INHIBITORY FACTORS IN CHEESE MILK.

By AILSA GILLIES, B.Sc. (App.), Dairy Research Laboratory, Division of Dairying.

SUMMARY.

Some milks were found to be inhibitory to one group of starters when tested by a 6½ hour activity test, but the phenomenon was not evident when the same milk was tested by a 6½ hour vitality test in which the milk is clotted with rennet.

Comparison of these tests with actual vat workings at a commercial cheese factory showed that the results of vitality testing correlate well with acidity production in the vat. In contrast, the results obtained from the activity test are markedly affected by inhibitory factors in the milk and show no correlation whatever with vat workings.

It is concluded that the activity test in pasteurized milk is a very unreliable measure of the ability of starter strains to work satisfactorily in the cheese vat and that the vitality test should be used for this purpose.

I. INTRODUCTION.

In the past few years, both in Australia and overseas, attention has been given to the so-called inhibitory winter milk supplies. Two sets of workers —Czulak and Meanwell (1951) in England and Jago (1954) in Australia— have found that, when grown in pasteurized milk for 6 hours at 30 deg. C., single strain starters fall into two categories on the basis of their acid production. One group comprises those starters which are inhibited in pasteurized milks; the other contains those that are unaffected. Both workers also found that sterilizing the milk eliminates the inhibitory effect.

In Queensland, the New Zealand vitality test (Whitehead and Cox 1932) has been used as a means of gauging acid-producing activity and no general incidence of inhibitory milk has been noticed with this test. It was only when using the activity test (Anderson and Meanwell 1942) as a quicker and less tedious method of examining acid-producing abilities that any evidence of an inhibitory phenomenon became apparent.

The two tests under consideration measure different things. The activity test is a measure of acidity production in heated or raw milk, whereas the vitality test measures acid production after the addition of rennet to the milk.

The present work was designed to establish any differences that may result when comparing acid production in the two tests. If these differences are marked, one or other of the tests cannot be used to predict the behaviour of starters in the vat.

II. LABORATORY COMPARISON OF TESTS.

(1) Methods.

In order to obtain a representative range of factory milks the bulk supply to four different factories close to the laboratory was sampled. In some cases mixed evening and morning milk was used and in some cases milk was held chilled at the factory overnight before it was pasteurized. All milks were sampled as soon as they were H.T.S.T. pasteurized, and testing was commenced immediately they were received at the laboratory. Each milk was tested in duplicate by both activity and vitality tests.

Six starters were tested. They divided into two groups: (a) those which were inhibited when cultured in pasteurized milk; and (b) those which were resistant to the inhibitory principle in the same milk. The six starters chosen were: (*a*) HP, R6, R1 (New Zealand strains), and (*b*) C1, C2, C13 (C.S.I.R.O. Laboratory, Victoria). All starters were active in sterilized milk and were quite distinct with regard to phage relationships. It is to be noted that strain C2 is a strain of *Streptococcus lactis;* all others are strains of *S. cremoris.*

The activity test as employed by Czulak and Meanwell (1951) and Jago (1954) was adapted so that 50 ml. of milk was dispensed in 2 oz. McCartney bottles, 0·5 ml. of starter added to each bottle, and the bottles incubated at 30 deg. C. for 6½ hours. Acidity was determined at 5½ hours and 6½ hours.

The vitality test was based on that of Whitehead and Cox (1932) and was as follows:—

One pint of milk at 86 deg. F. was placed in each jar, 6 ml. of starter added, and the jars placed in a water bath at 86 deg. F.

After 1 *hour,* diluted rennet was added in appropriate concentrations to give a clot in 15 min. and mixed thoroughly.

After 1½ *hours,* the curd was cut as evenly as possible in two directions and the temperature raised to cooking temperature (100 deg. F.) over a period of 30 min.

After 3½ *hours,* the whey was drained off. The temperature of the bath and contents was then allowed to fall to 86 deg F.

After 5½ *hours,* the whey was drained and the acidity determined.

