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A Study of Delayed Gas Formation in Cheddar Cheese

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A STUDY OF DELAYED GAS FORMATION IN CHEDDAR CHEESE

by

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Mark E. Oldham

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INTRODUCTION

Origin and nature of problem

During recent years, the dairy industry has been aware of a defect in Cheddar cheese known as "late gas". This causes the mechanical openings to enlarge and become shiny. In Cottage cheese the defect exhibits itself in the form of floating curd. Both of these defects result in a general lowering of the grade and quality of the finished product.

Workers in the industry have related this defect to the starter culture, although contamination of milk, equipment and cultures may also be a source. They conclude that with a source of good quality milk and proper manufacturing procedures and efficient sanitation methods, the defect may persist.

Whichever source the defect stems from, the conditions present a serious problem to the cheese industry.

Purpose of project

The purpose of this project is to establish a possible relationship between the defect and starter cultures which produce gas in any abnormal rates.

It will further try to classify the lactic acid cultures according to their gas producing ability in relation to the other desirable characteristics, flavor, aroma and activity.

The adaptability of such a system of classification will be fitted into a program of starter culture selection.

REVIEW OF LITERATURE

Floating curd in Cottage cheese

Sandine et al. (24) noted that there was a direct relationship between the floating curd defect in Cottage cheese and the volumes of CO_2 produced by the starter cultures used in the manufacture of the cheese which established the major cause of this defect. Further confirmation has been provided by the isolation of high gas-producing single strain lactic bacteria from the gassy starter and gassy Cottage cheese and the failure to isolate these types from the non-gassy mixed culture and satisfactory Cottage cheese. It was noted that the high gas-producing ability was also accompanied by the production of aroma compounds. None of the low gas producers formed aroma compounds to any extent. These cultures may be desirable in products like buttermilk, cultured sour cream and cream for churning butter. The aroma production is beneficial in Cottage cheese, but represents a hazard in production of floating curd defect.

They also noted that the gassy nature of cultures are susceptible to change depending on the individual strain composition and uniformity of culturing. Handling conditions including freezing, and over-ripening might allow high gas-producing strains to become more numerous.

Moore (17) observed the floating curd defect in Cottage cheese made from non-fat dry milk solids. Gas forming organisms were not found in the curd and whey. The problem was eliminated when the water was adjusted to a pH 6.8 with sulfuric acid. The use of water softened by base exchange method for reconstitution of the non-fat dry milk solids to be used in the manufacture of Cottage cheese, eliminated

gassy curd but resulted in an undesirable softness of the curd.

Hammer (12) reports that Escherichia-Aerobacter species produce floating curd to such an extent as to cause the curd to rise over the edge of the vat.

Apparatus for gas measurement

Some work was done by Allen (2) on an apparatus to measure the volume of gas produced by the lactic acid cultures. The apparatus which was used was a modification of one designed by Cranston (4). This apparatus could be adapted for anaerobic conditions and would measure the effect of developed hydrogen apart from carbon dioxide. It was found to be of use in general measurement but inaccurate in actual mathematical calculation. The apparatus is shown in Figure 1.

Hassouna and Allen (15) conducted a study (a) to measure the gas production (if any) by pure strains of lactic acid Streptococci and by starters growing in milk; (b) to measure the total gas evolved by Bact. coli in the early stages of growth in different media, particularly in milk; (c) to find the effect on gas production by Bact. coli of growing Str. lactis in the same medium with it and to measure the proportion of Str. lactis relative to Bact. coli required to inhibit gas production completely; (d) to find the influence on gas production by Bact. coli with and without the concomitant growth of Str. lactis, of subjecting it to anaerobic conditions from the outset; and (e) to measure the effect due to the hydrogen alone (by absorbing the carbon dioxide) on the gas produced by Bact. coli under different conditions.

Gas formation was noted in all cases except in tubes containing Str. lactis alone. In a second experiment, ranges of different inocula were used and the results are shown graphically in Figure 2.

