STUDIES ON THE EFFECTS OF TRANSPORT AND STORAGE ON THE BACTERIOLOGICAL QUALITY OF RAW MILK.

Part 2.—The Effect of the Milk Can on the Methylene Blue Reduction Time.

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SUMMARY.

Factors inherent in the milk can have been found to cause a marked fall in the reduction time of raw milk. The effect is accentuated by increased milk temperature and by prolonged transport. Furthermore, such factors result in a greater degree of injury to high quality milks than to low quality milks. No attempt has been made to differentiate between individual can factors.

INTRODUCTION.

In a previous paper (Smythe, 1945) data were presented showing the trends in the methylene blue reduction time of raw milk which follow various conditions of storage time and temperature. Since these data were obtained in the laboratory, where standardized conditions were attainable for the study of such trends, they do not represent entirely the conditions which apply during commercial handling and transport. Under actual transport conditions milk comes under the influence of factors such as contamination from can surfaces, agitation and aeration which may modify quality to a considerable extent. All such factors are intimately bound up with the milk container and for the purposes of this paper are collectively referred to as the "can factor." This paper presents an attempt to study the aggregate effect of these factors, thus affording a more complete interpretation of the effect of handling and transport conditions in Queensland on milk quality as judged by the methylene blue test.

An experiment was performed with the following aims:—

(*a*) To provide a measure of the effect of the can factor on the methylene blue reduction time of raw milk.

(*b*) To learn the extent to which the effect of the can factor may be modified by (1) the quality of the milk transported; (2) the period of holding prior to transport; (3) the duration of transport; (4) milk temperature at commencement of transport; and (5) mean air temperature of transport.

EXPERIMENTAL METHODS.

The effect of the can factor on the reduction of methylene blue has been measured by sampling a large number of milks at pick-up. Samples were drawn from cans of milk into sterile methylene blue test tubes fitted with rubber stoppers, and these tubes were placed in a basket above the cans in the milk truck. A further sample of each milk was taken at the depots and both series of samples were then brought to the laboratory for testing. In this way a sample of each milk was subjected to much the same conditions of transport as the filled cans, but was removed from further influence of the can container. Such samples served as controls. The difference between the reduction times of tube and can samples has been termed the fall in reduction time and has been taken as resulting from the can factor. It is to be noted that under the conditions of the experiment tube samples were more affected by transport temperature than were the larger bulk lots of milk in the cans. The unavoidable error which resulted will be discussed later.

Air temperatures in the truck were taken throughout the journeys with the thermometer placed in the basket of tubes. Usually such temperatures rose steadily during the mornings and as a result the mean air temperature during transport had to be calculated from the temperatures prevailing during the time each milk was in the truck. Milk temperatures were also taken at pick-up and again at depot.

The pre-transport period—i.e., the time elapsing from the completion of milking until pick-up—was obtained from information given by each

Table 1.

SUMMARY—MEAN VALUES.

No. of Samples.	Fall in Reduction Time. (Hrs.).	Reduction Time of Tube Sample (Control). (Hrs. and Mins.).	Time (Hrs. and Mins.). Transport.	Pre-transport.	Temperature °F. Milk at Pick-up.	Milk at Depot.	Mean Air Temp. in Truck.
	y	x_1	x_2	x_3	x_4	x_5	x_6
5 ..	0	3 : 27	2 : 40	0 : 44	82·8	83·8	81·5
12 ..	¼	3 : 58	2 : 48	0 : 57	81·4	81·3	78·6
14 ..	½	4 : 33	2 : 51	1 : 4	81·5	82·2	78·6
19 ..	¾	4 : 9	3 : 2	1 : 9	81·9	81·2	78·7
14 ..	1	4 : 49	3 : 4	0 : 49	82·6	82·2	79·6
16 ..	1¼	4 : 36	3 : 22	0 : 45	85·5	82·9	78·4
12 ..	1½	4 : 41	3 : 22	0 : 33	85·0	83·3	78·5
4 ..	1¾	4 : 53	3 : 56	0 : 29	82·8	81·0	77·1
5 ..	2	6 : 0	4 : 28	0 : 38	81·4	79·6	76·6
3 ..	2¼	5 : 15	3 : 34	0 : 32	85·0	82·7	77·4
2 ..	2½	5 : 15	4 : 36	0 : 9	87·4	82·0	75·9
0 ..	2¾	..	..	..	..	..	..
1 ..	3	7 : 30	3 : 30	0 : 24	77·5	79·0	77·4
0 ..	3¼	..	..	..	..	..	..
1 ..	3½	6 : 30	3 : 48	0 : 6	94·0	80·0	76·0
1 ..	3¾	6 : 15	3 : 54	0 : 30	89·0	84·0	75·8
Mean ..	1·057	4 : 36	3 : 13	49·32 min.	83·131	82·028	78·554
S.E. of mean	±·068	±7·24 min.	±5·48 min.	±3·39 min.	±·385	±·281	±·259