After 6½ hours, the whey was again drained and the acidity determined.

The vitality test has been used in Queensland because the conditions of the test approximate closely the cheese-making process. The starters were cultured at 86 deg. F. for the greater part of the test and were raised to 100 deg. F. only for a time comparable with cooking in the cheese vat. Thereafter, the temperature was allowed to fall back steadily to approximately 86 deg. F. A somewhat similar falling back occurs in practical cheese-making.

In both tests acidity was determined by titrating 9 ml. whey (vitality test) or 9 ml. milk (activity test) with O·1N NaOH, using phenolphthalein as indicator.

(2) Results.

Acidity determinations for both activity and vitality tests were obtained from titrations done after 5½ hours and 6½ hours. The rise in the final hour has been noted for both sets of tests in Tables 1 and 2.

Table 1.

ACIDITY PRODUCTION IN ONE MILK USING THE ACTIVITY AND VITALITY TESTS.

Starter.	Activity Test. Acidity Percentages.			Vitality Test. Acidity Percentages.		
	5½ hr.	6½ hr.	Rise in Final Hour.	5½ hr.	6½ hr.	Rise in Final Hour.
HP	·22	·28	·06	·34	·59	·25
R6	·22	·29	·07	·41	·67	·26
R1	·24	·33	·09	·34	·58	·24
C1	·35	·49	·14	·32	·46	·14
C2	·29	·50	·21	·36	·60	·24
C13	·34	·53	·19	·32	·52	·20

Table 2.

AVERAGE ACIDITY PRODUCTION OF SIX MILKS USING THE ACTIVITY AND VITALITY TESTS.

Starter.	Activity Test. Acidity Percentages.			Vitality Test. Acidity Percentages.		
	5½ hr.	6½ hr.	Rise in Final Hour.	5½ hr.	6½ hr.	Rise in Final Hour.
HP	·22	·26	·04	·34	·46	·12
R6	·23	·29	·06	·43	·56	·13
R1	·24	·30	·06	·36	·46	·10
C1	·36	·47	·11	·34	·45	·11
C2	·34	·51	·17	·38	·56	·18
C13	·35	·51	·16	·33	·46	·13

In both tests there were instances of variation between titrations of duplicate tests. In the vitality test 75 per cent. of duplicate titrations showed a difference of ·02 per cent.; in the activity test 77·7 per cent showed a difference of ·01 per cent. or less. In all instances, the mean value was taken as the true one. A typical set of results is given in Table 1.

The averages of results from tests carried out on six different days are set out in Table 2.

These results clearly demonstrate that there were marked differences in the acidity production of some strains in the two tests. These differences are such that the activity test would tend to give a false impression of the acid-producing ability of these strains under conditions of renneting. It is unlikely, therefore, that this test would be an accurate guide to vat workings.

III. FACTORY COMPARISON OF TESTS.

Following the results obtained in the laboratory when comparing the activity and vitality tests, it was decided to extend the work to factory conditions and compare starter behaviour in the cheese vat with both tests.

Accordingly, arrangements were made with one Dairy Association to use only one single strain starter in the vat on the days when trial tests would be run.

(1) Methods.

For each series of tests a drip sample from a sterilized sampler was taken so that a representative sample of the milk in the vat was obtained. The milk was collected in a sterile can and the milk immediately transported to the laboratory, where tests were started immediately.

For each day the tests were run the cheesemaker kept complete vat records. In addition, a sample of the bulk starter used in the vat was collected and brought back to the laboratory for testing with the laboratory starters. The work was so arranged that one of the same six starters used in the laboratory test was used in the vat. When the milk samples arrived at the laboratory, sub-samples were taken for vitality and activity tests.

(2) Results.

The results representing tests run on two days are set out in Tables 3 and 4.