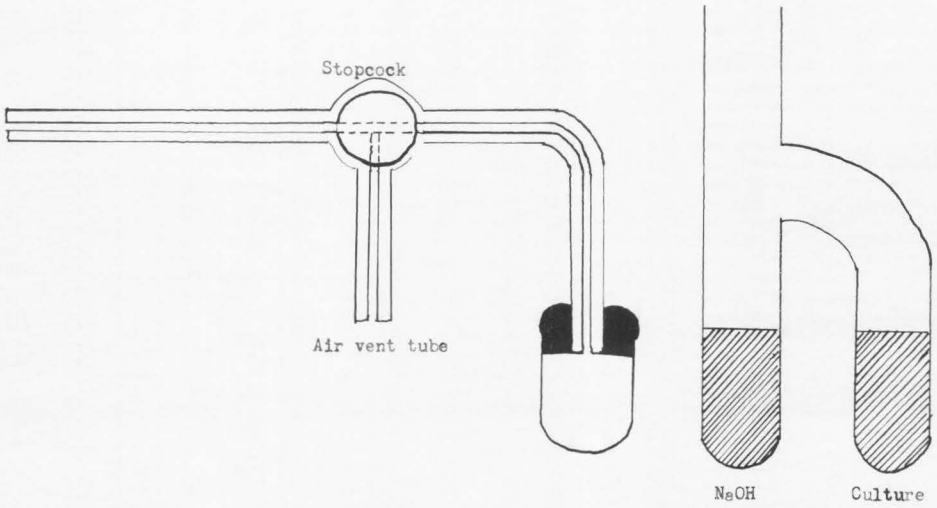


Figure 1. An apparatus to measure gas production starter cultures and a tube to measure H_2 evolution

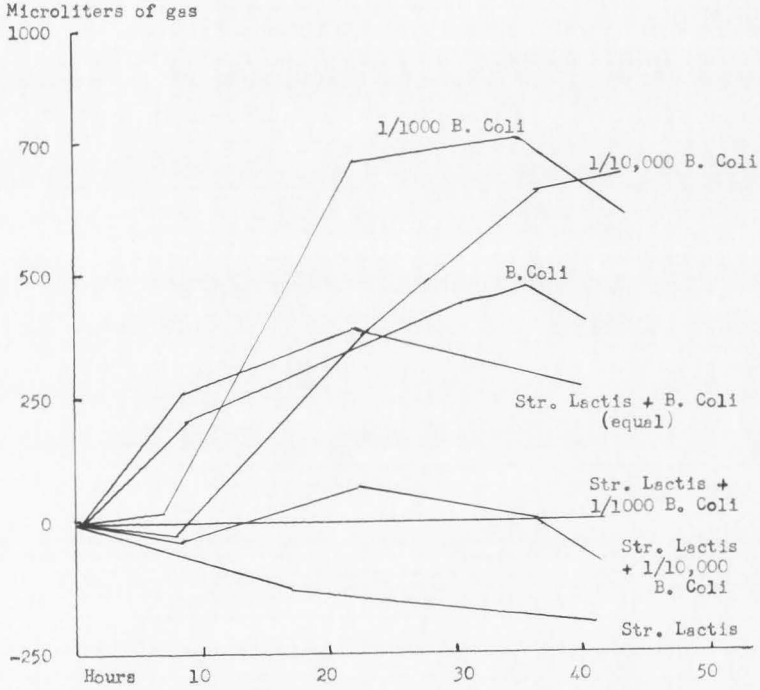


Figure 2. Rate of gas production with different rates of inoculum of B. Coli and S. Lactis

Tests showed slightly more gas in glucose broth than in lactose broth. Gas production under anaerobic conditions was of little or no importance, in sterile skim milk the gas evolved by Bact. coli was found to be carbon dioxide, and when this was absorbed by alkali no gas evolution could be detected.

In a series of experiments it was noted that both Str. lactis and Str. paracitrovorus produced small but definite amounts of gas. When this gas is produced too fast to difuse through the cheese, the defect becomes evident.

