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## A COMPARISON OF THE ACID TEST AND THE RENNET TEST FOR DETERMINING THE CONDITION OF MILK FOR THE CHEDDAR TYPE OF CHEESE.

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In the making of the Cheddar type of cheese it is desirable that the condition of the milk be such that the whey can be drawn from the curd at the expiration of approximately the same time each day. To control this the cheese maker must be able to ripen the milk to approximately the same point each day. Two tests are employed to determine the ripeness of the milk, the rennet test in one or the other of its modifications, and the acid test or the titration of the milk. The work herein reported was done to determine the relative value of these two tests as means of determining the condition of the milk.

It is evident that the value of these tests lies in enabling the individual maker to follow approximately the same methods from day to day with the hope that this constancy in method will result in a cheese more uniform in quality than could be obtained without this control, rather than to enable one maker to compare his methods with those of another working under different conditions.

### THE ACID FERMENTATION.

In the making of Cheddar cheese it is essential that a large amount of acid be formed during the process of manufacture. The initial acidity of milk fitted for Cheddar cheese may range from 0.18 to 0.20 per cent. At the time of salting, the whey coming from the curd should have an acidity of 0.9 per cent or above. This rapid formation of acid in the curd is possible only when the milk contains large numbers of lactic-acid-forming bacteria. The development of acid within the curd by the bacteria that were present in the milk and were enmeshed in the curd is an essential condition in the manufacture

of this kind of cheese, since the increasing acid is a factor of much influence in the firming of the curd, or the expulsion of moisture therefrom, as has been shown by Sammis.[1] Cheddar cheese is often termed a sour or acid curd cheese in contradistinction to other hard cheeses, such as Swiss, in which little or no acid is formed during the making process proper. The expulsion of the whey is here obtained by high temperatures and stirring the curd until it is finely divided.

There seems to be no doubt that the relation between the amount of acid formed in the milk and in the curd before pressing is of much importance in determining the quality of Cheddar cheese with reference to both texture and flavor, and that the success of the Cheddar cheese maker is largely dependent on his ability to control the rate of acid formation in the milk and curd.

The initial phase of the acid fermentation has been called the "period of incubation." This comprises the time when the increase in bacteria is not accompanied by an apparent increase in acid. It has often been stated that during this period of rapid cell growth acid formation does not occur. This is certainly not correct. As shown by Rahn, the amount of acid is at first so small that it can not be detected by the ordinary methods. The acidity may remain apparently stationary and the acid-forming bacteria increase many hundredfold, as shown in the following table:

TABLE 1.—*Changes in milk during the initial period of the acid fermentation.*

| Hours. | Sample 1. | | Sample 2. | | Sample 3. | |
|---|---|---|---|---|---|---|
| | Acidity. | Bacteria. | Acidity. | Bacteria. | Acidity. | Bacteria. |
| | *Per cent.* | *Per cubic centimeter.* | *Per cent.* | *Per cubic centimeter.* | *Per cent.* | *Per cubic centimeter.* |
| 0 | 0.14 | 240,000 | 0.13 | 610,000 | 0.13 | 610,000 |
| 6 | .14 | 1,600,000 | .12 | 7,700,000 | .12 | 63,000,000 |
| 12 | .14 | 33,000,000 | .13 | 170,000,000 | ..... | ..... |

According to Rahn,[2] each cell will form 0.0 000 000 018 milligram of acid per hour. In the case of sample 1, the number of cells forming acid between the sixth and the twelfth hour would be the average of the numbers found at the end of these respective hours, or 17,600,000 per cubic centimeter. This number acting during 6 hours would form 9.5 milligrams of acid, or approximately 0.02 per cent of 50 cubic centimeters of milk, as large a quantity as is ever taken for an acid determination.

In order to determine the error in the titration method, known quantities of acid were added to milk. The acidities of these samples were determined by one who had no knowledge of the amount of

[1] Hart, E. B., Suzuki, S. K., and Sammis, J. L. Some improved methods of dairy chemistry analysis. Wisconsin Agricultural Experiment Station, Research Bulletin 10. Madison, May, 1910.

[2] Rahn, Otto. The fermenting capacity of the average single cell of bacterium lactis acidi. Michigan Agricultural Experiment Station, Technical Bulletin 10. East Lansing, June, 1911.

acid added. The average error in these determinations was approximately 0.01 per cent, even when 50 cubic centimeters of milk was titrated. It should be remembered that these determinations were made under constant conditions as to light, etc. With a less experienced operator working under less constant conditions, the error will be at least 0.02 per cent, as has been determined by actual trial. It is thus evident that the number of bacteria may vary most widely and the cheesemaker be unable to detect any change in the acidity.

