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A Study of a Bacterial Cause of the Late Gas Defect in Cheddar Cheese

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A STUDY OF A BACTERIAL CAUSE OF THE LATE GAS DEFECT IN CHEDDAR CHEESE

by

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INTRODUCTION

More of the manufacturing milk purchased from Utah's dairy farms is used for the production of cheese than for any other product. Approximately eleven and one-half million pounds of cheese were produced in Utah in 1959, of which more than half was cheddar. Many problems are encountered in the production of A-grade cheddar cheese. One of the most important causes of the reduction in grade is the late gas defect which accounts for about 50 percent of the cheese grading below A-grade. To increase the percentage of A-grade cheddar cheese in Utah, the late gas problem should be solved. The objective of this study is to learn some of the causes of the late gas defect and point the way to its prevention.

REVIEW OF LITERATURE

The late gas defect in cheddar cheese is characterized by excessive openness and gassy formation in the mechanical openings or weak points in the cheese mass. The defect may appear at the first official grading or later in storage. This defect has been more prevalent since the increased use of pasteurized milk. Previously the defect had been observed in sweet curd types of cheese like brick and Colby. Europeans have described it in Edam and Gouda (Price, 1954).

In cheddar cheese the defect usually appears during the period from one to twelve weeks in storage. The defect appears earlier in cheese with a high moisture content. The flavor of the cheese may or may not be affected. In cheddar it may appear as a smothered, fermented or unclean flavor. The holes may be scarcely noticeable, but sometimes are large enough to become quite objectionable. Mechanical openings are usually enlarged as a result of gas pressure and the walls become shiny (Price, 1954).

The underlying factors involved in the development of the late gas defect include development of gas within the cheese and the character of the cheese body and texture which retains the gas, thus giving rise to the defect. The normal development of gas is more rapid during the early stages of ripening and decreases as the cheese ages. It can be accelerated again by warming the cheese. If the production of gas occurs too rapidly during the curing process, the mechanical openings are enlarged and some round gas holes may appear.

Price (1954) lists some factors which cause over-development of gas as:

1. Inferior milk
2. Raw milk
3. Low acid production
4. Temperatures of cooling above 50 F.

Overcast and Albrecht (1952) in work with gas producing associate starter organisms have shown that a pure culture of Streptococcus lactis did not produce the defect. When they added the associate organisms (Leuconostocs) to commercial starters the defect was enhanced. Albrecht and Ashe (1955) found that incubation of bulk starters containing these organisms at temperatures slightly higher than the normal (70-72 F) produced a marked reduction in the growth of the Leuconostoc citrovorum. This also resulted in a decrease in the occurrence of the defect.

Sherwood (1939) studied the late gas defect in New Zealand and concluded that the abnormality is caused by gas-producing lactobacilli. He also stated that since some cheddar cheese starter cultures contain L citrovorum, this might also be a cause of the trouble. Galesloot (1950) confirmed Sherwood's findings, but he states that a rapid acid-producing culture can eliminate the problem. Prouty and Golding (1946) in their study of the effect of the associate organisms in vacuum packed cheddar cheese reported that greater loss of vacuum was observed with Leuconostoc dextranicum than with L citrovorum. This indicates greater gas production by L dextranicum.

Price (1954) attributes most of the present difficulty to gas forming organisms which can survive pasteurizing treatments. These are the anaerobic spore formers of the genus Clostridium. Clostridium butyricum is a typical representative found in this defective cheese.

Foster et al. (1957) give as causes of gas formation in cheese:

1. Coliform bacteria (species of Aerobacter are more active gas formers than species of Escherichia).
2. Lactose-fermenting yeasts.
3. Gas-producing, aerobic, spore-forming rods. Primarily Bacillus polymyxa and Bacillus mascerans.
4. Species of Closdridium, including Clostridium sporogenes, Clostridium lentoputrescens, Clostridium pasteurianium, Clostridium butyricum and many others.
5. Leuconostoc citrovorum and Leuconostoc dextranicum.