Prevention of floating curd defect

Sandine, Anderson and Elliker (25) made a study of methods to follow to avoid the floating curd defect in Cottage cheese. The Warburg respirometer was used to measure the CO_2 produced in microliters. Results showed that by this method gassy and non-gassy lactic acid cultures could be separated. It was noted that eight hour tests magnified the results, but the same relative gas producing properties could be demonstrated using the four hour period. It was further observed that all cultures causing "floating curd" defect produced three to five times as much CO_2 than those from normal batches of cheese. (See Table 1.)

Commercial cultures were also studied to determine their gas producing characteristics. Table 2 summarizes this work. It was found that the same commercial cultures from different sources did not show comparable results, but when each culture was kept on daily transfer gas production remained constant over quite a period of time, up to five months.

A mixture of different non-gassy strains, consisting of 75 percent lactic acid producers and 25 percent associate organisms exhibited

Table 1. Gas production by lactic acid cultures obtained from Cottage cheese manufacturing plants

Culture	History of Culture	CO_2 Produced Microliters
1 R	Used in plant with no evidence	167
2 R	of excessive gas production	150
5 R		214
3 R	Curd floated in all vats	632
4 R		549
6 B		615
7 B		554
3 R-1[a]	Single strain isolates from 3 R	472 + b
3 R-2		32 - c
3 R-3		20 -
3 R-4		417 +
3 R-5		50 -
3 R-6		45 -
3 R-7		633 +
3 R-8		43 -
4 R-1	Single strain isolates from 4 R	534 +
4 R-2		50 -
4 R-3		35 -
4 R-4		46 -
4 R-5		727 +
4 R-6		517 +
4 R-7		53 -

a Represents one determination. All others are average of triplicate.
- b Aroma producers.
- c No aroma.

Table 2. Gas produced by various commercial lactic acid starter cultures. Five percent inoculum incubated four hours at 30°C

Culture	CO_2 Produced Microliters	Rating based on Gas production
3 D	209	low
8 D	225	low
9 D	196	low
10 D	179	low
11 D	201	low
12 D	133	low
13 D	195	low
14 D	141	low
15 D	65	low
16 D	195	low
17 D	220	low
18	324	medium
22	184	low
23	857	very high
24	179	low
25	212	low
26	1008	very high
27	806	very high
28	198	low
29	207	low
30	1027	very high
31	301	medium
32	273	medium
33	598	high

high aroma production, but without noticeable gas production. It appeared that aroma production may be realized without excessive gas production by combining non-gassy lactic cultures with non-gassy Leuconostocs.

In actual plant use, the direct relationship between the floating curd defect and large volumes of CO_2 produced by the starters establishes the major cause of the defect.

Simple apparatus to measure gas

The same workers (26) set about to design a simple apparatus for the measurement of gas production and activity of lactic acid cultures.

The facts noted above established the importance of having a simple laboratory method for the measurement of CO_2 production by lactic acid starter cultures. A gasometer was designed for this purpose and its construction is described in Figure 3.

Ten representative mixed strain cultures were selected for this study. The results are shown on Table 3. It was concluded from further trials that cultures producing more than 150 microliters invariably caused the floating curd defect. The use of this method of selection will reduce, if not eliminate, the incidence of floating curd defect.

Sandine, Elliker, Wilster, Stein and Anderson (27) demonstrated that a beaker test for gas production can be used to identify many lactic cultures which produce floating curd defect.

Factors affecting gas production

In work done at Minnesota, Myers (16) studied the factors affecting the growth and gas production of lactic acid cultures. It was noted that heating the milk to 180°F for ten minutes caused a slight increase in CO_2 production while heating in excess of ten minutes had an inhibitory effect on CO_2 production. The optimum pH for CO_2 production was noted to be between 5.0 and 5.5. Over-ripening of the cultures increased the total CO_2 production. Storage up to three days resulted in an increased CO_2 production, while up to five days a decrease in CO_2 production was observed. When two or more cultures were grown together, larger amounts of CO_2 were produced due to a lowering of pH to near the optimum for gas production. Jezeski (14) noted that age and acidity of culture, interval between transfer and time and temperature of incubation had an effect on CO_2 output. A selection of starters which produce acid