The larger part of the milk used by Cheddar cheese makers is in the "period of incubation." The content in bacteria may be in the thousands per cubic centimeter or in the millions. When it is low a larger per cent of starter must be used if it is desired to draw the whey at the expiration of a given time, while if the content in acid-forming bacteria is high the per cent of starter must be decreased. In the making of cheese from a thousand pounds of milk a certain amount of work has to be done in the formation of lactic acid. The rate at which this acid will be formed is dependent on the number of acid-forming bacteria initially present, other conditions being constant. If the number is approximately the same from day to day, and other conditions, such as temperature, are kept constant, the results will be much the same from day to day so far as the rate of firming the curd is concerned.

## THE RELATION OF BACTERIAL CONTENT TO EXPULSION OF MOISTURE.

The following experiment shows that differences in bacterial content which can not be detected by the determination of the acidity do exert an influence with reference to the rate of firming of curd from milk having apparently the same initial acidity but a different bacterial content. A sample of milk having an acidity of 0.17 per cent was divided. To one portion rennet was at once added; the second was kept at 86° F. for one hour. At the end of this period the acidity was still 0.17 per cent, but the bacterial content must have been several times greater than in the first sample. The rate of expulsion of the whey from the two curds is illustrated in Table 2.

TABLE 2.—*The moisture content of curds from milk containing varying numbers of bacteria at time of adding rennet.*

| Time after cutting curd. | Moisture of curd. | | Time after cutting curd. | Moisture of curd. | |
|---|---|---|---|---|---|
| | Sample 1. | Sample 2. | | Sample 1. | Sample 2. |
| *H. m.* | *Per cent.* | *Per cent.* | *H. m.* | *Per cent.* | *Per cent.* |
| 0 00 | 87.0 | 87.0 | 3 00 | 49.4 | 45.8 |
| 0 30 | 82.0 | 81.2 | 4 00 | 43.5 | 42.9 |
| 1 30 | 67.5 | 67.5 | 6 00 | 40.3 | 40.0 |
| 2 30 | 63.0 | 60.8 | 6 20 | 38.0 | 36.7 |
| 2 45 | 54.0 | 50.5 | | | |

It will be seen from this experiment, typical of a series, that in the case of sample 2 made from milk kept 1 hour longer at a temperature favorable for the growth of lactic bacteria there is a constant tendency for the curd to lose moisture more rapidly than in the curd from the milk containing less bacteria. Differences in the rate of firming of curds from milk with the same acidity may often be much greater than are here shown.

That this thing is noted by the practical maker is shown in the following, quoted from the Cheese Makers' Discussion Club: [1]

> In handling milk on straight cheese-factory work we do not strive to get just the same acidity on each vat of milk, having learned by experience that hardly any two vats of milk will work up just the same, even when one starts with the same degree of acidity.

The explanation of this lies in the varying bacterial content with apparently the same acidity.

It is therefore evident that the maker should employ the most delicate means at his command for the determination of the condition of the milk, since the information thus obtained will indicate how the milk should be handled in the subsequent operations.

## COMPARISON OF THE RENNET AND ACID TESTS.

Each of the ways used for determining the bacteriological condition of milk has certain errors and disadvantages. For example, the alkaline solution used in the titration gradually decreases in strength, while the results obtained with the rennet test depend on the strength of the extract, which varies from sample to sample. These errors can be avoided to some extent by the careful maker. The titration of the milk with an alkaline solution is subject to error, because the appearance of the end point or the development of the pink color is not sharp, an error that is largely avoided in the rennet test.

With the idea of determining whether the rennet test was not capable of detecting smaller quantities of acid, and of giving more certain results than the titration method, a series of comparative tests were made. Fresh milk was maintained at 36.5° C. At intervals acid and rennet tests were made. The rennet was added at the rate of 1 part to 5,000 of milk, or approximately the proportion used in cheese making. The curdling of the milk was determined by sprinkling a little carbon on the surface of the milk and noting when these particles ceased to follow a stirring rod as it was passed slowly through the milk. The results of the two tests are given in Table 3.

[1] Jenks, W. W. New York Produce Review, vol. 33, No. 9, p. 404. New York, Dec. 20, 1911.

TABLE 3.—*The relative delicacy of the rennet and acid tests when rennet is added at the rate of 1 part to 5,000 of milk.*

| Sample 1. | | | Sample 2. | | |
|---|---|---|---|---|---|
| Time of incubation of milk. | Time required to curdle. | Per cent of acid. | Time of incubation of milk. | Time required to curdle. | Per cent of acid. |
| *H. m.* | *M. s.* | *Per cent.* | *H. m.* | *M. s.* | *Per cent.* |
| 0 00 | 14 30 | 0.180 | 0 00 | 15 00 | 0.184 |
| 1 00 | 13 45 | .180 | 1 30 | 14 30 | .180 |
| 1 30 | 13 30 | .180 | 2 00 | 13 45 | .181 |
| 2 00 | 12 45 | .189 | 3 00 | 13 00 | .192 |
| 2 30 | 12 00 | .180 | 3 30 | 12 00 | .191 |
| 3 00 | 10 00 | .189 | 4 00 | 10 00 | .198 |
| 3 30 | 8 30 | .189 | 4 30 | 8 30 | .196 |
| 4 00 | 7 30 | .207 | 5 00 | 7 00 | .202 |
| 4 30 | 6 00 | .220 | 5 30 | 5 30 | .229 |
| 5 00 | 5 00 | .234 | ..... | ..... | ..... |

A second trial was made with such quantities of milk and rennet as are used in the Monrad rennet test, since it was desirable to ascertain whether the same results would be obtained when the proportion of rennet was much greater and the curdling period shortened. The results are given in Table 4.