It is obvious from these results that strain R6 works satisfactorily in the vat even though it shows little activity as judged by the activity test. On the other hand, strain C13 gave satisfactory acid production in the cheese vat and during both tests. This strain was apparently resistant to the inhibitory factor in pasteurized milk and its vat behaviour correlates well with results of the activity test. One may conclude that the activity test is not a reliable indicator of the acid-producing ability of a cheese starter in the vat.

These results clearly demonstrate also that there were marked differences in the acidity production in the two tests. These variations are such that the activity test tends to give a false impression of the acid-producing ability of many strains under conditions of renneting. It is unlikely, therefore, that this test would be an accurate guide to acid production under normal cheese-making conditions.

Table 3.

ACID PRODUCTION OF STARTERS IN PASTEURIZED MILK USING (*a*) ACTIVITY TEST, (*b*) VITALITY TEST, AND (*c*) COMMERCIAL CHEESE-MAKING CONDITIONS.

Starter.	Activity Test. Acidity Percentages.			Vitality Test. Acidity Percentages.			Vat Records (C13 Starter used 1·9%).		
	5½ hr.	6½ hr.	Rise in Final hour.	5½ hr.	6½ hr.	Rise in Final hour.	Stage.	Acidity.	Temperature.
							Starter added (at start)		
HP	·21	·24	·03	·27	·47	·20	Set (7 min. after start))	·185	88°F.
R6	·21	·23	·02	·42	·64	·22	Cut (37 min. after start)	·13	
R1	·25	·30	·05	·32	·51	·19	Cooked	·13	100°F.
C1	·37	·50	·13	·37	·50	·13	Run off (2 hr. 17 min. after start) ..	·16	
C2	·40	·52	·12	·32	·46	·14	Dry	·21	
C13	·32	·45	·13	·27	·46	·19	Milled (4 hr. 17 min. after start) ..	·62	
C13 B.S.* ..	·39	·54	·15	·33	·51	·18	Salted (4 hr. 42 min. after start) ..	·65	

* B.S. = Bulk starter.

Table 4.

ACID PRODUCTION OF STARTERS IN PASTEURIZED MILK USING (*a*) ACTIVITY TEST, (*b*) VITALITY TEST, AND (*c*) COMMERCIAL CHEESE-MAKING CONDITIONS.

Starter.	Activity Test. Acidity Percentages.			Vitality Test. Acidity Percentages.			Vat Records (R6 Starter used 1·3%).		
	5½ hr.	6½ hr.	Rise in Final hour.	5½ hr.	6½ hr.	Rise in Final hour.	Stage.	Acidity.	Temperature.
							Starter added (at start)		
HP	·23	·25	·02	·30	·52	·22	Set (6 min. after start)	·185	88° F.
R6	·22	·25	·03	·40	·62	·22	Cut (35 min. after start)	·13	
R1	·24	·25	·01	·30	·55	·25	Cooked	·13	100° F.
C1	·30	·41	·11	·35	·56	·21	Run off (2 hr. 10 min. after start)..	·16	
C2	·36	·43	·07	·34	·52	·18	Dry	·22	
C13	·29	·40	·11	·32	·50	·18	Milled (3 hr. 55 min. after start) ..	·65	
R6 B.S.* ..	·25	·29	·04	·37	·60	·23	Salted (4 hr. 25 min. after start) ..	·70	

* B.S. = Bulk starter.

IV. EFFECT OF TEMPERATURE.

It was thought possible that the difference in results obtained from the two tests might be explainable on a temperature basis because the tests are not performed at the same temperature. Tests were conducted in order to examine this possibility.

(1) Methods.

Pasteurized milk was dispensed in 50 ml. quantities into McCartney bottles and 1 per cent. of starter added to each bottle. Starters HP, R6, R1, C1, C2, C13 were used and all tests were carried out in duplicate. Two series of bottles of milk were inoculated. Series A was incubated at 30 deg. C. for 6½ hours, and Series B was placed in a water bath at vitality test temperatures for 6½ hours.

(2) Results.

Acidity production in one milk, using (*a*) an incubation temperature of 30 deg. C. for 6½ hours (Series A), and (*b*) a temperature of 37 deg. C. for 2 hours during the course of 6½ hours' incubation (Series B), is shown in Table 5.