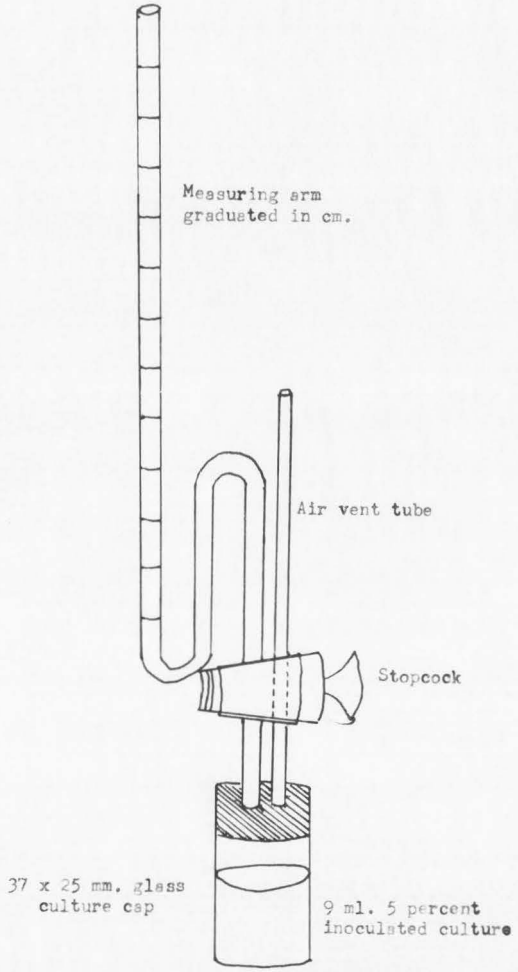


Figure 3. An apparatus for measuring gas production in starter cultures

Table 3. Microliters of CO_2 produced by ten representative mixed strain lactic cultures in four hours at 30°C as measured by the laboratory gasometer. Activity of cultures is indicated by titratable acidity determinations.

Culture	Microliters of CO_2 produced in Following separate trials						Titratable Acidity
	Trial Number						
	1	2	3	4	5	Ave.	
1 R	56	48	54	74	48	56	.50
2 R	32	48	50	70	48	50	.41
3 R	382	360	424	438	384	398	.52
3 D	86	88	102	112	68	91	.53
4 R	452	364	360	390	338	381	.48
D	216	238	234	230	182	220	.53
6 B	442	400	448	494	408	434	.50
7 B	130	176	182	190	174	170	.53
8 D	58	72	78	98	72	76	.47
9 C	338	282	298	264	242	285	.48

only and no flavor and aroma compounds, mixed strains of starter cultures of low CO_2 producing power and the proper programming of transfer and time and temperature relationships, kept the late gas under control. Davis and Thiel (5) observed gas to be at a minimum at the extreme pH ranges of 3.5 and 10.5 while the optimum for its production is usually pH 6.5 to 7.5 the same as for growth.

Loss of vacuum due to gas production

Prouty and Golding (21) concluded from work done with vacuum packed American Cheddar cheese into which known quantities of S. Citrovous or S. paracitrovous were used in conjunction with S. lactis or L. bulgaricus, that cheese manufactured from starters containing

S. lactis or L. bulgaricus alone showed little or no loss of vacuum over periods ranging up to six months, whereas the addition of S. citrovous or S. paracitrovorus caused a greater loss in vacuum; more loss being associated with S. paracitrovorus than with S. citrovorus.

In a study on the case of gas production by lactic acid bacteria, Gibson and Abdel-Malek (10) concluded that glucose fermented to gas much more rapidly than lactose. Some factors which determine the amount of CO_2 produced are: (a) type of sugar, (b) the reaction and buffering capacity of the medium, (c) the concentration of sugar present up to an optimum of 5 percent.