TABLE 4.—*The relative delicacy of the rennet and acid tests when rennet is added at the rate of 1 part to 320 of milk (Monrad test).*

| Sample 1. | | | Sample 2. | | |
|---|---|---|---|---|---|
| Time of incubation of milk. | Time required to curdle. | Per cent of acid. | Time of incubation of milk. | Time required to curdle. | Per cent of acid. |
| *H. m.* | *M. s.* | *Per cent.* | *H. m.* | *M. s.* | *Per cent.* |
| 0 00 | 3 37 | 0.166 | 0 00 | 2 17 | 0.183 |
| 0 45 | 3 30 | .171 | 0 30 | 2 14 | .183 |
| 1 30 | 3 15 | .169 | 1 00 | 2 6 | .186 |
| 2 15 | 3 15 | .175 | 1 30 | 1 59 | .189 |
| 2 45 | 3 5 | .173 | 2 00 | 1 43 | .195 |
| 3 15 | 2 50 | .177 | 2 30 | 1 20 | .199 |
| 3 45 | 2 28 | .180 | 3 00 | 1 7 | .202 |
| 4 15 | 1 55 | .177 | 3 30 | 0 47 | .211 |
| 4 45 | 1 30 | .184 | 4 00 | 0 35 | .216 |
| 5 15 | 1 8 | .200 | 4 30 | 0 28 | .234 |
| 5 45 | 0 47 | .216 | ..... | ..... | ..... |
| 6 15 | 0 37 | .229 | ..... | ..... | ..... |

It will be noted that in all the trials the curdling time has decreased continuously from the beginning, while the acidity has remained more or less stationary for some time. In a general way it may be stated that the decrease in curdling time has been about 50 per cent before such an increase in acid has taken place as could be detected by the ordinary titration. It has been assumed that the errors of titration under cheese-factory conditions are at least 0.02 per cent. This assumption is well based, as shown by the data obtained when a sample of milk was divided into a number of portions which were then titrated by different persons accustomed to making acid determinations.

It is clear that the rennet test is a much more delicate index of the bacteriological condition of the milk during the initial phase of the acid fermentation than is the titration method. In other words, the curdling time of milk by rennet under otherwise constant conditions is profoundly influenced by the most minute quantities of acid. It is not conceivable that anyone would be unable to differentiate between a milk curdling in 3 minutes and 37 seconds and one curdling in 1 minute and 30 seconds, as shown in Table 4, sample 1. On the other hand, the difference in the acidity of these milks was approximately only 0.02 per cent. This difference is equal to that previously mentioned as within the limits of error of titration. It should be remembered also that the determinations given in the tables for each sample were made at the same time and under such constant conditions that the error would be much less than under practical conditions in the cheese factory.

In the cases of sample 2 in Tables 3 and 4 determinations were made of the number of bacteria present at the varying times. The data thus obtained, together with the results of the rennet tests and the acidity, are given in Table 5. It is to be noted that the rennet test parallels very closely the change in bacterial content.

TABLE 5.—*The relative delicacy of the rennet and acid tests according to number of bacteria in the milk.*

| Sample 2 from Table 3. | | | Sample 2 from Table 4. | | |
|---|---|---|---|---|---|
| Time required to curdle. | Per cent of acid. | Bacteria per c. c. | Time required to curdle. | Per cent of acid. | Bacteria per c. c. |
| *M.* *S.* | *Per cent.* | *Number.* | *M.* *S.* | *Per cent.* | *Number.* |
| 15 00 | 0.184 | 270,000 | 2 17 | 0.183 | 1,600,000 |
| 14 30 | .180 | 1,425,000 | 2 14 | .183 | 3,800,000 |
| 13 45 | .181 | 2,250,000 | 2 6 | .186 | 6,700,000 |
| 13 00 | .192 | 5,520,000 | 1 59 | .189 | 11,200,000 |
| 12 00 | .191 | 27,200,000 | 1 43 | .195 | 51,000,000 |
| 10 00 | .198 | 57,400,000 | 1 20 | .199 | 128,000,000 |
| 8 30 | .196 | 94,000,000 | 1 7 | .202 | 195,000,000 |
| 7 00 | .202 | 143,000,000 | 47 | .211 | 275,000,000 |
| 5 30 | .229 | 125,000,000 | 35 | .216 | 395,000,000 |

It is evident from the data presented that for the purpose of determining the ripeness of milk for cheesemaking the rennet test is superior to the acid test. The latter test can be used to advantage in following the rate of acid formation in the curd by the titration of the whey, but for the milk the rennet test is preferable.

Approved:

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*Secretary of Agriculture.*

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