A METHOD FOR THE DETERMINATION OF THE KEEPING QUALITY OF MILK.

By JOHN C. BAKER and LUCIUS L. VAN SLYKE.

(*From the Chemical Laboratory of the New York Agricultural Experiment Station, Geneva.*)

(Received for publication, October 8, 1919.)

INTRODUCTION.

Keeping quality or keeping power is an expression used to indicate the length of time milk remains sweet and otherwise palatable and suitable for direct consumption. This is obviously an important factor in estimating the commercial value of market milk, since milk that is sour or otherwise unpalatable is comparatively valueless for direct use, however rich it may be in fat and other solids.

Various methods have been proposed for measuring the keeping quality of milk but these have been found unsatisfactory in actual practice. Therefore, a method which can be utilized to furnish consistent results in measuring, even though only approximately, the keeping quality of different milks is needed.

Proposed Method.

We have found that the brom-cresol purple test (see page 359) can, with simple modifications in technique, be applied to the measurement of certain factors affecting the keeping power of milk. In applying the test for this purpose, the test-tubes and pipettes must be sterilized before use and the milk in the test-tubes must be incubated for a stated length of time at a definite temperature. Examination of the milk after incubation furnishes evidence in respect to the keeping power of milk as shown by one or more of several possible changes that may take place in the milk. Such changes can be divided into two classes, first, those affecting the color of brom-cresol purple, which show a

change of reaction in the milk due to the production of acid or less often to the formation of alkali salts; and, second, other accompanying or succeeding changes, such as curdling of the milk due to coagulation of casein, digestion of casein, changes in the character of the coagulated or curdled milk, production of gas, and the development of abnormal odor and taste.

Non-Germicidal Effect of Brom-Cresol Purple Solution.

In order to be of value as a means of measuring the keeping quality of milk by the reaction, it is essential that the brom-cresol purple solution should not, under the conditions used, show any germicidal effect sufficient to interfere with the growth of bacteria in milk. In order to test this fundamental requirement, pure cultures of *Bacterium lactis acidi* were added to freshly pasteurized skim-milk; one portion of this was treated with brom-cresol purple solution and both portions were incubated at 20°C. At intervals the brom-cresol purple test was applied to samples taken from the incubated portion of milk containing none of the indicator, and comparison was made with the portion to which brom-cresol purple had been added at the start. Also samples of the two portions of milk were titrated with alkali. These tests were made many times with different milks, but in no case was there observable any difference in behavior. The same tests were also applied in numerous cases to two portions of a milk undergoing the process of natural souring, using both unheated and pasteurized milk, without showing any difference. The results all go to show that the brom-cresol purple solution has no germicidal effect under the conditions used. However, it is advisable to take precaution to use for this test only brom-cresol purple that is wholly free from the odor of phenol or cresol, as suggested by Clark and Lubs.[1] In our experience it is not difficult to obtain this.

Production of Acid.

In considering the application of the brom-cresol purple test to the measurement of the keeping quality of milk as shown by the formation of acid in milk, we will present the subject under

[1] Clark, W. M., and Lubs, H. A., *J. Agric. Research*, 1917, x, 105.

the two heads, localization of acid production and degrees of acid production.

Localization of Acid Production.—In the natural souring of milk standing undisturbed, the formation of acid rarely proceeds uniformly through the body of the milk but is largely localized, especially in the earlier stages of the process. Acid is usually first formed in appreciable amount at the upper surface next the cream layer, or less often in the layer at the bottom of the container, or it may appear in some cases simultaneously in both the top and bottom layers. Less frequently it may start in the layer of milk next the side walls of the container.

When acid is formed first at the upper surface of the milk, it is probably due to the fact that the organisms are enmeshed and carried upward with the rising fat-globules and are thus concentrated in the upper layer. The bacteria left in the body of the milk after the rising of the fat-globules would tend, under the downward pulling effect of gravity, to settle at the bottom of the container. Generally, the number carried up is apparently greater than that carried down. Such a concentration of bacteria in the top or bottom layer of the milk would have the effect of making the brom-cresol purple test more sensitive as a result of more rapid formation of acid. The effect of acid development is more commonly shown first in the upper layer, though sometimes in the lower or less often in the side layer. But whether it starts at the top or bottom or side, the process of acid production works from the starting area or areas through the main body of the milk.