Albrecht and Ashe (1955) suggest the possibility that milk produced under certain conditions may yield cheese that is more susceptible to the late gas defect than cheese from normal milk. Sommer (1948) states that the calcium and magnesium content of milk increases during the last third of the lactation period and that citric acid is highest when cows receive pasture feeds. Greenberg (1944) found calcium citrate to be soluble in water but not ionizable; therefore, it is possible that the calcium ions are not as available in milk during the pasture feeding periods as during fall or winter months. The work of Van Slyke and Bosworth (1916) indicates the calcium ions must be removed before monobasic paracasein would be soluble in a 5 percent salt solution. If this is so one might conclude that despite the fact that calcium ions are necessary for the formation of the typical cheese curd, excessive amounts might be detrimental to the proper fusing of the curd into a single mass. Because of this it might be easier for the gas produced during the early stages of ripening to rupture the partially fused curd particles in finished cheese made from milk that is not produced on pasture feed or milk produced during the last third of the lactation period.

Various investigators have given recommendations for control of the late gas defect. Foster et al. (1957) state that coliforms can be controlled by using good quality milk and proper pasteurization. Lactose fermenting yeasts are supposedly eliminated by proper pasteurization. As a group the Clostridia are sensitive to acid and salt, and most of them have a fairly high optimum temperature as compared with that of the desirable organisms. Consequently they do not grow readily in normal cheese. Thus their control in cheese is best assured by keeping them out of the milk, by insuring vigorous acid formation in the curd, and by adequate salting. Hopeful results have been obtained in attempts to control them by using a nisin-producing strain of S lactis (Foster et al. 1957).

Albrecht and Ashe (1955) studied the effect of variation in acidity at milling. Four vats of curd were allowed to develop acid up to 0.35, 0.40, 0.45, and 0.50 percent respectively, before they were milled. The results showed that the degree of acid development before the curd was milled had no effect upon the development of slit-openness. In another experiment the amount of salt added to the milled curd was varied in an attempt to control the slit-openness defect. In the first trial, salt was added to four vats of cheese curd at the rate of 2.00, 2.50, 3.00 and 3.50 pounds of salt per 1,000 pounds of milk. At the level of 3 percent or higher, the defect did not occur. Four additional trials were run in which the amounts of salt were reduced to 2.25, 2.50, 2.75 and 3.00 pounds per 1,000 pounds of milk. Variations in the amount of salt added to the milled curd showed that the defect could be controlled. In the 20 vat-lots of cheese in this series of experiments, the slip-openness defect was not found in any lot containing 1.7 percent salt or more (Albrecht and Ashe, 1953).

No attempt was made to explain whether the added salt in the cheese retards the growth of the organisms or improves the fusibility of the curd particles so that the gas does not rupture the curd particles.

Price (1954) lists measures to be taken to prevent development of the late gas defect. This list includes:

1. Use chlorine sanitizing agents on the farm at a concentration of 200 to 400 ppm with five minutes or more contact.
2. Use selected milk of low bacterial content and caution patrons against possible contamination.
3. Use only active starters.
4. Develop the maximum acidity in the cheese without producing acid defects.
5. Keep the lowest practical moisture content in the cheese for the variety being manufactured.
6. Incorporate not less than 1.7 percent of salt (by actual analysis) in the finished cheese.
7. Maintain the lowest possible temperatures during curing.
8. High temperatures essential for certain steps in curing or wrapping should be maintained for the least possible time.
9. Maintain careful checks during ripening.

PROCEDURE

Samples of cheese showing the late gas defect were obtained from the storage rooms of Brooklawn and Nelson Ricks creameries in Salt Lake City, Utah, and from the Utah State University creamery. Before taking each sample the trier was carefully washed with 95 percent ethyl alcohol. The plugs were taken as nearly aseptically as possible and placed in one-half pint glass bottles that had previously been sterilized. The samples showing the late gas defect were taken to the laboratory where the shiny walled holes were swabbed with sterile cotton swabs. The swabs were streaked on a defined area of the plates of standard agar[1] and in a like manner on plates of thioglycolate agar[2]. The uninoculated area of the plates was then streaked in a manner that would cause isolation of the separate organisms obtained from the cheese. The standard agar plates were incubated aerobically at 98.6 F until the colonies appeared large enough to show distinguishing characteristics. The thioglycolate agar plates were incubated anaerobically in a nitrogen atmosphere at 86 F for seven days. The different types of colonies on each plate were given identifying numbers. Each of the different types of colonies were then transferred to litmus milk and sterile skim milk. A Gram stain was made of each type of colony on each plate. Observations on activity were made in litmus and regular milk. Cultural characteristics, morphology

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[1]Baltimore Biological Laboratory M-P H Medium, Standard Plate Count Medium.

