OCCURRENCE AND CONTROL
OF THE BRITTLE PRECIPITATE DEFECT
IN MAKING COTTAGE CHEESE

by

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OCCURRENCE AND CONTROL
OF THE BROWN PRECIPITATE DEFECT
IN MAKING COTTAGE CHEESE

INTRODUCTION

Cottage cheese consumption has shown a great increase in recent years. In 1954, 535 million pounds of creamed cottage cheese were manufactured in the United States (18 p. 10). This is a per capita consumption of 3.1 pounds. In 1957 the annual production was 694.0 million with a per capita consumption of over 4.0 pounds. (19 p. 19). The three western states, Washington, Oregon and California account for over 20% of the national production of cottage cheese.

Improved quality resulting from better technical control during manufacture has been responsible for much of the increased consumption. The body, texture and flavor of cottage cheese have been improved and standardized in recent years so that the consumer can depend on a uniform product. In striving for better processes for handling the raw and finished products much of the "art" of making cottage cheese has been replaced with scientific methods.

New problems have been brought about by modern methods of handling milk. Milk can be stored for longer periods of time by using refrigerated tanks. The problem involved here was first noticed locally when farm tanks were installed. The farm tank is an insulated refrigerated tank large enough to hold from four to six complete milkings. The tanks are installed on the farm where the milk can be stored until the procurement tank truck picks up for delivery to the processor. The milk received at the plants
was of excellent quality according to conventional analysis. The bacteria count was low, the titratable acidity normal and the flavor apparently was unaffected.

Simultaneously with the use of the farm tank and longer storage periods, a brown precipitate began to appear in the bottom of the cottage cheese vats during the coagulation period. The precipitate appears either as a slight thickening of the milk on the bottom of the vat in the mild cases or as a brown viscous layer in the severe cases. In the latter the milk must be discarded. In borderline cases the offending milk often has not been discarded and the resulting cottage cheese curd does not have the desired body and texture.

Cottage cheese curd is produced by acid coagulation of skim milk usually with the aid of a coagulator or rennet extract. The flavor of cottage cheese is due largely to the milk constituents and products of bacteria during coagulation. The organisms used in cottage cheese develop acid necessary for coagulation and a certain amount of diacetyl for flavor. Many of the commercial cultures contain organisms that give off CO₂ and other gases that are associated with high flavor. Cottage cheese may also be made without an added enzyme. From a chemical point of view the acid curd will show several points of difference from rennet curd, the chief difference being that the acid curd retains a smaller amount of mineral matter and undergoes less digestion than rennet curd (10 p. 101).
In this study several possible causes of the precipitate have been investigated. Some of the factors investigated were: aging of milk, effect of certain coagulating agents, state in which milk was stored (whether as skim or whole milk) and the effect of certain starter cultures.
Consultants in the cottage cheese industry have reported prevalence of the brown precipitate defect in making cottage cheese. Daines (4) said: "The defect has appeared occasionally in various parts of the country and for no readily apparent reason disappears after a few days." Lawrence (9) said: "Regarding the precipitate in making cottage cheese, we have encountered this quite often in consultation work with dairymen throughout the United States. We have found this to be true - that the precipitate can come about in most any type of milk. We have encountered it in fresh milk, milk that was stored overnight and in reconstituted milk."

To understand the chemical and physical changes involved in acid coagulation of milk, a knowledge of the physical state in which the main constituents exist must be understood. The greater part (85 - 88%) of milk is water in which the milk solids are either suspended or in solution. The following outline classifies the more important constituents according to their state of dispersion: (14, p. 661)

A. In true solution

1. Lactose

2. Ions as follows:

   a. Cations

      H   (hydrogen ion)

      Na  (sodium ion)
K (potassium ion)
Ca (calcium ion)
Mg (magnesium ion)

b. anions

OH⁻ (hydroxyl ion)
Cl⁻ (chlorine ion)
$H_2PO_4^-$ (primary phosphate ion)
$HPO_4^{--}$ (secondary phosphate ion)
$PO_4^{---}$ (tertiary phosphate ion)
Citrate⁻ (primary citrate ion)
Citrate⁻⁻⁻ (secondary citrate ion)
Citrate⁻⁻⁻⁻ (tertiary citrate ion)
$HCO_3^-$ (bicarbonate ion)

3. Undissociated salts representing all possible combinations of the above ions.

B. In colloidal suspension

1. casein (in combination with bases, mostly calcium)
2. albumin (in combination with bases)
3. globulin (in combination with bases)

C. In emulsion

1. milk fat

Sommer (14, p. 662) described the particles in true solution as very small, appearing as ions or molecules. The difference between true solutions and colloidal suspensions is largely one of
degree of dispersion. There are borderline cases such as lactoglobulin and lactalbumin. He further added that protein molecules may reach colloidal size, making a fine distinction difficult. Colloids are large enough to reflect light and be removed by ultracentrifugation.