From these results it is evident that there is little effect due to the temperature difference. It is clear also that the starter strains again fall into two distinct groups with regard to acidity production.

Table 5.

EFFECT OF INCUBATION TEMPERATURE.

Starter.	Series A.			Series B.		
	Acidity Percentages.			Acidity Percentages.		
	5½ hr.	6½ hr.	Rise in Final Hour.	5½ hr.	6½ hr.	Rise in Final Hour.
HP	·26	·30	·04	·23	·27	·04
R6	·28	·30	·02	·28	·30	·02
R1	·28	·30	·02	·30	·34	·04
C1	·58	·67	·09	·61	·64	·03
C2	·55	·68	·13	·54	·70	·16
C13	·58	·68	·10	·50	·67	·17

V. DISCUSSION.

The experiments reported herein were undertaken to compare the acid-producing abilities of starter cultures in the activity test and the vitality test, and the relationship of the results of these tests to starter behaviour in the cheese vat.

When the activity test was used to determine the acid production of the cultures, it was found that the starters were divided into two groups—the fast acid producers, and strains which were susceptible to some inhibitory factor in the pasteurized milk. This finding is similar to that of Czulak and Meanwell (1951) and Jago (1954). These workers divided their starter cultures into susceptible and resistant groups and used the resistant types in cheese-making to overcome the inhibition which had been found to cause marked vat slowness. However, the inhibitory principle which was investigated by these workers apparently differs in some way from that detected by the activity test in the present work, even if only in degree; for it was found that, although a culture was inhibited in the activity test, acid production in the cheese vat was quite normal.

When a vitality test was conducted using pasteurized milk which inhibited some starter strains in the activity test, all starters produced normal amounts of acid and there was no evidence of any inhibitory factor. It was also found that the vitality test results in the laboratory correlated very well with commercial vat workings, and that the inhibitory factor revealed by the activity test was without effect when rennet was added, as in the vitality test and in cheese manufacture. Therefore, it is apparent that the activity test in pasteurized milk may be a most unreliable indicator of the suitability of a starter for cheese-making, and it should not be used as a basis for starter strain selection. The selection of strains on the basis of resistance to an inhibitory principle, such as was present in the pasteurized milk used in these investigations, is not justified. The vitality test is a more satisfactory means of selecting or differentiating starter strains suitable for cheese-making.

No attempt has been made in the present work to study the exact nature of the inhibitory factor in milk, but it is suggested that the inhibition may be a manifestation of the natural bactericidal property of milk, which retains greater activity during the cooler winter weather and quickly passes off under the high temperatures of the Queensland summer.

It was considered that the differences between the results of the activity and vitality tests using pasteurized milk might be due, at least in part, to differences in the temperatures at which the tests are conducted. However, it has been found that this was not the case, and, consequently, it would seem that the suppression of the inhibitory principle in the vitality tests and in the cheese vat is a result of the clotting of the milk with rennet.

VI. ACKNOWLEDGEMENT.

It is desired to thank the manager of the Pittsworth Co-operative Dairy Association, Pittsworth, for his co-operation in keeping vat records.

REFERENCES.

ANDERSON, E. B., and MEANWELL, L. J., 1942. The problem of bacteriophage in cheese making. Part I. Observations and investigations on slow acid production. J. Dairy Res. 13: 58-72.

CZULAK, J., and MEANWELL, L. J. 1951. Seasonal variation in cheese starter activity. Proc. Soc. Appl. Bact. 14: 1-6.

JAGO, G. R. 1954. Factors influencing the lactic-acid producing properties of streptococci used in the manufacture of cheddar cheese. I. Observations relating to inhibitory and stimulatory phenomena. J. Dairy Res. 21: 111-121.

WHITEHEAD, H. R., and COX, G. A. 1932. A method for the determination of vitality of starters. N.Z. J. Sci. Tech. 13: 304.