RESULTS WITH THE ASCHAFFENBURG–MULLEN PHOSPHATASE TEST FOR PASTEURIZED MILK.

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SUMMARY.

Good agreement between results using the Aschaffenburg-Mullen phosphatase test and the Kay-Graham test to detect under-pasteurization of milk was obtained. Higher atmospheric temperatures did not influence the performance of the test, provided the substrate and solutions were kept in the refrigerator to prevent hydrolysis.

The Aschaffenburg-Mullen test is of considerable use for testing certain flavoured pasteurized milks by diluting the test milk in an equal quantity of boiled normal milk.

As the Aschaffenburg-Mullen test is simple to perform, gives accurate results in two hours, and is unaffected by phenolic-type substances which interfere with the Kay-Graham test, it is very suitable for routine testing for pasteurization efficiency.

I. INTRODUCTION.

A rapid phosphatase test for pasteurized milk was introduced by Aschaffenburg and Mullen (1949), using di-sodium p-nitrophenylphosphate as substrate. This substance is rapidly hydrolysed to p-nitrophenol by milk phosphatase, and the test can be completed in two hours. Tramer and Wight (1950) suggested the use of permanent colour standards, and found that results obtained in the Aschaffenburg-Mullen test compared favourably with those obtained by the official Kay-Graham method.

The purpose of the present investigation was to determine the suitability of the test for use under Queensland conditions, and also to examine its suitability for the testing of flavoured milk. Before the completion of this work, the results of a similar investigation into the use of the test under Australian conditions were published by Marriner (1955).

II. METHODS.

The substrate di-sodium p-nitrophenylphosphate was unobtainable in Australia and was obtained from the Astell Laboratory Service Co. Ltd. of England. Though the powder when received showed slight yellowing, it was found to give a satisfactory substrate solution. For all experiments the carbonate-bicarbonate buffer suggested by Aschaffenburg (1953) was used, and the buffer-substrate solution was considered to be suitable for use if the colour did not exceed 10 on the APTW disc when comparison was made in a 25 mm. cell (Wight and Tramer 1952).

The method used was that outlined by Wight and Tramer (1952), using 5 ml. of buffer substrate and 1 ml. of milk for each test. The colour developed in the test was compared, after incubation at 37°C. for 30 min. and again after 2 hours' incubation, with a control containing boiled milk, using the APTW Lovibond disc which is calibrated in terms of μg. p-nitrophenol per ml. milk.

The substrate in the powder form has been kept at approximately 5 deg. C., with only short exposures to higher temperatures, for over four months without any deterioration. The prepared buffer-substrate solution, kept at refrigeration temperatures, remained serviceable for at least a week. However, if it was left at room temperature, considerable hydrolysis took place in 24 hours and a colour reading greater than 10 was obtained. As the substrate deteriorates quite rapidly at room temperature, it was necessary to ensure that the buffer-substrate solution was kept out of the refrigerator for as short a time as possible.

III. COMPARISON OF THE ASCHAFFENBURG-MULLEN AND KAY-GRAHAM TESTS.

The milks examined comprised samples of commercially pasteurized milk, and pasteurized milk with various amounts of added raw milk, to give a series of results over the critical range. All milks were examined by both tests, the Aschaffenburg-Mullen test being read after 30 min. and after 2 hours. The results obtained are set out in Tables 1 and 2. It can be seen from these results that there was good agreement between the two tests.

Table 1.

COMPARISON OF 30 MIN. ASCHAFFENBURG-MULLEN TEST AND KAY-GRAHAM TEST.

Reading on APTW disc.	Lovibond blue units produced in Kay-Graham test.					
	<2·3.	2·3.	>2·3.	<6·0.	6·0.	>6·0.
<6 ..	113	3	2	..	..	..
6 ..	1	2	15	2	..	..
10 ..	..	..	5	7	3	..
14 ..	..	..	..	1	8	2
18 ..	..	..	..	..	5	9
25 ..	..	..	..	..	1	3
42 ..	..	..	..	..	..	..
>42 ..	..	..	..	..	..	19

Table 2.

COMPARISON OF 2 HR. ASCHAFFENBURG-MULLEN TEST AND KAY-GRAHAM TEST.