Casein, the main protein in milk, is a key constituent in cottage cheese manufacturing. Cottage cheese curd is largely casein. The quality of the finished product depends upon the condition in which the curd is recovered.

Casein is identified by Van Slyke and Price as a (21, p. 6) phosphoprotein because it contains phosphorus. It is present in milk in association with calcium and calcium phosphate as a calcium caseinate - calcium phosphate complex.

Calcium caseinate exists in milk as minute particles suspended in the liquid. It can be seen with the ultramicroscope and can be removed by ultracentrifugation or by a special porcelain filter. Other methods of isolating casein usually involve one of several methods of coagulation.

Casein can be removed by acid coagulation. In making cottage cheese, lactic acid is formed from lactose by action of "starter" bacteria. As the hydrogen ion concentration increases more and more calcium is removed from the caseinate resulting in calcium-free protein. At about pH 4.7, near the isoelectric point of casein, the calcium-free protein forms a coagulum and the characteristic cottage cheese curd appears.
The most important method of coagulating casein for cheese making is through the combined use of acid and rennet extract. Rennet extract contains the enzyme rennin, which has the ability to coagulate milk. Coagulation by rennin is of particular interest in the dairy field since the process is so widely used in cheese making. Hammersten proposed the theory that rennin coagulation occurs in two stages and since 1897 this theory has been accepted. (8 p. 310). The first stage is the enzymatic stage and has specific action on the caseinate. The second stage is the coagulation stage when the curd is formed.

Very small quantities of rennet extract are needed in the manufacture of cottage cheese. The advantages of both acid and rennin coagulation are used in coagulating the milk. Price (11, p. 38) illustrated the sensitivity of milk when small differences of rennet were used.

**FIGURE 1**

Relation of acid and amount of rennet at coagulation

<table>
<thead>
<tr>
<th>Amount of Rennet in 1000# milk</th>
<th>% Titrable Acidity</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.0 cc</td>
<td>0</td>
</tr>
<tr>
<td>4.0 cc</td>
<td>0.2</td>
</tr>
<tr>
<td>3.0 cc</td>
<td>0.3</td>
</tr>
<tr>
<td>2.0 cc</td>
<td>0.4</td>
</tr>
<tr>
<td>1.0 cc</td>
<td>0.5</td>
</tr>
<tr>
<td>0.0 cc</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>0.7</td>
</tr>
</tbody>
</table>
It will be noted that a very small amount of rennet is capable of coagulating the milk regardless of the acidity. The rennet-acidity relationship permits the calculation of the amount of rennet that may be added without causing coagulation much before the isoelectric point is reached.

Tuckey (16, p. 52) states: "The isoelectric point of casein is pH 4.6. On either side of this pH value the casein tends to go into solution. So far as cottage cheese is concerned, casein exhibits most desirable physical characteristics between pH 4.6 and 4.5 when rennet is used. At pH values greater than pH 4.6, casein exhibits marked cohesive properties. At pH values less than 4.6 the curd tends to go into solution. The cheese becomes soft and mushy; it loses its cohesive properties and shatters."

Davis (3, p. 15) referred to the proper cutting acidity of cottage cheese as falling between pH 4.5 to pH 5.5.

Baker (2, p. 74) found the pH range of pH 4.75 to pH 4.65 was most desirable.

Fricke (5, p. 84) investigated the slow coagulation of milk in relation to the nutrition and heredity of the cow, particularly under conditions of intense feeding and high yield. The addition of a calcium phosphate - vitamin D3 preparation to the daily feed of milking cows improved the renneting ability of the milk. Slow coagulation seemed to be more influenced by nutritional factors rather than by heredity.
Henson and Miller (6, p. 211) found when comparing Friesian and Jersey milk that Friesian milk was unsatisfactory for making cottage cheese. Blending the two milks and the addition of calcium chloride did not improve the condition. Walker test readings were sufficiently high, but apparently the nitrogen distribution was not normal. The formol titrations were not consistent with the total protein as determined by the Kjeldahl.

