Microcalorimetric estimation of bacteria in milk

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The rapid detection of bacterial contamination is of practical importance to all organizations handling large quantities of milk. Dye reduction tests are frequently used but they require considerable incubation time unless the milk is heavily contaminated, and they have other disadvantages, whereas a sufficiently sensitive calorimeter would speedily indicate the level of bacterial metabolism.

The production of heat by bacteria varies widely (see, e.g., Bayne-Jones & Rhees, 1929; Forrest, 1969) but, taking one of the lower values observed by Bayne-Jones & Rhees we have 10^{-9} \text{ cal/h/cell} of \textit{Staphylococcus aureus}. This is equal to 5 \mu\text{ cal ml}^{-1} \text{ min}^{-1} in a culture containing 300,000 bacteria/ml. The sensitivity of the flow-through version of a microcalorimeter is of about this value, i.e. 6 \mu\text{ cal/min} (Monk & Wadsö, 1968).

However, many of the bacteria likely to be present in milk produce acid and the heat of neutralization will be added to the heat of metabolism. Thus, it was interesting to use a microcalorimeter (LKB Instruments Ltd., 232 Addington Road, South Croydon, Surrey) to compare the heat production of a sample of milk during the growth of its natural flora, with the number of bacteria determined by the plate count, and with the methylene blue reduction time.

A sample of bulked single herd milk was incubated at 25°C during three days and kept in ice during the intervening nights. From time to time a sub-sample was diluted and plated on ‘Yeastrel’ milk agar, a second sub-sample was subjected to the methylene blue test with incubation at 37°C and a third sub-sample was pumped through the calorimeter.

The calorimeter contains a heat exchanger by which the temperature of the inflowing liquid is brought to that of a metal heat sink surrounding the measuring cell and the thermocouples. From the heat exchanger the sample passes to the measuring cell and any heat produced in the sample while it is in the cell passes through the thermocouples into the surrounding heat sink. The amplified signal from the thermocouples is recorded and compared with that produced from a known quantity of electricity passed through a known resistance in the measuring cell under the same conditions.

In this experiment, sterile separated milk was pumped continuously through the calorimeter between the sampling periods, to provide a base line and during electrical standardization. Previous comparison had shown no heat effect when fresh pasteurized whole milk was pumped through in place of sterile separated milk. It seemed unlikely therefore that a serious error would arise from the use of separated milk to provide a base line against which heat production in full-cream milk could be measured.

The results are shown in Fig. 1 from which it is clear that the milk had a basal metabolism of about 200 \mu\text{ cal/ml/min} when the bacterial count was very low. This could have been due to leucocytes. An easily-discernible rise occurred at 16:00 h on the second day when the bacterial count was 275,000/ml and the methylene blue reduction time was 2\frac{1}{2} h. Since the calorimeter measurement required 0.5 h the result was obtained 2 h in advance of the methylene blue result. It is, moreover, possible that the time for the calorimetric measurement could be much reduced. Further, it is clear from the figure
that the heat output followed the bacterial count quite closely. Another sample of the same milk was kept in ice for two days and then incubated, being tested at intervals as the first sample. The results, which are shown in Fig. 2 confirm those for the first sample.

The result of this preliminary experiment suggests that further exploration of possible correlations between the hygienic status of milk and its output of heat might be useful.

REFERENCES

BRITISH STANDARDS

WAXED BOARD FOR ICE-CREAM PACKAGING

A new specification from the British Standards Institution, BS 4879 Waxed board for packaging ice-cream and frozen confectionery has been prepared at the suggestion of the National Association of Creamery Proprietors and Wholesale Dairymen.

The board specified is manufactured with either the traditional wax coatings or the more recently developed hot-melt coatings used to give a highly glossy surface. These coatings incorporate polymers and resins which provide heat-sealing properties. The standard is written in terms of both the composition of the individual components which make up the final product and the performance requirements of the final product. It has not been considered necessary to include limits for moisture penetration and absorption since existing products are satisfactory in this respect.

An appendix describes the composition of ice-cream for the guidance of board and carton manufacturers.

BS 4879 may be obtained from BSI Sales Branch, 101 Pentonville Road, London N1 9ND. Price 90p.