Degrees of Acidity.—It would be desirable, if it were possible, to distinguish different degrees of increasing acidity by preparing a color standard representing different values of hydrogen ion concentration, similar to the method described on page 363 for determining the approximate hydrogen ion concentration of milk when its acidity is less than that of normal milk. This is impossible for several reasons and especially because, as pointed out above, the production of acid is localized and not distributed uniformly through the body of the milk. However, we have found that it is possible, with some experience, to distinguish readily not less than four degrees or stages of acidity by changes of color, varying from the grayish-blue observed with normal

fresh milk to a pure yellow occurring in milk sufficiently sour to undergo coagulation, which occurs at about pH 4.65. These four stages or degrees of acidity can be distinguished by the following description.

(1) The first stage or beginning of acid production (A_1) is indicated by the first sign of change from the grayish-blue color of normal milk to a lighter shade observable in any portion of the milk. This is most often distinguishable at the upper surface of the milk just under the cream layer, though it may occur at the bottom layer of the milk or less frequently at the side walls of the containing vessel.

(2) The second stage (A_2) shows distinct acid production and is indicated when the milk in a test-tube gives evidence of more extensive and marked change of color than in case of A_1. The main body of the milk, however, still retains a grayish-blue color more or less interspersed with, but predominant over, yellowish or greenish-yellow shades. The prevailing color may be bluish or a dull shade of bluish-green.

(3) The third stage (A_3) shows marked acid production and is indicated when the color of the milk in the test-tube appears greenish to greenish-yellow; the yellow is predominant through the body of the milk, though not complete, but is more or less interspersed with shades intermediate between dull green and yellow.

(4) The fourth stage (A_4) of acid production is easily observable, since the color is a pure, fairly uniform yellow, free from every trace of bluish or greenish tints. The curdling of the milk usually occurs at this stage and is generally, though not always, readily seen.

It can easily be understood that these divisions are somewhat arbitrary and not always capable of sharp separation but they afford a practical basis for differentiating milks, furnishing a test which greatly exceeds in delicacy and ease of application the usual titration methods. With experience in distinguishing shades of color, it is easily possible to carry the division of classes further if desired; but usually the four broad classes described above suffice for most purposes.

Other Changes in Milk.

Changes other than those produced by acid formation can also be observed, and to these attention will be briefly called. Such changes may occur only after somewhat prolonged incubation in the case of good market milks, but they appear more quickly in the case of milks which have been drawn more than 24 hours before incubation, or in the case of milks drawn under unfavorable conditions as to cleanliness and not kept at a sufficiently low temperature. It should be stated here that while these changes have been studied by bacteriologists, it is essential that they be given special attention and further study under the conditions of the proposed test.

1. Production of alkali during incubation is shown by decreased acidity and is indicated by increase of depth of the grayish-blue color given with normal milk by the brom-cresol purple solution.

2. Digestion of casein is observable just below the cream layer and is indicated by the appearance of a layer of more or less clear solution.

3. Gas production is easily observed, indicating the presence of gas-producing organisms. This test is especially valuable in connection with milk to be used for cheese-making.

4. The contraction or shrinking of the curd or coagulated casein into a smaller mass is easily seen when it occurs. This is accompanied by the separation of more or less clear whey.

5. Any abnormal odor or taste is readily ascertained by any one having well developed senses of smell and taste. Such abnormal conditions have been noticed in our experience only in the case of milks which had shown marked change in reaction as indicated by the color given with brom-cresol purple solution.

RESULTS OF APPLICATION OF THE METHOD.

In applying the brom-cresol purple test to the measurement of acid production in relation to the keeping quality of milk, two separate series of experiments will be presented. In the first series, the samples used were taken from individual cans of milk as delivered by producers at the two collecting stations handling the supply of the city of Geneva. In the second series,

the samples were obtained from the regular milk supply of New York City.