Seekles and Smeets (12, p. 7) reported flocculation in apparently normal milk. Stability was improved with an increase in pH, but impaired by the addition of calcium chloride. Oral or subcutaneous administration of sodium citrate to the cows tended to increase stability, as did addition of sodium citrate to the milk. Observations on starvation vs. ample rations indicated that both showed the presence of excessive calcium salts in relation to the other stabilizing properties, contributed to the defect. Addition of citrates, phosphates and oxalates decreased calcium ion activity and increased stability of the milk.

Walker, (22, p. 37) reported the occurrence of abnormal curd formation in making cheese in the southern counties of England. He found that with modern breeding programs the yield per cow was increased, but solids-not-fat content of the milk was decreased. During the season that the abnormal coagulation occurred the animals were being fed poor quality hay and pastured on short pastures. Both the pasture and hay were low in protein, which resulted in the
production of milk of low casein content. He concluded that the producers should have their hay and silage analyzed so that any deficiency can be noted and corrected by the addition of other foods to the rations.

Hostettler and Ruegger (7, p. 711) investigated the influence of calcium chloride on rennet coagulation. Increased calcium chloride resulted in acceleration of the coagulation. They found that increased concentration of H-ions brought about by addition of calcium chloride, was more responsible for accelerating coagulation than the coagulating effects of the calcium ions. They also reported that addition of calcium chloride in increasing amounts caused accelerated coagulation to a point beyond which further additions would cause slight inhibition.
MATERIALS AND METHODS

Starter Cultures

Starter cultures were obtained from two sources: commercial manufacturers and the Department of Bacteriology at Oregon State College. The cultures were identified as M, K, and SCl. M was selected because of its known ability to produce gas and K and SCl because they were known to be low gas producers.

All cultures were Streptococcus lactis and associative organisms typical of those commonly found in any cottage cheese plant. Cultures were carried in sterile reconstituted milk. Non-fat dry milk solids were reconstituted in water at the rate of 10% dry milk solids and autoclaved in 500 ml. flasks for 15 minutes under 15 pounds of pressure. The sterile blanks were cooled and stored at 40°F. until used. The cultures were transferred daily, using a 1% inoculum, and incubated for a period of 15 hours at 70°F. Upon completion of the incubation period the cultures were removed from the incubator, inspected and stored at 40°F. When the cultures were to be used immediately, they were not cooled. Inspection of the cultures involved titrating to determine if the desired acidity of at least .80% acid had been reached, checking firmness of coagulum and, in the case of the gas forming culture, checking for formation of gas. The test for gas will be described later.
A Hafis titrometer, calibrated to read in per cent acid, was used for titratable acidity. A solution of N/10 sodium hydroxide was used with a 1% solution of phenolphthalein as indicator. Nine grams of milk, or filtered cottage cheese whey, is pipetted into a beaker and titrated to a faint pink.

Coagulating Agents

Commercial coagulators were in the liquid form with the exception of one which was in the tablet form. Rennet was obtained from one source and will be referred to as rennet B, while the coagulators were from several sources and will be referred to as "A", "B", "C" and "D". The exact composition of the coagulators is not known, but according to the labels and the correspondence with the manufacturers, they contain calcium salts, such as calcium chloride and calcium lactate. They also contain enzymes such as rennin and pepsin. (1, p. 9)

Milk

Pooled milk from the Oregon State College dairy herd of Jersey and Holstein cows was used, except in specific cases when it was obtained from individual cows. Milk for part of the trials was stored in two refrigerated farm tanks in the laboratory and milk for the remainder of the trials was stored in cans in a 40°F. walk-in cooler, since the quantity was not large enough to be agitated and held in the farm tanks.
The whole milk was heated to 105°F., agitated, and separated with a laboratory-sized DeLaval separator. The skim milk was pasteurized or stored at 40°F., depending on whether the trial was to follow immediately after separation or after aging of the skim milk.