Galesloot (9) studied the genus Betacocci as a source of gas in cheese. He demonstrated that Betacocci were able to produce the early gas defect. In the presence of active lactose fermenting bacteria, the defect did not seem to appear. In comparison with other literature, the Betacocci were also called Leuconostac. It was believed that the Betacocci occuring in normal starters did not present any dangerous problem.

Evans (7) studied the Streptococci concerned with cheese ripening and noted that of all the bacteria which played a part, Streptococci kefir was able to produce large quantities of CO_2 when grown in suitable media. It was further noted that Streptococci kefir may be found in some commercial cultures.

Storage temperatures and open texture

Price (20) observed that in cheese ripened at temperatures of $50^{o}F$ or higher there is a rapid development of CO_2. With this rapid development, openness in the body was noted. Some factors which aid in this development are: Inferior milk supply, raw milk, low acid production and high curing temperature. It was concluded that the

general type of organism responsible for this excessive CO_2 development is anaerobic spore forming bacteria, Clostridium bulyricum being an example. The main source of this contamination is mud, soil, and unclean utensils.

Albrecht and Ashe (1) are in general agreement with Price (20) in that high storage temperatures lends to the development of excessive CO_2 and open texture. Several other factors were studied in relation to CO_2 production and openness in cheese. It was noted that the salt balance showed no relation to the abnormality. The variations in temperatures of pasteurization showed no effect on control over the slit openness defect. Cooking procedure had no control on the defect. It was further noted that milling acidity had no effect on the development of slit openness. Salt seemed to be the only factor which could control the slit openness.

Sherwood (29) summarized his working in the relation of lactic acid culture to open texture in Cheddar cheese: (a) Open cheese evolved more CO_2 than close-bodied cheese. Lactobacilli or Betacocci could be isolated from this open cheese, (b) the most common types of organisms causing this slit openness appeared to be in the lactobacilli group.

Overcast and Albrecht (19) reported a comparison between cheeses made from various culture organisms and the degree of openness after a ripening period of 30 days at various temperatures. A summary of the results are shown in Table 4. The exhibiting of slit openness in cheese made from the commercial strains demonstrates that these strains do contain some gas producing organisms, such as L. destranicum or L. citrovorum.

Table 4. The degree of slit openness in cheese made from various cultures and ripened for 30 days at 40°F, 50°F, and 58°F

Cheese	Culture used	Degree of slit openness at ripening temperature of 40°F	50°F	58°F
1	S. Lactis (712)	-	-	-
2	Commercial cheese culture #1	***	***	****
3	S. Lactis (712)	-	-	-
4	Commercial cheese culture #1	**	**	***
5	S. Lactis (712)	-	-	-
6	S. Lactis (712) and Citrovorus	**	***	****
7	Commercial cheese culture #2	**	***	****
8	Commercial cheese culture #2 and L. Citrovorus	****	****	****

\- No evidence of slits.
* Very slight evidence of slits.
** Slight evidence of slits.
*** Moderate evidence of slits.
**** Distinct slits.

Robertson (22) reported on twenty-seven cheeses made in New Zealand as to their CO_2 content. Twenty-one were made with single strains and six with commercial mixed strains. It was noted that the cheese made from mixed cultures showed a rapid increase in CO_2 during the first two weeks of ripening, while cheese made from single strain cultures did not change in CO_2 content during this same period, but eventually did contain as much on further ripening. High concentrations of CO_2 immediately after manufacture resulted in an open texture while storage at temperatures lower than usual retarded the CO_2 formation.

Source of gas

Van Slyke and Hart (30) observed that milk sugar was the principle source of CO_2 in the cheese. The respiration processes of the micro-organisms during the early period of ripening account for the major portion of the CO_2 produced. Dorn and Dahlberg (6) concluded that CO_2 production in Cheddar cheese came from several sources; enzymatic breakdown of proteins, the organisms in the culture used for the cheese making and the dissolved CO_2 in the milk, although much of this is lost in handling and pasteurization. Yale and Marquardt (33) noted gas development in cheese containing large numbers of coliform bacteria. In pasteurized milk, recontamination was the principle source of these organisms and they would be sufficient to produce the gas defect if no attempt was made to control it.