1. The Geneva Samples.—In applying our method to 389 samples obtained in Geneva, we have had the cooperation of the city bacteriologist, Miss Mildred C. Davis, who classified the samples into groups by microscopical examination, using the direct-counting method.[2]

The results are summarized in Table I.

In interpreting the results of the microscopical examination with reference to the fitness or keeping quality of milk for domestic use, milks in Class I are regarded as excellent, in Class II as satisfactory, in Class III as unsatisfactory, and in Class IV as

TABLE I.

Comparison of Results of Brom-Cresol Purple Test with Classification by Microscopical Examination.

Class.	No. of individual bacteria per cc. of milk.	No. of samples examined.	No. of samples changing color.	No. of samples not changing color.	Milk showing good keeping quality.	Milk showing poor keeping quality.
					per cent	*per cent*
I	Below 350,000	283	41	242	85.5	14.5
II	Between 350,000 and 1,000,000	21	6	15	71.5	28.5
III	" 1,000,000 and 10,000,000	52	33	19	36.5	63.5
IV	Over 10,000,000	33	27	6	18.0	82.0

very unsatisfactory. While there is a general correspondence between the results obtained by the microscopical examination and by the brom-cresol purple test, the agreement is not complete. In Class I, representing milk of excellent quality by microscopical examination, 242 samples out of 283 show no change by the brom-cresol purple solution, thus confirming the results of the microscopical examination; but 41 samples out of the 283, or 14.5 per cent, show sufficient increase of acidity to be detected by the brom-cresol purple solution. In Class II, of the 21 samples graded as satisfactory by microscopic examination, 6 samples, or 28.5 per cent, show increase of acidity with brom-cresol purple solution. In Class III, 52 samples are graded as unsatisfactory

[2] Breed, R. S., *New York Agric. Exp. Station, Techn. Bull. 49*, 1916.

by the microscopic method, while 19 samples, or 36.5 per cent, fail to show increased acidity. In Class IV, 33 samples are graded as very unsatisfactory by the microscopical method, but of these there are 6, or 18.0 per cent, which show no increase of acidity. These observed differences of interpretation in the application of these two methods to the determination of keeping quality in milk are what might be expected under the conditions and are easily explained. The brom-cresol purple test is here applied to detect increase of acidity while the microscopical examination includes all kinds of bacteria and not merely those capable of producing acid. It is obvious that in the case of bacteria producing no acid or only very small amounts under the conditions of the test, the brom-cresol purple test would not be expected to apply as it does in the case of marked acid producers.

2. The New York City Samples.—These samples were obtained in the regular inspection work of the city milk supply, through the courtesy of Dr. Wm. H. Park, Director of the Laboratories of the Department of Health. We are indebted also to Dr. Hazel Hatfield for the work done in making the bacteriological examinations of the samples. There were examined 220 samples of unheated or raw milk and 186 of pasteurized milk. The bacteriological examination was made by the official plate method after incubation at 37°C. The tests with brom-cresol purple solution were made in all cases on samples incubated at 18°C. for 24 hours. This temperature is higher than that found in efficient household refrigerators, but it may be regarded as representing approximately the average temperature at which milk is kept after delivery to the consumer.

The division into classes on the basis of bacterial content has been carried farther than in case of the Geneva milks, providing fifteen different divisions as shown in Table II.

The results with the raw milks examined in New York City show, in general, that when the numbers of bacteria increase, there is an increase in the percentage of samples showing increase of acidity. Milks with high bacterial content usually show poor keeping quality by the brom-cresol purple test, while those containing small numbers of bacteria generally show good keeping quality by the color test. However, some milks with fairly high

TABLE II.

Results of Examination of New York City Milks.