The skim milk was pasteurized by heating to 143°F. in ten-gallon cans and holding for 30 minutes at that temperature. At the end of the holding period the milk was cooled to 90°F. and the culture was added.

Cheese Making Equipment

Part of the trials were made using two laboratory-sized stainless steel cheese vats of 200 pound capacity. The vats were jacketed with steam and water so that any temperature can be maintained. Long-stem thermometers were used to determine the temperature of the water in the steam jacket and floating thermometers were used to determine the temperature of the cheese milk in the vats. Agitation was manual, using stainless steel paddles. All cheese-making equipment was sterilized using boiling water and all glassware was sterilized with a 200 ppm sodium hypochlorite solution.

Later in the trials, large test tubes were substituted for the cheese vats since the important part in obtaining the desired results was to have at least an 18 inch depth of milk. This could be accomplished as easily by using a column of milk in a 20 inch test tube as with a cheese vat of equal depth. Pyrex test tubes, 2\(\frac{1}{2}\) inches in
diameter and 20 inches high with a volume of 1500 ml., were used. Racks that could accommodate six large test tubes were used in order to observe the changes taking place in the milk. The racks could be placed in a controlled temperature room and checked periodically for precipitate without disturbing the coagulum.

Cheese Making Procedure

Two methods of making cottage cheese were used in the following trials: In one known as the short set, the milk is pasteurized and cooled to 90°F.; starter is added at the rate of 5% of the weight of the milk. The milk and starter are allowed to incubate for one hour, at which time the coagulator or rennet is added at the rate recommended by the manufacturer. The coagulating agent is diluted with about 5 times its volume of cold sterile water and added slowly while the milk is being agitated. Stirring is discontinued after five minutes and the mixture is allowed to coagulate. A period of about five hours from the time of adding the culture is allowed for the desired firmness of coagulum to be attained and the proper cutting acidity to be reached. The coagulum is then cut into 1/4 inch cubes by the use of curd knives which consist of wires 1/4 inch apart. These are drawn through the coagulum horizontally and vertically. After a period of twenty minutes the cooking process begins. This process expels moisture from the curd particles and causes them to shrink. The process requires about 1 1/2 hours at the end of which time the temperature of 120°F. is reached. The whey is drained until the curd
barely shows above the whey. Tap water is added to increase the contents to the original volume. This should cool the curd to about 85°F. Two more washings are given the curd using chilled water so that it is cooled to about 40°F. or lower. After the curd has been allowed to drain one hour the dressing is added.

The second method of making cottage cheese is called the long-set method. The main differences between the long-set and short-set are the per cent of starter culture and the time and temperature of incubation. In the long set, 1% starter culture is used and a temperature of 70°F. is maintained for a period of about 13 hours. When the desired acidity and firmness of curd is reached, the coagulum is cut and the process is identical to that described in the short-set method. These two methods are similar to those used in commercial cottage cheese making.
RESULTS

Effects of Aging

Since the brown precipitate was first associated with milk held for extended periods of time the first trial involved holding the milk for varying periods before use. Cottage cheese was made using the two methods described earlier as the long-set and short-set.

Fresh milk was received from the college herd. Sufficient milk was obtained to provide for one trial per day for seven consecutive days. It was separated at 105°F. and an aliquot for the first day's trial was pasteurized at 143°F. and held for 30 minutes. The skim milk was then cooled to 90°F. and inoculated with 5% starter. The remainder was cooled to 40°F. and stored at that temperature for the succeeding trials. Cottage cheese was made by removing daily aliquots from the cold milk. Starters K and L were used at the rate of 2.5% each and coagulator A was used at the rate of 1 oz. per 1,000 pounds of skim milk. This was repeated for seven consecutive days. At the end of seven days fresh milk was obtained and the process repeated. The samples were examined periodically for formation of the precipitate and results were recorded. Results of this trial appear in Table I.
TABLE I

The effects of age of the milk on occurrence of the precipitate.

<table>
<thead>
<tr>
<th>Age of milk in Days</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Samples</td>
<td>18</td>
<td>16</td>
<td>18</td>
<td>16</td>
<td>18</td>
<td>16</td>
<td>18</td>
</tr>
<tr>
<td>Occurrence of Defect</td>
<td>2</td>
<td>0</td>
<td>10</td>
<td>11</td>
<td>9</td>
<td>8</td>
<td>2</td>
</tr>
</tbody>
</table>

The defect occurred in various degrees of intensity throughout the seven day period with the exception of the second day. The appearance of the defect was most regular on the third through the sixth day of storage. These data are similar to those reported by commercial cheese makers. (17, p. 3).