Marshall (15) observed that definite gassy cheese was a direct result of organisms present in the milk from contamination. The Bacillus coli group were among the more numerous bacteria present and their ability to produce large volumes of gas resulted in gassy cheese. Moore and Ward (18) further observed that the gassy curds were a direct result of certain species of bacteria being introduced into the milk at the time of milking. The organism is closely related to Bacillus coli communis which is commonly found in the intestinal tract. Russell (23) noted that the organisms capable of producing gas in the cheese rapidly declined after a storage of 30 days. If storage is at 50°F or lower, these organisms are too slow in activity to cause the gassy defect. Foster, et al. (8) ascribed gassy defects in cheese to such organisms as Cl. pasteurianum, Cl. butyricum and Cl. sporogenes. Bacillus polymxa has in some cases, been found to cause gassy defects in cheese.

EXPERIMENTAL PROCEDURE

Material

Commercial lactic acid cultures from leading laboratories were used in this experiment. The mother cultures were carried in Grade A whole milk which had been heated in the autoclave to a temperature of 240°F for ten minutes. Good activity was maintained by transferring the culture daily, using one percent inoculum. The cultures were incubated at a temperature of 70°F for 12 to 16 hours. Bulk starter was prepared in one gallon stainless steel buckets using Grade A skim milk and the same method of sterilization, percent inoculum and temperature of incubation used in the preparation of the mother cultures. The starter usually developed an acidity of about 0.80 percent and possessed a smooth texture and pleasing flavor typical of good lactic acid starter.

The apparatus shown in Figure 4 was designed to give a rapid measurement for gas production and the sample from the gasometer was used to determine the lactic acid producing activity by the cultures. The basic design was developed by Elliker and associates (26).

A few modifications were made to adapt the Elliker design to our needs. Capillary tubing with an inside diameter of approximately 1.0 mm. was used in place of soft glass tubing. A ruler in centimeters was installed behind the measuring tube to measure and to add to the rigidity of the apparatus. The apparatus was welded into a 14 x 35 standard taper tube, which in turn fitted into a 20 ml. sample bottle with a standard taper ground glass mouth. Each gasometer was calibrated as follows: Mercury was drawn into the side arm (see Figure 4)

