

# FACTORS INVOLVED IN THE CONTROL OF GELATINOUS CURD DEFECTS OF COTTAGE CHEESE.

## I. STORAGE TEMPERATURE AND pH

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### METHODS

Fruitiness, caused by growth of *Pseudomonas fragi*, was the defect that most commonly limited the storage life of commercial cottage cheese. Cultures of *Pseudomonas fragi*, *Pseudomonas viscosa*, and *Alcaligenes metalcaligenes* produced typical defects in cottage cheese at initial pH values as low as 4.6 and at temperatures as low as 3.5° C. Low temperature was very effective as a means of retarding the development of defects. Of the species studied, *P. fragi* was retarded least by low temperature. Violet red bile agar was found satisfactory for enumeration of the three species of bacteria.

The development of gelatinous curd defects on the surface of cottage cheese is an important bacteriological problem. Parker *et al.* (4) studied this type of spoilage, and demonstrated that three species of bacteria, *Pseudomonas fragi*, *Pseudomonas viscosa*, and *Alcaligenes metalcaligenes*, produced defects that were typical and distinguishable. The chief sources of the organisms were thought to be contaminated equipment and water. A limited control of surface spoilage was accomplished by regulating the pH of the final product. Recently, Davis and Babel (2) associated certain unidentified species of *Proteus*, *Pseudomonas*, *Aerobacter*, *Achromobacter*, and *Alcaligenes* with surface spoilage of cottage cheese.

The present study was made to determine the bacteria responsible for surface spoilage of cottage cheese in this locality and to investigate the influence of storage temperature and initial pH upon the development of defective odor or appearance in cottage cheese inoculated with bacteria capable of causing surface spoilage. Parker *et al.* (4) found that none of the species studied produced a defect during 72 hours at 15° C. when the initial pH was below 5.0. But since the "fruity" defect resulting from the growth of *P. fragi* has been observed to develop during prolonged storage of cottage cheese with initial pH as low as 4.65, it appeared desirable to have additional data on this point.

### *Enumeration of spoilage bacteria.*

Violet red bile agar, recently suggested by Elliker (3) for detecting surface spoilage bacteria, was used for plating cultures of *P. fragi*, *P. viscosa*, and *A. metalcaligenes* in preference to several other media recommended for culturing Gram negative bacteria. An incubation period of 48 hours at 25° C. was selected. Subsurface colonies were reddish purple to greyish purple with grey edges, round to elliptical, and usually 0.5 mm. or less in diameter. *A. metalcaligenes* formed colonies that were slightly redder than those formed by *P. fragi* and *P. viscosa*. Representative species of genera *Streptococcus*, *Micrococcus*, *Lactobacillus*, *Leuconostoc*, and *Bacillus* did not form colonies on violet red bile agar during the 48-hour incubation period.

*Isolation and initial study of cultures.* Samples of commercial cottage cheese that exhibited surface spoilage, and samples of tap water from a plant experiencing such difficulty, were plated on violet red bile agar. Isolated colonies were grown in litmus milk at 25° C. Several cultures that produced visible changes and/or gave undesirable odors in litmus milk, and pure cultures of *Pseudomonas*, *Alcaligenes*, and *Xanthomonas*, were grown in litmus milk for 48 hours and inoculated into cottage cheese at pH 5.2. (Commercially-prepared large-curd type cheese was used.) Ten ml. of a 1:100 dilution of each culture was added to each of two petri dishes containing cottage cheese. The excess inoculum was poured off, and the inoculated samples were observed for changes at 10° C.

*Preparation of experimental cheese for use in studying the influence of temperature and initial pH upon the development of surface spoilage.* Cottage cheese curd was cut into particles about 1/8 in.