The Effect of Coagulators

In cottage cheese making a firm curd is desired and in efforts to attain this, coagulators have been used. These trials were conducted to compare the performance of four commercial coagulators. The trials involved long-set and short-set methods and the use of large test tubes instead of cheese vats. Pasteurization was at 143°F for 30 minutes. Starters K and L were used at the rate of 2.5% each in the
short-sets and .5% each in the long-sets. Coagulants A, B, C and D were used at the rates recommended by the manufacturer and were as follows:

- **A**: 0.5 oz. per 4,000 pounds of skim milk
- **B**: 1.0 oz. per 1,000 pounds of skim milk
- **C**: 1.0 oz. per 100 gallons of skim milk
- **D**: 1 tablet per 1,000 pounds of skim milk

Fresh milk received from the college dairy herd was separated and the skim milk was pasteurized and cooled to 90°F. Trials were run on fresh skim milk and three-day-old milk. On the day of each trial the milk was pasteurized and divided into two equal parts; one part was used for a long-set and the other half for a short-set. Results of this trial appear in Table II.

**TABLE II**

The effects of coagulants on occurrence of the brown precipitate

<table>
<thead>
<tr>
<th>Number of Samples</th>
<th>Control</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Occurrence of Precipitate</td>
<td>18</td>
<td>19</td>
<td>18</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>0</td>
<td>2</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>
Milk, to which two of the coagulators (A and B) had been added, showed the defect, while the control milk and milk with coagulators C and D did not show the defect. There appeared to be little difference whether the long-set or short-set was used. These trials indicated that the occurrence of the defect is related to the type of coagulator.

Effects of the Concentration of the Coagulator and the Age of the Milk Upon the Intensity of the Precipitate

An experiment was designed to determine what effect the concentration of the coagulator and the age of the milk had on the intensity of the precipitate. The intensity of the defect was judged by the darkness of color and viscosity. The brown color deepened as the viscosity of the precipitate increased.

Fresh milk was used on the first day and an aliquot of the same milk was used each succeeding day. The whole milk was stored at 40°F and a portion was separated and the skim pasteurized just prior to use. The short-set method was used. Starters K and L were used at the rate of 2.5% each. Since previous trials indicated coagulator B was more apt to show the defect, it was used in this experiment. In recording the results it was necessary to indicate how intense the defect was. If the precipitate was just heavy enough that a difference could be seen between the precipitate and the balance of the milk, it was called "low". If the precipitate was apparent in both color and consistency, it was called "medium", and if there was
separation with marked discoloration, the defect was called "high". Two concentrations of coagulator were used: the concentration recommended by the manufacturer and four times the recommended amount. The control consisted of starter and milk only. Test tubes of 1,500 ml. capacity were used. The skim milk was pasteurized, inoculated with 5% starter and incubated at 90°F. for 5 hours. Results of this experiment are shown in Table III.

Table III shows that little sediment was noticed until after two days of aging. These results were similar to those of earlier trials.

The Effects of pH on the Occurrence of the Brown Precipitate

The acidity of freshly drawn milk is about pH 6.4 to pH 6.6. After the starter cultures have been added and during the incubation period the pH will gradually drop. As the acidity approaches pH 4.6, the isoelectric point of casein, the skim milk will gradually thicken until a firm coagulum is formed. It is at this point in making cottage cheese that the coagulum is cut and the cooking process begins.

This experiment was designed to determine at what pH value the brown precipitate appeared. The short set method was used. Starter cultures were added at the rate of 5% of the weight of the skim milk and incubated for five hours at 90°F. The pH values were determined before the starter cultures were added and every half hour after
TABLE III

Effects of concentration of coagulator and age of milk upon the intensity of the defect.