producer. As producers were not always waiting with their milk and as some who were waiting may not have been accurate with the information they supplied, the data in this respect had to be treated with caution. The duration of transport was taken as the period from pick-up until commencing the methylene blue test. On no occasion were samples iced. As in previous experiments the technique of Wilson (1935) was used for methylene blue testing.

EXPERIMENTAL DATA.

The fall in reduction time from the tube sample to the can sample has been taken as an index of the effect of the can factor. In no instance was a negative fall observed, while in only 5 cases out of a total of 109 was the fall found to be zero. Thus, in such instances, no deterioration in reduction time can be attributed to the cans. In order to express the great bulk of results obtained a summary of mean values has been set out as a frequency table in Table 1. In this summary a class range of $\frac{1}{4}$-hour fall in reduction time has been used.

In order to find whether the observed fall in reduction time was related to any of the six variables measured the data obtained for each sample have been taken separately and a statistical analysis performed using the method of partial regression.

The following variables were measured:—

y = fall in reduction time from tube to can sample.
x_1 = reduction time of tube sample (control).
x_2 = transport time.
x_3 = pre-transport period.
x_4 = milk temperature at pick-up.
x_5 = milk temperature at depot.
x_6 = mean air temperature of transport.

A partial regression equation of the following type was fitted:—

$$y-\bar{y}=b_1(x_1-\bar{x}_1)+b_2(x_2-\bar{x}_2)+b_3(x_3-\bar{x}_3)+b_4(x_4-\bar{x}_4)+b_5(x_5-\bar{x}_5)+b_6(x_6-\bar{x}_6)$$

The analysis of variance is given in Table 2, and a summary of regression coefficients with corresponding "t" values is given in Table 3.

Table 2.

ANALYSIS OF VARIANCE.

Source of Variation.	D.F.	Sum of Squares.	Mean Square.
Regression	6	457·67	76·28
Residual	102	411·60	4·035
Total	108	869·27	..

F for regression = 18·9, which is very highly significant.

Table 3.

SUMMARY.

Regression Coefficient.	"t"
$b_1 = \cdot 233 \pm \cdot 040$	5·82
$b_2 = \cdot 764 \pm \cdot 280$	2·73
$b_3 = -\cdot 560 \pm \cdot 375$	1·49
$b_4 = \cdot 324 \pm \cdot 068$	4·75
$b_5 = -\cdot 011 \pm \cdot 115$	<1
$b_6 = -\cdot 222 \pm \cdot 098$	2·26

The interpretation of partial regression coefficients is such that the significance tested is actually that of the additional amount of variation in fall in reduction time accounted for by the variable under consideration over and above that accounted for by the remaining variables. Thus, in the case of variable x_5 (milk temperature at depot), all that may be said is that the fit of the regression line ignoring this variable is not significantly worse than the fit of the regression line on all six variables. In other words, the data supply evidence that any effect on the reduction time resulting from the temperature of the milk at the depot is adequately represented by the effects of the remaining variables.

Regression coefficients b_3 and b_5 are not significant. Coefficients b_3 and b_6 are negative, indicating that the fall in reduction time is less for higher values of variables x_3 and x_6. As noted previously, the data for x_3 were not trustworthy, and as the value is not significant the result may be attributed to this feature.

Since the reliability of the data for x_3 possibly affects the other coefficients, a second analysis was carried out omitting this variable together with x_5, which was found insignificant in the first analysis. The results of this second analysis are set out in Tables 4 and 5.

Table 4.

SECOND ANALYSIS OF VARIANCE.

Source of Variation.	D.F.	Sum of Squares.	Mean Square.
Regression	4	448·67	112·17
Residual	104	420·60	4·044
Total	108	869·27	..

F for regression = 27·7, which is very highly significant.