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in diameter or smaller, heated, washed, and drained in the usual manner. To each of five 2-lb. quantities of curd enough sterile distilled refrigerated water was added to give a layer about 1 in. deep above the curd, after the mixture had been stirred and permitted to settle. Sufficient sterile dilute lactic acid or sodium carbonate to give pH values of approximately 4.6, 4.8, 5.0, 5.2, and 5.4 then was added, and the curd was stored overnight at 3.5° C. A Beckman model K potentiometer was used for determining pH. A half a day was required to make final adjustments in pH, after which sterile cheese cloth was used for draining the curd. Five 450-ml. quantities of refrigerated creaming mixture also were adjusted to the same pH

values and then inoculated with 10 ml. of a 1:100 dilution of the desired bacterial species (grown 40 hours in litmus milk). Adding the creaming mixtures to the appropriate curds inoculated each with about  $10^5$  bacteria per gram. After each curd and creaming mixture combination had been stirred and permitted to stand 1 hour, the excess creaming mixtures were poured off, so that the consistency of the finished product approached that of commercial cheese. Quantities of the creamed curd were stored in sterile petri dishes at 3.5°, 10°, and 15° C. and checked daily for defective odor or appearance.

#### RESULTS AND DISCUSSION

**Bacteria found to cause surface spoilage.** The defect most commonly encountered in samples of commercial cottage cheese was fruitiness, caused by growth of *P. fragi*. One sample was slightly putrid and slightly moldy, as a result of the growth of *Geotrichum candidum*. Cultures of *P. viscosa* and *A. metalcaligenes* were not isolated from any of the samples of cottage cheese examined.

Defects similar to that produced by *P. viscosa* (4) were produced by four cultures isolated from water provided by a plant that had experienced difficulty. Two of these cultures were classified as *P. viscosa* and the others were *P. jaegeri* and *P. geniculata* (1), respectively. Although the culture of *P. jaegeri* after prolonged incubation in litmus milk gave an odor resembling jasmine (which was not produced in cottage cheese), it was motile and therefore probably not *P. smaragdina*. Although these species were present in the plant water supply, only the fruity defect caused by growth of *P. fragi* was encountered in cottage cheese manufactured in the plant. This was due, at least in part, to contamination of the cottage cheese with comparatively large numbers of *P. fragi* from unsanitary cream cans.

In view of the possibility that other species could cause surface spoilage, 60 cultures of *Pseudomonas*, three of *Alcaligenes*, and five of *Xanthomonas* were inoculated into cottage cheese of pH 5.2. The experiment is noted because of the infrequency of resulting defects. Within 5 to 7 days at 10° C. four

cultures produced a defect similar to that produced by a control culture of *P. viscosa*, and 13 cultures produced gelatinous curd similar to that produced by *A. metalcaligenes*. It was somewhat surprising to find that 51 of the 68 cultures did not produce defects during storage for 10 days. It is likely that some of the cultures had not grown well in litmus milk, which undoubtedly gave variations in the level of inoculation. However, such differences in numbers of bacteria were not considered the primary limitation that determined whether or not a species produced surface spoilage.

**Influence of temperature and initial pH upon the development of surface spoilage.** Experiments were run to determine the time required for cultures of *P. fragi*, *P. viscosa*, and *A. metalcaligenes* to produce defective odor or appearance in cottage cheese at different temperatures and different initial pH levels. With cultures of *P. fragi*, a fruity odor was normally the first evidence of defect development; with cultures of *P. viscosa*, small gelatinous areas usually appeared simultaneously with a slightly putrid odor, or just before; with cul-

tures of *A. metalcaligenes*, small gelatinous areas were the first evidence of defect.

The experimental cheese was prepared as described above because large curd particles proved difficult to adjust to the desired pH values with accuracy. This procedure may have influenced the time required for defects to develop, for the resulting particles were considerably smaller than those of country-style cheese. It also is possible that storage life was influenced by the numbers of bacteria in the inocula. Davis and Babel (2) demonstrated with cultures of the genus *Aerobacter* that large initial numbers of bacteria produce a defect sooner than smaller numbers at 21° C. In the present experiments the numbers of bacteria were greater than those expected in freshly-made cottage cheese.