[2]Difco Laboratories, Anaerobic Agar.

and Gram staining properties were studied and compared. A partial grouping of the organisms was made from these observations. It was concluded that the aerobically incubated plates contained the same types of organisms as the anaerobically incubated plates.

Each of the colony types was transferred to tubes of milk prepared to detect gas production under near anaerobic conditions. The method of excluding air was to:

1. Drive off oxygen from medium by autoclaving for ten minutes.
2. Cool rapidly to keep oxygen absorption to a minimum.
3. Inoculate; cover with a layer of petroleum jelly (Bryan and Bryan, 1953).

The tubes were incubated at around 70 F for seven days and examined for evidence of gas production, which is indicated by a bubble of gas between the liquid medium and the petroleum jelly plug. A loopful of the medium from those tubes showing gas production was streaked on plates of agar. The plates were incubated at 70 F until the colonies were large enough to show distinguishing characteristics. The colonies and Gram stains from the colonies were examined for the purpose of grouping into groups of like organisms and to detect any mixing of types. The gas-producing organisms were placed in four groups of culturally and morphologically related organisms. These were further combined into two primary groups. These two groups were identified as Coliforms and Leuconostocs.

Trials were run to find the effect of different levels of pH and salt concentration on the coliform and leuconostoc organisms obtained from the defective cheese. The medium used was milk with the pH adjusted with fresh lactic starter and buffered at the desired pH with 5 percent of an acetic acid sodium acetate buffer. In the first trial the pH of the milk medium was adjusted to 4.5, 5.0 and 5.5. The lots of milk with the pH adjusted were divided into three portions at each pH level.

Sodium chloride was added to the three portions at levels of 0.0, 0.5 and 1.0 percent. This resulted in nine different pH level and salt concentration combinations. The medium was then dispensed in 75 mm test tubes, 2 ml per tube. The tubes of the medium were prepared as previously described for near anaerobic conditions. The tubes were then inoculated with the different types of organisms and incubated at 70 F for seven days. Three replications were run on each pH and salt combination. At the end of the incubation period the tubes were examined and the amount of gas produced was noted. The volume of gas produced in each tube was observed and given a value of 0, 1, 2, 3, 4 or 5.

The second trial was run exactly the same as the first except for the pH levels and salt concentrations. The pH levels for the second trial were 5.0, 5.2 and 5.4. The salt concentrations were 1.0, 1.5 and 2.0 percent. The results for both trials were set up for statistical analysis by the factorial design with two treatments and three levels of each treatment. However, in the second trial there were four replications instead of three.

Three vats of cheese were made to determine whether the late gas defect could be produced in the cheese by the two types of organisms studied. The first vat was designated as a control. The second vat was inoculated with a 24 hour culture of Leuconostoc organisms. The third vat was inoculated with a 24 hour culture of Aerobacter aerogenes. The inoculations were made in the milk just prior to the addition of the starter. The defect was produced in the cheese and the types of organisms used in the inoculation were isolated from the shiny walled holes or mechanical openings.

Figure 1 shows the method used to detect the amount of gas produced by bacteria under near anaerobic conditions. Note the position of

the petroleum jelly plug in each tube between the cotton plug and the milk. The position of the petroleum jelly plug gives an indication of the volume of gas produced. In some cases the plug was pushed entirely out of the tube by gas pressure.

Figure 1. Method used to detect the production of gas under near anaerobic conditions

RESULTS AND DISCUSSION

Fourteen samples of cheese grading B and C and showing the late gas defect were examined. Yeast and Leuconostocs were isolated as the gas producing organisms in one sample, gram positive rods probably of the genus Bacillus or Clostridium were found in one sample, coliform type organisms were found in five samples and organisms of the genus Lauconostoc were found in seven of the samples. This indicates a predominance of leuconostoc and coliform type organisms in the late gas defect in the cheese from which the samples were taken. The samples came from factories throughout Utah and in southern Idaho.