Class.	R = raw P = pasteurized.	No. of colonies developed per cc. of milk.	No. of samples.	No. of samples showing change of reaction.	No. of samples not showing change of reaction.	Milk classed as poor by test.	Milk classed as good by test.
						per cent	*per cent*
I	R	Below 1,000	0	0	0	0	—
II	"	Between 1,000 and 5,000	4	0	4	0	100
III	"	" 5,000 " 10,000	6	0	6	0	100
IV	"	" 10,000 " 20,000	4	3	1	75	25
V	"	" 20,000 " 30,000	10	5	5	50	50
VI	"	" 30,000 " 60,000	17	14	3	82.4	17.6
VII	"	" 60,000 " 100,000	23	22	1	95.7	4.3
VIII	"	" 100,000 " 150,000	25	20	5	80	20
IX	"	" 150,000 " 250,000	57	54	3	94.7	5.3
X	"	" 250,000 " 400,000	16	14	2	87.5	12.5
XI	"	" 400,000 " 600,000	16	16	0	100	0
XII	"	" 600,000 " 1,000,000	20	20	0	100	0
XIII	"	" 1,000,000 " 1,500,000	10	9	1	90	10
XIV	"	" 1,500,000 " 3,000,000	7	7	0	100	0
XV	"	Over 3,000,000	5	5	0	100	0
I	P	Below 1,000	4	0	4	0	100
II	"	Between 1,000 and 5,000	16	1	15	6.2	93.8
III	"	" 5,000 " 10,000	9	1	8	11	89
IV	"	" 10,000 " 20,000	32	0	32	0	100
V	"	" 20,000 " 30,000	32	2	30	6.2	93.8
VI	"	" 30,000 " 60,000	28	3	25	10.7	89.3
VII	"	" 60,000 " 100,000	20	5	15	25	75
VIII	"	" 100,000 " 150,000	15	9	6	60	40
IX	"	" 150,000 " 250,000	13	12	1	92.3	7.7
X	"	" 250,000 " 400,000	4	2	2	50	50
XI	"	" 400,000 " 600,000	5	5	0	100	0
XII	"	" 600,000 " 1,000,000	3	3	0	100	0
XIII	"	" 1,000,000 " 1,500,000	5	5	0	100	0
XIV	"	" 1,500,000 " 3,000,000	0				
XV	"	Over 3,000,000	0				

bacterial content show good keeping quality, while some with a low content show poor keeping quality, by the brom-cresol purple test.

With pasteurized milks we obtain similar results, except that a large proportion of the milks with the higher bacterial content show good keeping quality by the brom-cresol purple test.

The results obtained with the New York City milks are not properly comparable with those obtained with the Geneva milks, especially for two reasons. In the first place, the two methods of obtaining the bacterial content, Breed's direct-counting method and the so-called official plate method, do not give results sufficiently comparable for our purpose. In the second place, the New York City milks average probably not less than 24 hours old when the samples are used for laboratory work, while the Geneva samples are not more than 6 to 16 hours old.

In the summary of the results here presented, we do not give the varying degrees of acidity developed on incubation but only the general fact of an increase. Data in greater detail are being collected.

Additional Work.

While some observations have been made on the relation of the other factors to keeping quality, much additional work remains to be done along the following lines: (1) Digestion of casein, (2) production of alkali, (3) production of gas, (4) taste and odor, (5) relation of age of milk to temperature and length of time of incubation.

SUMMARY.

1. Brom-cresol purple can be used to measure approximately and relatively the keeping quality of milk. The test is applied in the manner described in the article preceding (page 357), with the modification that the pipettes and test-tubes used are sterilized before sampling the milk, and, further, the samples of milk in the test-tubes must be incubated a given time at a given temperature (usually 18° to 20° C.). The milk is examined for changes of color at 24 hour intervals. The main factor shown by this test as related to keeping quality is production of acid, but

additional factors to be observed are coagulation of casein, digestion of casein, production of alkali, production of gas, development of abnormal odor and taste.

2. In showing the development of acidity, four stages of progress are distinguishable through change of color, varying from the grayish-blue of brom-cresol purple in normal milk to a final clear yellow, the intermediate stages showing mixtures of color. In comparing this test with the bacterial count, it is found that in general large numbers of bacteria and increase of acidity are in fair correlation.

3. The other factors related to keeping quality, such as digestion, gas, alkali production, and abnormal odor and taste, are readily observable, but frequently not until after 24 hours of incubation.

A METHOD FOR THE DETERMINATION OF THE KEEPING QUALITY OF MILK

John C. Baker and Lucius L. Van Slyke

J. Biol. Chem. 1919, 40:373-382.