<table>
<thead>
<tr>
<th>Age of Milk In Days</th>
<th>Number of Samples</th>
<th>Control</th>
<th>1 x Normal Coagulation Number of times appeared</th>
<th>4 x Normal Coagulation Number of times appeared</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Low</td>
<td>Med</td>
</tr>
<tr>
<td>1</td>
<td>6</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>-</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>9</td>
<td>-</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>-</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>-</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>6</td>
<td>-</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td><strong>Total . . . .</strong></td>
<td><strong>48</strong></td>
<td>-</td>
<td><strong>13</strong></td>
<td><strong>3</strong></td>
</tr>
</tbody>
</table>
inoculation. Every half hour after inoculation the vat of skim milk was examined for formation of the precipitate. The method employed for detecting formation of the precipitate was by sliding the hand down the wall of the vat and feeling for the viscous sediment in the bottom of the vat. Results of this experiment appear in Figure II.

FIGURE II

Time from inoculation to formation of the precipitate and pH at which the precipitate was first detectable

Time in hours

<table>
<thead>
<tr>
<th>pH</th>
<th>Time (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.2</td>
<td>0</td>
</tr>
<tr>
<td>6.1</td>
<td>0</td>
</tr>
<tr>
<td>6.0</td>
<td>0</td>
</tr>
<tr>
<td>5.9</td>
<td>0</td>
</tr>
<tr>
<td>5.8</td>
<td>0</td>
</tr>
<tr>
<td>5.7</td>
<td>0</td>
</tr>
<tr>
<td>5.6</td>
<td>0</td>
</tr>
<tr>
<td>5.5</td>
<td>0</td>
</tr>
<tr>
<td>5.4</td>
<td>0</td>
</tr>
<tr>
<td>5.3</td>
<td>0</td>
</tr>
<tr>
<td>5.2</td>
<td>0</td>
</tr>
<tr>
<td>5.1</td>
<td>0</td>
</tr>
<tr>
<td>5.0</td>
<td>0</td>
</tr>
<tr>
<td>4.9</td>
<td>0</td>
</tr>
<tr>
<td>4.8</td>
<td>0</td>
</tr>
</tbody>
</table>
The Relative Effects of Coagulators and Rennet

Some coagulators contain a certain amount of rennet and some processors have reported the difficulty when rennet is used. The following experiment was designed to compare results of trials in which rennet and a coagulator at two different concentrations were used. The procedure was similar to that of previous trials. The short-set method was used with cultures K and L and rennet B and coagulator B. The recommended amounts and four times the recommended concentration was used for the rennet and coagulator. Skim milk was held three days and used on the third, fourth and fifth days. A control was used that contained starter culture and skim milk only. Results of this experiment appear in Table IV.

TABLE IV

The relative effect of coagulator B and rennet B on the defective coagulation

<table>
<thead>
<tr>
<th>Control</th>
<th>Rennet</th>
<th>Coagulator</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 cc.</td>
<td>4 cc.</td>
</tr>
<tr>
<td></td>
<td>1000#</td>
<td>1000#</td>
</tr>
<tr>
<td>Number of Samples</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Occurrence of Defect</td>
<td>2</td>
<td>4</td>
</tr>
</tbody>
</table>
There was an increase in the occurrence of the defect as the concentration of either the rennet or the coagulator was increased.

**Effects of the State in Which Milk is Stored**

The effect of aging as whole milk or as its skim milk was studied. The procedure was similar to that of the previous trials. The short-set method was used. Coagulator B, at the rate of one ounce per 1,000 pounds of milk and starters K and L, at the rate of 2.5%, each were used. A quantity of fresh milk was stored for three days at 40°F. At the end of three days one-half of the whole milk was separated and the skim and the other half were stored at 40°F. Each day a portion of the whole milk was separated, pasteurized and a trial begun. A portion of the skim milk was similarly treated. This was continued through the sixth day. Results of this trial appear in Table V.

**TABLE V**

Effects of storing milk as whole milk and as skim on occurrence of the precipitate

<table>
<thead>
<tr>
<th>Stored as</th>
<th>Stored as</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Skim</strong></td>
<td><strong>Whole</strong></td>
</tr>
<tr>
<td>Number of Samples</td>
<td>29</td>
</tr>
<tr>
<td>Occurrence of Defect</td>
<td>22</td>
</tr>
</tbody>
</table>
There was little difference in method of storage and occurrence of the sediment. Of the 29 samples, 22 showed the defect in the milk stored as skim milk and 20 of the 29 samples showed the defect in the milk stored as whole milk.