Coefficients b_1, b_2 and b_4 are significant; b_6 is negative and significant at the 5 per cent. level. The negative value of this coefficient would indicate that, when other factors are constant, the fall in reduction time is greater when the mean air temperature of transport is lower. Such a result is anomalous.

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Table 5.

SUMMARY.

Regression Coefficient.	"t"
$b_1 = \cdot 244 \pm \cdot 039$	6·18
$b_2 = \cdot 910 \pm \cdot 245$	3·72
$b_4 = \cdot 344 \pm \cdot 050$	6·81
$b_6 = - \cdot 188 \pm \cdot 089$	2·12

The explanation of this result obviously lies in the fact that the smaller bulk of the tube samples was more affected by air temperature in the truck than was the larger bulk of milk in the cans. In every instance the milk in cans was higher in temperature than the truck atmosphere, with the result that a fall in milk temperature was inevitable. The tubes of milk quickly fell to air temperature, inducing a slightly prolonged reduction time. The lower the air temperature, the more prolonged would the reduction time become. This lengthening would be reflected not only as a prolonged control value, but as an increased fall in reduction time from the tube to the can samples. The only way to overcome this defect in technique would have been to immerse the tube samples in the cans of milk, thus producing equality in the temperature trends of both samples and bulk. As this would have been most undesirable for many reasons, the error had to be accepted. Unfortunately, however, this error was very far reaching and no doubt caused a general increase in all observed falls in reduction time.

DISCUSSION.

The results obtained show that factors inherent in the milk can may be responsible for a considerable fall in the methylene blue reduction time of raw milk quite apart from the fall resulting from time and temperature of storage. A mean fall in reduction time of 1 hour 3 minutes was observed with 109 milks stored and transported under actual commercial conditions when the mean period of transport from pick-up to testing was 3 hours 13 minutes. This fall is independent of the direct effect of temperature and time, but is influenced by the effect of these two factors on the can factor. It is suggested that this fall in reduction time is the result, either directly or indirectly, of improperly sterilized can surfaces.

Observations made on the condition of cans returned to farmers from Brisbane milk depots suggest that bacterial contamination from cans is probably the greatest contributing agency to the can factor. It is obvious that organisms closely associated with a can surface which has been improperly cleansed and sterilized will provide constant contamination of the contained milk when conditions for bacterial growth are favourable. Particularly would this be true when minute pitting of the can surfaces harbours sporing bacilli, incapable of sterilization under farm conditions. The effect of aeration and agitation cannot, however, be completely ignored. Following the work of

Wilson (1935), Davis and Newland (1944) and Davis *et al.* (1945) it would appear that agitation and aeration may considerably modify the quality of milk samples. It is probable that similar phenomena occur in the case of milk in cans and particularly in partly filled cans.

The effect of the can factor may, however, be influenced to a great extent by a number of contributory factors. As would be expected, particularly in the light of the results previously obtained (Smythe, 1945), temperature and time exert a marked influence. Within the temperature ranges prevailing at the time of experiment the effect of milk temperature was found to be greater than that of time. The temperature of the milk at pick-up was found to be directly related to the fall in reduction time. This places emphasis on the need for rapid cooling of milk on the farm. No relation could be found between the milk temperature at the depot and the fall in reduction time. Such a result would be expected to follow the levelling-out of milk temperatures during transport.

The duration of transport has been shown to affect the fall in reduction time in a significant manner. The transport period has been taken as extending from the time of pick-up of the milk until commencing the methylene blue tests. Actually this period was longer (approximately 1 hour) than the true time of transport, that is, from pick-up to tipping, since milks were sampled at tipping and brought to the laboratory for testing. Data concerning the pre-transport period from completion of milking to pick-up must be regarded as being largely unreliable, and as a consequence no importance was attached to them.

Probably the most interesting feature of the results is the marked positive relation between the quality of the milk, as shown by the reduction time of the tube sample which is taken as a control, and the fall in reduction time. Other factors being constant, the fall in reduction time is much greater when milk quality is high. This result is a parallel to that obtained in the previous work (Smythe, 1945) in respect to temperature and time, but the effect is even more marked in this instance. No further explanation can be offered than was tendered then, namely, that poor quality milks allow comparatively less scope for bacterial growth and consequent reduction by virtue of the heavy bacterial populations they already carry than do milks of better quality.

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