored at 3·5° C. (Fig. 1). With Tekinsen & Rothwell, it was found that there was very considerable variation in the colony counts at 30° and 5° C. However, in the present work, it was found that these two counts were of the same order when spoilage was due to bacterial action. Tekinsen & Rothwell (1974) do not consider the possibility of non-bacterial spoilage, which could account for the variation in colony count that these workers found at spoilage.

Non-bacterial taints develop readily in products, such as cream, which have a high fat content. The oxidation of unsaturated fatty acids is an autocatalytic reaction caused by the production of free radicals. Unstable hydroperoxides are formed which readily decompose to give highly flavoured carbonyl compounds (Downey, 1969). The taints that can be produced in this manner cannot be distinguished from those that result from bacterial action without both chemical and bacteriological test at the time of spoilage, and this was not done. Because the creams were not tasted daily, it was only possible to allocate the period of keeping quality for 154 of the 231 creams that were examined (Table 1). The water agar test proteolytic count correctly identified 87 % of samples that remained palatable for 14 days or more. It is interesting to note that when a proteolytic count was obtained in this group of creams, the proteolytic organisms were sporeformers in 4 out of the 6 samples. Although psychrotrophic sporeformers were not isolated in the present work, these organisms have been found in other creams and their presence reported by other workers (Grosskopf & Harper, 1974). It must be presumed that the sporeformers isolated in the present work were mesophilic, not

growing at 5° C. The only meaningful methylene blue reduction time was not less than 7½ hr. and this test identified only 71·7 % of creams that had keeping qualities of 14 days or longer. Since peroxide values were not measured, and because colony counts were not made at spoilage, it is not possible to attribute the relative importance of the development of bacterial and non-bacterial taints to the off-flavour at spoilage. This was particularly true for those creams with keeping qualities of 3 days or more, but less than 14 days. In those creams where the keeping quality was less than 3 days, spoilage must be presumed to be due to non-bacterial causes in 40–43 % of these samples. This was established for two samples in which the initial colony count was less than $10^4$/g. and which had not altered appreciably at spoilage. Proteolytic organisms could not be detected in either of these creams and the methylene blue reduction time was greater than 7½ hr. for both. Non-bacterial spoilage was most commonly found in pasteurized creams rather than cream made from pasteurized milk. The most common taint was rancid, but fruity, sour, bitter, metallic and cooked taints were also found.

These results show that off-flavours in cream are not necessarily due to bacteriological growth. Although the water agar test can never be more accurate than any other bacteriological test that demonstrates the absence of growth, it is more reliable and more suitable as a practical test for bacteriological spoilage than the methylene blue test. Psychrotrophic sporeformers were not isolated from any of the creams but the presence of these organisms should be considered in the context of public health.

## REFERENCES

BRITISH STANDARDS INSTITUTION. Methods of microbiological examination for dairy purposes. *British Standard* 4285: 1968.

BRITISH STANDARDS INSTITUTION. Methods of microbiological examination of milk products. *Supplement No.* 1 (1970) *to British Standard* 4285: 1968.

COX, W. A. (1970). Methylene blue keeping quality test and flora of market cream. *Journal of the Society of Dairy Technology* **23**, 195.

CROSSLEY, E. L. (1948). Studies on the bacteriological flora and keeping quality of pasteurized liquid cream. *Journal of Dairy Research* **15**, 261.

DOWNEY, W. K. (1969). Lipid oxidation as a source of off-flavour development during the storage of dairy products. *Journal of the Society of Dairy Technology* **22**, 154.

DRUCE, R. G. & THOMAS, S. B. (1968). Preliminary incubation of milk and cream prior to bacteriological examination: A review. *Dairy Science Abstracts* **30**, 291.

GROSSKOPF, J. C. & HARPER, W. J. (1974). Isolation and identification of psychrotrophic sporeformers in milk. *Milchwissenschaft* **29**, 467.

LIGHTBODY, L. G. & SMYTHE, V. R. (1962). Effect of heat treatment on bacteriological quality and phosphatase activity of market cream. *Queensland Journal of Agricultural Science* **19**, 77.

REPORT (1958). The bacteriological examination and grading of fresh cream. Report of a working party of the Public Health Laboratory Service. *Monthly Bulletin of the Ministry of Health and the Public Health Laboratory Service* **17**, 77.

REPORT (1971). The hygiene and marketing of fresh cream as assessed by the methylene blue test. Report of a working party of the Public Health Laboratory Service. *Journal of Hygiene* **69**, 155.

STATUTORY INSTRUMENT 1571: 1963. *The Milk (Special Designation) Regulations*, 1963.

TAYLOR, M. M. (1971). The water agar test for rapid estimation of psychrotrophs. *Dairy Industries* **36**, 400.

TAYLOR, M. M. (1975). The water agar test, a new test to measure the bacteriological quality of cream. *Journal of Hygiene* **74**, 345.

TEKINSEN, O. C. & ROTHWELL, J. (1974). A study of the effect of storage at 5° C. on the microbial flora of heat-treated market cream. *Journal of the Society of Dairy Technology* **27**, 57.

THOMAS, S. B., DRUCE, R. G., DAVIES, A. & BEAR, J. S. (1966). Bacteriological aspects of bulk milk collection. *Journal of the Society of Dairy Technology* **19**, 161.

WILSON, G. S. (1935). The bacteriological grading of milk. *Medical Research Council Special Report Series* No. 206, p. 232.