**Gassy and Non-gassy Cultures**

Small bubbles of gas evolved from the sediment as it formed in the bottom of the test tubes. The next trial entailed the use of gas-forming organisms. Two cultures were tested for gas production. The test used to detect gas formation is simple and easy to perform under commercial conditions. A quantity of starter in question is carefully pipetted into a small test tube. About 25 ml. of starter in a 50 ml. test tube is sufficient. The test tube is immersed in a beaker of water at 160°F. for five minutes. If the starter is gaseous, bubbles will form and the starter will expand in the tube. A high-gas producing culture will show many bubbles and expand quite extensively. In this test the quantity of gas evolved was not measured. Gas production was recorded as positive or negative.

Culture M9 was found to produce large quantities of gas and culture K produced negligible quantities. The procedure for this trial was similar to that used in trial V. The short-set was used and the milk was aged three days before use. The trials were run on the third, fourth and fifth days. Coagulator B was used at the rate of one ounce per 1,000 pounds skim milk and starters K and M9 were
used at the rate of 2.5% each. The trials were repeated three times.

Results of this experiment appear in Table VI.

**TABLE VI**

Comparison of high-gas and low-gas producing cultures.

<table>
<thead>
<tr>
<th></th>
<th>M9 Culture</th>
<th>K Culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Samples</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Occurrence of Defect</td>
<td>6</td>
<td>0</td>
</tr>
</tbody>
</table>

No precipitate was found when the low-gas producing culture was used. Of the nine samples, six showed the defect when the high-gas producing culture was used.
DISCUSSION

The abnormal coagulation of milk is not new in the cheese industry. Reports have referred to abnormalities in milk coagulation that range from the spontaneous curdling of fresh milk to the complete failure of milk to curdle even though the acidity is near the isoelectric point. The brown precipitate in cottage cheese is new. It was first noticed when milk for cottage cheese was stored for a period of time before use. Milk stored for relatively long periods of time under ideal conditions is being used for many dairy processes with, apparently, little loss in quality. However, when this milk is used for cottage cheese an abnormal curd would sometimes develop.

Abnormal coagulation of milk has been reported in England (22), New Zealand (6), The Netherlands (20), and Switzerland (7). One factor remains common to all reports; there is not a simple solution to the problem.

Comparison of Age of Milk and Occurrence of the Defect

Findings of this report show that aging of milk has a marked effect on the tendency of the brown precipitate to appear. One possible reason for this might be that a slight change in pH will cause a change in ionic calcium. The calcium of milk appears in association with the proteins, phosphates and citrates and also in the ionic form. Ionic calcium is probably the most responsible
factor in determining the rate of coagulation in milk (20 p. 1) If the lowering of the pH were to occur during storage the ionic calcium would increase. Tessier and Rose (15, p. 355) reported that normal skin milk will show between 2.5 and 3.4 millimoles calcium ions per liter and Smeets (13, p. 257) found that with calcium added to the level of four millimoles per liter, milk would become unstable. Any factor that would change the ionic calcium could bring about instability and abnormal coagulation.

Comparison of Coagulators

Samples in which coagulators A and B were used were more prone to show the precipitate. The exact composition of the coagulators is not known but these two were known to contain added calcium. Also, these two coagulators contained added proteolytic enzymes, but it is not known if the added enzymes are other than rennin.

Of the two trials showing the defect with coagulator A, one was in the short-set and the other was with the accompanying long-set. Of the seven samples showing the defect with coagulator B, four were by the short-set and three were by the accompanying long-set. There was little difference whether the long or short-set was used.
Effects of Concentration of the Coagulator and Age of Milk Upon the Intensity of the Defect

The results showed that in only one case did the defect occur on the day-old milk and then only slightly. With two-day old, two samples showed the precipitate in an intense form. By the third day samples with the normal amount of coagulator and samples with four times the normal amount of coagulator showed the defect. It appears, as shown in Table III, that the occurrence of the defect is about the same whether the normal amount of coagulator or four times the normal amount of coagulator is used. The intensity, however, is greater with the increased concentration of coagulator.

As in previous trials the defect appeared starting with the third day. From the third day through the seventh the incidence and intensity of the precipitate was quite constant.

Small bubbles of gas appeared in the sediment. The size and number of the bubbles seem to increase as the viscosity of the precipitate increased.