The tests to determine the effect of decreasing the pH and increasing the salt concentration on the volume of gas produced were run on Aerobactor aerogenes and Leuconostoc dextranicum as typical representatives of the two groups.

The volume of gas produced by A aerogenes and L dextranicum was measured and given a numerical value for ease in statistical analysis. The values given were:

1. Slightest evidence of a gas bubble.
2. Up to 10 mm displacement of the plug.
3. Up to 20 mm displacement of the plug.
4. Up to 30 mm displacement of the plug.
5. Plug pushed clear out of the tube.

Table 1. Gas production by A aerogenes as affected by varying levels of pH and salt concentration in trial 1

Replicate	A_1			A_2			A_3		
	b_1	b_2	b_3	b_1	b_2	b_3	b_1	b_2	b_3
1	0	0	0	0	1	2	4	4	4
2	0	0	0	2	2	2	4	4	4
3	0	0	0	2	2	3	4	4	4

A_1=pH 4.5, A_2=5.0, A_3=5.5, b_1=0.0% NaCl, b_2=0.5%, b_3=1.0%

The observations were given values of one through five depending on the volume of gas produced.

Table 2. Gas production by A aerogenes as affected by varying levels of pH and salt concentration in trial 2

Replicate	A_1			A_2			A_3		
	b_1	b_2	b_3	b_1	b_2	b_3	b_1	b_2	b_3
1	3	2	3	4	4	4	4	3	4
2	4	4	4	4	4	5	4	4	4
3	5	4	4	5	5	5	4	4	4
4	4	4	5	4	5	4	4	5	4

A_1=pH 5.0, A_2=5.2, A_3=5.4, b_1=1.0% NaCl, b_2=1.5%, b_3=3.0%

The observations were given values of one through five depending on the volume of gas produced.

Table 3. Gas production by L dextranicum as affected by varying levels pH and salt concentration in trial 1

Replicate	A_1			A_2			A_3		
	b_1	b_2	b_3	b_1	b_2	b_3	b_1	b_2	b_3
1	0	0	0	0	0	0	2	3	4
2	0	0	0	0	0	0	4	3	1
3	0	0	0	0	0	0	2	3	4

A_1=pH 4.5, A_2=5.0, A_3=5.5, b_1=0.0% NaCl, b_2=0.5%, b_3=1.0%

The observations were given values of one through five depending on the volume of gas produced.

Table 4. Gas production by L dextranicum as affected by varying levels of pH and salt concentration in trial 2

Replicate	A_1			A_2			A_3		
	b_1	b_2	b_3	b_1	b_2	b_3	b_1	b_2	b_3
1	0	0	0	4	0	5	4	4	5
2	4	2	1	2	4	5	5	1	1
3	4	0	4	4	4	4	2	3	1
4	4	0	1	4	4	4	4	1	4

A_1=pH 5.0, A_2=5.2, A_3=5.4, b_1=1.0% NaCl, b_2=1.5%, b_3=2.0%

The observations were given values of one through five depending on the volume of gas produced.

Table 5 shows that as the pH was lowered from 5.5 to 5.0 to 4.5, there was a highly significant decrease in the volume of gas produced by A aerogenes. Increasing the salt concentration from 0.0 to 0.5 to 1.0 percent gave no significant decrease in the volume of gas produced. There was no significant interaction between the two factors.

Table 5. Analysis of variance on the volume of gas produced by A aerogenes under various levels of pH and salt concentration in trial 1

S.O.V.	d.f.	S. sq.	M. sq.	F.
Replicates	2	1	.50	
Treatments	8	73.9	9.24	
pH	2	72.3	36.15	190.26**
Salt concentration	2	.6	.30	1.58
Interaction	4	1.0	.25	1.32
Error	16	3.0	.19	
Total	26	77.9	3.0	

** Significant at 1 percent level.

Table 6 shows a significant decrease in the volume of gas produced by A aerogenes when the pH was lowered from 5.4 to 5.2 to 5.0. There was no significant decrease in the volume of gas produced when the salt concentration was raised from 1.0 to 1.5 to 2.0 percent. The interaction between the two factors was not significant.