The results in Table 1 show that the lower pH values and storage temperatures retarded the appearance of typical defects but did not prevent them. Cultures of *P. fragi* and one culture of *A. metalcaligenes* were retarded by low pH to a slightly greater extent than was the culture of *P. viscosa*. Data are not available to show whether or

TABLE 1—INFLUENCE OF TEMPERATURE AND INITIAL pH ON THE PRODUCTION OF DEFECTS IN COTTAGE CHEESE BY *Pseudomonas fragi*, *Pseudomonas viscosa* AND *Alcaligenes metalcaligenes*

Culture	Minimum number of days required for development of defective odor or appearance at:				
	pH 4.6	pH 4.8	pH 5.0	pH 5.2	pH 5.4
Storage temperature of 15° C.					
<i>P. fragi</i> A	7	4	4	3	3
<i>P. fragi</i> B	6	4	4	3	3
<i>P. fragi</i> C	5	4	3	3	3
<i>P. fragi</i> D	5	4	3	3	3
<i>P. viscosa</i>	5	4	3	3	3
<i>A. metalcaligenes</i>	5	4	4	3	3
Storage temperature of 10° C.					
<i>P. fragi</i> A	12	8	7	4	4
<i>P. fragi</i> B	9	7	6	4	4
<i>P. fragi</i> C	—	—	—	—	—
<i>P. fragi</i> D	8	6	5	4	4
<i>P. viscosa</i>	8	6	5	5	5
<i>A. metalcaligenes</i>	7	6	6	5	5
Storage temperature of 3.5° C.					
<i>P. fragi</i> A	16	11	9	8	8
<i>P. fragi</i> B	21	17	10	11	10
<i>P. fragi</i> C	18	14	13	12	12
<i>P. fragi</i> D	17	15	10	10	10
<i>P. viscosa</i>	16	13	12	12	12
<i>A. metalcaligenes</i>	18	16	13	13	13

not pH increased during the time required for defects to develop. Relative to this point, Davis and Babel (2) reported that some unidentified species of five genera caused surface spoilage at pH 4.75. They found that inoculated cottage cheese samples held at 4.4° C. showed little change in pH; samples held at 10° C. showed no change or an increase in pH; and all samples held at 21° C. showed an increase in pH.

The data indicate that low temperature is very important as a means of retarding the production of defects by the species of bacteria studied. The development of defective odor or appearance required longer storage periods at 10° than at 15° C. and considerably longer storage periods at 3.5° C. Of the three species studied, *P. fragi* was retarded least by the lower temperatures. However, under the experimental conditions only one culture of *P. fragi* produced defective cheese in less than 10 days at 3.5° C., even at initial pH values above 5.0. Obviously a considerable advantage may be gained by careful maintenance of low temperature as early as possible during the processing of cottage cheese curd and during storage of the finished product.

*Use of violet red bile agar for routine enumeration of bacteria.* Bacteria in products from a plant experiencing difficulty with fruity cheese were enumerated by means of violet red bile agar, with an incubation period of 48 hours at 25° C. The creaming mixture and

the finished product contained large numbers of bacteria from which cultures of *P. fragi* were easily isolated. Contamination of the creaming mixtures proved to be the result of inadequate sanitation of cans. Subsequent enumeration on violet red bile agar was practiced routinely. Counts on creaming mixtures and cottage cheese normally were very low, but in a few instances large numbers of bacteria warned of unsanitary practices.

Counts at about 10-day intervals on the plant water, obtained from two wells about 350 ft. deep and stored in a metal tank, eliminated the possibility that seasonal variations caused differences in the numbers of bacteria capable of forming colonies on violet red bile agar. The average count for 36 samples was eight colonies per milliliter.

#### SUMMARY

Prolonged incubation permitted cultures of *Pseudomonas fragi*, *Pseudomonas viscosa*, and *Alcaligenes metalcaligenes* to cause surface spoilage of cottage cheese at initial pH values as low as 4.6 and at temperatures as low as 3.5° C. The defects developed slowly at low pH values and very slowly at 3.5° C. Low initial pH values did not retard *P. viscosa* quite as much as they retarded *P. fragi* and *A. metalcaligenes*; low temperatures did not retard *P. fragi* quite as much as they retarded the other two species.

The defect most commonly found to limit the storage life of com-

mercial cottage cheese in this locality was fruitiness, caused by growth of *P. fragi*. Other species of bacteria were found to cause surface spoilage, but they were not isolated from the samples of cottage cheese examined.

Violet red bile agar was found satisfactory for enumeration of *P. fragi*, *P. viscosa*, and *A. metalcaligenes*. Subsurface colonies formed by these species in 48 hours at 25° C. were round to elliptical, usually 0.5 mm. or less in diameter, and reddish purple to greyish purple with grey edges. Enumeration of bacteria with violet red bile agar was found helpful in the prevention and control of surface spoilage.

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