Comparison of a Coagulator and Rennet

There was an increase in the occurrence of the defect as the concentration of either the rennet or coagulator was increased. The coagulator appeared to cause the defect more often than rennet and milks with either coagulator or rennet showed the defect more often than the control. It is possible that the non-enzyme portion of the
coagulator may contain a substance that contributes to the formation of the precipitate. Since it is known that the coagulator contains added calcium, and since an increase in the calcium ion concentration is conducive to instability, it is suggested that the added calcium may be the culprit.

**State in Which the Milk is Stored**

There appeared to be little difference whether the milk is stored as whole or as skim since 22 of the 29 samples were positive in the case of milk stored as skim, while 20 of the 29 were positive in milk stored as whole milk.

**Comparison of High-gas and Low-gas Producing Cultures**

No precipitate was found when the non-gassy culture was used. Of the nine samples, six showed the defect when the gassy cultures were used. However, this may not be true of all gas formers. The change in citrate content of the milk was measured during incubation.* The high-gas formers eliminated the citrate while the low-gas formers did not. Utilization of the citrate would cause an increase in ionic calcium. This might have been responsible for the precipitate formation found in trial VI as shown in Table VI.

* Citrate analysis done by Seshadri Rajan.
The Effects of pH on the Occurrence of the Brown Precipitate

Results from this trial show that the precipitate formed between pH 4.95 and pH 6.05, well above that needed for making cottage cheese. The commonly recommended pH for cutting cottage cheese curd is 4.6 to 4.7. On either side of pH 4.6, the isoelectric point of casein, the casein tends to go into solution and does not exhibit the desired firmness of curd. No explanation is offered for the appearance of the precipitate at a pH so far from the isoelectric point of casein.

In making cottage cheese by the short-set method a period of 5 hours is normally used from the time starter is added until a firm coagulum is formed. In this trial the precipitate formed between 2.5 and 4 hours from the addition of the starter. The short incubation period and high pH at which the precipitate formed indicates the precipitate is something other than whole calcium caseinate.
A brown precipitate has been noticed in the bottom of the cheese vats during the process of coagulating milk for making cottage cheese. The precipitate has not been reported until recent years. Apparently, the cause is to be found in methods not used a few years ago. One change in handling of milk is the farm tank, where milk is stored on the farm and shipped every other day instead of the former daily pick up. This was investigated using actual and simulated procedures. The precipitate was found to appear more often after the milk was stored prior to use in the cottage cheese making.

In recent years rennet and patented coagulating agents have been used to shorten coagulating time of the milk. Four of these coagulating agents were investigated and results showed the defect occurred more often when certain of the coagulators were used. A relationship was established between the concentration of the coagulator used and the severity of the precipitate.

Milk was stored as skim milk and as whole milk to see if a difference could be established in the occurrence of the defect between the two milks. Little difference was observed in the occurrence of the defect.
Starter cultures containing high-gas forming bacteria were used and compared with cultures containing low-gas forming organisms. The precipitate appeared more often when the high-gas producing cultures were used.

Skim milk used in making cottage cheese will coagulate as the pH approaches 4.6 and will be ready to cut at about pH 4.65. The precipitate formed at a pH well above that needed for normal coagulation of milk. The precipitate formed much sooner than the normal five-hour incubation period used in the short-set method of making cottage cheese.
CONCLUSIONS

1. In making cottage cheese, chances of the precipitate occurring increase upon storage of the milk, starting about the third day of storage.

2. Some commercial coagulants tend to cause the defect more than others.

3. There is a negligible difference in the occurrence of the defect between the long-set or short-set method.

4. The intensity of the defect does not necessarily increase with increasing age of the milk. In some cases, however, it may increase in intensity up to the third or fourth day.

5. Intensity of the defect increases with the increased concentration of coagulants or rennet extract.

6. The defect is more likely to occur when using a coagulator than when using rennet extract.

7. There is little difference in the occurrence of the defect whether the milk was stored as whole milk or skim milk.

8. The precipitate is more likely to occur when a high-gas producing starter culture is used than when a low-gas producing culture is used.

9. The precipitate forms between pH 4.95 and pH 6.05 and between 2.5 and 4 hours after inoculation.
BIBLIOGRAPHY


3. Davis, H. E. Quality of cottage cheese as effected by some manufacturing and storage factors. Masters thesis. Columbus, Ohio State University, 1950. 50 numb. leaves.