Table 6. Analysis of variance on the volume of gas produced by A aerogenes under various levels of pH and salt concentration in trial 2

S.O.V.	d.f.	S. sq.	M. sq.	F.
Replicates	3	5.41	1.80	
Treatments	8	3.00	.38	
pH	2	2.17	1.09	4.19*
Salt concentration	2	0.17	.09	.35
Interaction	4	0.66	.17	.65
Error	24	6.34	.26	
Total	35	14.75		

* Significant at 5 percent level.

Table 7 shows a highly significant decrease in the volume of gas produced by L dextranicum when the pH was lowered from 5.5 to 5.0 to 4.5. The effect of increasing the salt concentration from 0.0 to 0.5 to 1.0 percent was insignificant. There was no significant interaction between the two factors.

Table 8 shows a highly significant reduction in the volume of gas produced by L dextranicum which was observed when the pH of the milk medium was lowered from 5.4 to 5.2 to 5.0. There was also a significant reduction in the volume of gas produced when the salt concentration was increased from 1.0 to 1.5 to 2.0 percent. There was no significant interaction between the two factors.

Table 7. Analysis of variance on the volume of gas produced by L dextranicum under various levels of pH and salt concentration in trial 1

S.O.V.	d.f.	S. sq.	M. sq.	F.
Replicates	2	.1	.05	
Treatments	8	50.3	6.28	
pH	2	50.1	25.05	86.38**
Salt concentration	2	.1	.05	.17
Interaction	4	.1	.025	.09
Error	16	4.6	.29	
Total	26	55.0	2.12	

** Significant at 1 percent level.

Table 8. Analysis of variance on the volume of gas produced by L dextranicum under various levels of pH and salt concentration in trial 2

S.O.V.	d.f.	S. sq.	M. sq.	F.
Replicates	3	1.19	.40	
Treatments	8	46.50	5.81	
pH	2	24.50	12.25	6.25**
Salt concentration	2	14.00	7.00	3.57*
Interaction	4	8.00	2.00	1.02
Error	24	47.06	1.96	
Total	35	94.65		

* Significant at 5 percent level.
** Significant at 1 percent level.

In every case of the late gas defect studied there was isolated from the cheese an organism or organisms which produced the defect. This seems to indicate that most of the trouble in this area is of a bacterial nature. The types of organisms appearing most frequently indicate a need for:

1. Careful selection of low gas producing starters.

2. Making sure the proper pasteurizing temperature is reached and maintained for the correct period of time.

3. Careful sterilization of each piece of equipment that comes in contact with the milk or cheese after the milk has been pasteurized.

4. Maintaining good starter activity in order to produce the acid necessary in the manufacturing process. An acceptable pH for cheddar cheese is around 5.1.

5. Salting to at least 1.7 percent by actual analysis (Price, 1954; Albrecht and Ashe, 1955).

6. Maintaining the curing temperature as low as possible to achieve the desired ripening qualities in flavor, body and texture.

SUMMARY AND CONCLUSIONS

Summary

Fourteen samples of cheddar cheese showing the late gas defect were studied. The microorganisms from the gas holes and enlarged mechanical openings were subcultured on prepared bacterial medium. The gas producing organisms were determined and classified as to genus. In five of the fourteen samples, bacteria of the coliform type were present. Seven of the fourteen samples had leuconostocs as the only gas producing organisms isolated from the openings or holes showing evidence of gas pressure. It was found that there was a significant reduction in the volume of gas produced by both organisms when the pH was taken from around 5.5 down to 5.0 or lower. There was a significant reduction in the volume of gas produced by L dextranicum when the salt concentration was raised to around 2.0 percent. No significant interaction between salt and acid was observed.

Conclusions

1. There should be careful selection of low gas producing starters.

2. There was a reduction in the volume of gas produced by both A aerogenes and L dextranicum when the pH was lowered to around 5.0.

3. There was a reduction in the volume of gas produced by L dextranicum when the salt concentration was increased to around 2.0 percent.

4. The effect of acid was neither increased or decreased by variation in the salt concentration from 0 to 2 percent. The action of each of these two factors seems to be independent of the other.

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