Reading on APTW disc.	Lovibond blue units in Kay-Graham test.					
	<2·3.	2·3.	>2·3.	<6·0.	6·0.	>6·0.
<6 ..	102	..	..	..	..	..
6 ..	8	..	..	..	..	..
10 ..	4	5	2	..	..	..
14 ..	..	1	10	..	..	..
18 ..	..	..	11	3	..	..
25 ..	..	..	1	6	7	..
42 ..	..	..	..	1	7	11
>42 ..	..	..	..	..	3	24

The interpretation suggested by Wight and Tramer (1952) is as follows:—

30 min. incubation.	μ g. p–nitrophenol per ml. milk.	2 hr. incubation.
Properly pasteurized	0 or trace	Properly pasteurized
Doubtful	6	Properly pasteurised
Under-pasteurized	10	Properly pasteurized
	Over 10 to 18	Slightly under-pasteurized
	Over 18 to 42	Under-pasteurized
	Over 42	Grossly under-pasteurized.

Using this system of interpretation, it can be seen from Table 2 that two samples which passed the 2-hr. Aschaffenburg-Mullen test failed the Kay-Graham test, but the Lovibond blue units were only slightly greater than 2·3. One sample which passed the Kay-Graham test was classified as slightly under-pasteurized on the Aschaffenburg-Mullen test, and it did in fact contain approximately 0·2 per cent. raw milk.

Generally, it was possible to detect 0·2 per cent. raw milk by both tests, but it was found that there was some variation with different samples of raw milk, as was reported by Marriner (1955).

With samples of raw milk, or pasteurized milk containing 10 per cent. or more of raw milk, a definite yellow colour developed within a few minutes, and a reading of more than 42 was obtained in 30 min. The rapidity with which raw milk can be detected is an important feature of this test.

IV. USE OF THE ASCHAFFENBURG-MULLEN TEST WITH FLAVOURED MILK.

Experience over many years in this State (V. R. Smythe, personal communication) has shown that many flavoured milks cannot be tested for efficiency of pasteurization by the Kay-Graham test, as the syrups containing the flavouring and colouring are usually preserved with benzoic acid, which interferes with the test and gives very dark controls. The Aschaffenburg-Mullen test is not affected by the presence of phenolic-type substances, but the yellow colour developed in the test may be masked if the flavoured milk contains too much colour.

Experiments were made with chocolate-flavoured milk and strawberry-flavoured milk, in both of which the colour was quite intense. Although the same milk was used for the control, colour matching in the comparator was not easy and considerable inaccuracy might result.

In order to eliminate some of the interfering colour, tests were conducted using equal quantities of the flavoured milk and boiled normal milk. This dilution of the flavoured milk in normal milk considerably reduces the intensity of the colouring. When 1 ml. of the mixture was added to 5 ml. of buffer-substrate, the colour did not interfere with readings. The control tube contained 1 ml. of a boiled mixture of equal parts of normal milk and flavoured milk.

Dilution of the test milk as described reduces by half the sensitivity of the phosphatase test. However, the test should still detect 0·4 per cent. added raw milk in the sample, and the greater ease of colour matching with the diluted samples is considered to warrant the use of the smaller sample.

V. DISCUSSION.

As the Aschaffenburg-Mullen phosphatase test is simple to carry out and gives accurate results in a short time, it is very suitable as a routine control test for pasteurizing efficiency. The substrate is specific and therefore there is little likelihood of errors because of contaminating substances. This is one of the disadvantages of the Kay-Graham method, in which a series of controls have to be performed to check against false positives. In the case of a high control value in the latter test there is no method of testing the sample.

Comparisons between results obtained in the Aschaffenburg-Mullen test and the official Kay-Graham test have been made by Tramer and Wight (1950), Webb and Humphries (1954), and Marriner (1955). These authors all found good agreement between the two tests, as is also seen in the results quoted herein.

The extension of the test to flavoured pasteurized milk should prove a useful control measure, which is especially necessary in view of the fact that a large proportion of this milk is consumed by children. It is suggested that the flavoured milk be diluted in an equal quantity of normal milk which has been boiled for the purposes of this test. Though the sensitivity is thereby reduced, the test can still prove very useful in the examination of flavoured milks. Some flavoured milks may not contain very much colour and in such cases it may be quite satisfactory to examine them without dilution.

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