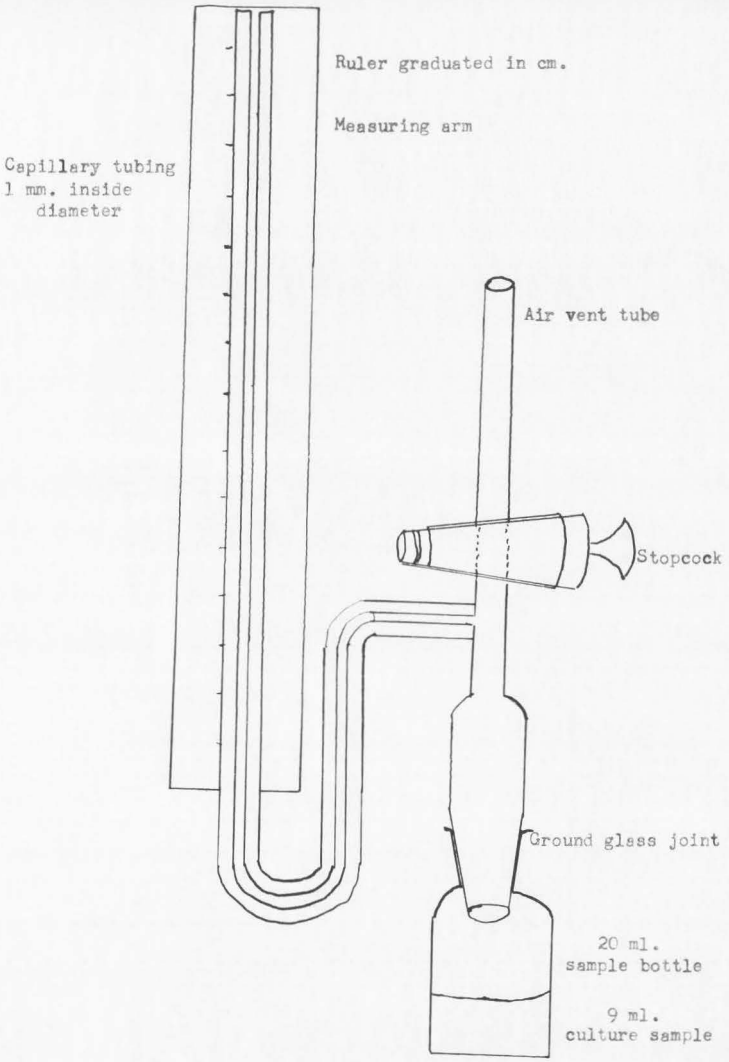


Figure 4. A re-designed apparatus to measure gas production of starter cultures

and measured for length. The mercury was then placed in a tared container. The weight of the mercury delivered per centimeter of tube length was noted, and this was converted to microliters per volume per centimeter using the density of mercury at that temperature. Gasometers calibrated in this manner were found to contain approximately 22 microliters volume per centimeter on the gas-measuring arm. This is termed the conversion figure.

Brodies solution was used as the liquid in the gas-measuring arm. It was made up as follows: 23 grams of NaCL and 5 grams of Sodium Cholate (Ox-gall) were dissolved in 500 milliliters of distilled water and the solution was colored blue with a small quantity of Evans Blue dye (Eastman Kodak). Specific gravity of the fresh solution was approximately 1.033. This solution was drawn into the measuring arm by inverting the apparatus, closing the stopcock and applying suction to the tube until it reached a mark 14 centimeters from the tip of the tube. The apparatus was then turned right side up and the solution allowed to settle in the measuring arm. The height of the solution was marked as a base point.

The medium used for testing the cultures was sterile skim milk containing nine percent of solids. Sixteen-hour cultures were inoculated at the rate of five percent into the sterile milk and 9 milliliter volumes of the inoculated milk were dispensed into the clean, dry 20 milliliter bottles. One control bottle containing 9 ml. of sterile milk was included with each experiment. The ground glass joints were fitted air tight with the use of vaseline as a seal.

Preliminary trials indicated that when the air space was small and uniform between the bottles, accurate measurements were obtained.

The assembled gasometers were attached to a piece of plywood by boring holes in rubber stoppers, inserting the stopcock tubes into the stopper through a hole in the plywood. This was mounted over a 30°C (86°F) water bath so the water level was just above the glass joint.

A period of 15 minutes was allowed for temperature adjustment of the inoculated milk, then the stopcock was closed and the apparatus was left undisturbed for four hours. Some cultures produced enough gas to force the level of the liquid over the top of the measuring tube. In this case it was necessary to record the level of the liquid at the top, and then open the vent and reset the solution, then close the vent to allow the measurement to continue.

The microliters of gas produced was determined by subtracting the increase of the control flask from the increase of the other flasks, and multiplying by the conversion factors.

The nine milliliter sample was then titrated to determine starter activity with 0.1 N. NaOH to the phenolphalein end point.

Procedure

The cultures were inoculated from the original form into the mother culture milk and transferred until the desired activity was obtained. Samples were then taken on successive days and all tests were run in duplicate for gas production. A total of six trials was made on each culture. The cultures were classified into high, medium and low gas producers with the following relation to gas volume: 0-200 microliters as low, 200-400 microliters as medium, over 400 microliters as high. This classification follows that suggested by Dr. Elliker (26). The cultures were then labeled, frozen, and stored at -15°F for use in cheese manufacture later. Samples of

these cultures were reactivated at the end of thirty days and a second series of tests were run. The cultures were again frozen and stored for 30 days and again reactivated and tested for gas production. All trials were run in duplicate.

A test for gas production was made on the cultures selected for cheese making, on mother culture, on the bulk starter, and on a sample of the milk used in the cheese manufacture. Figure 5 shows the details of the experimental procedure.

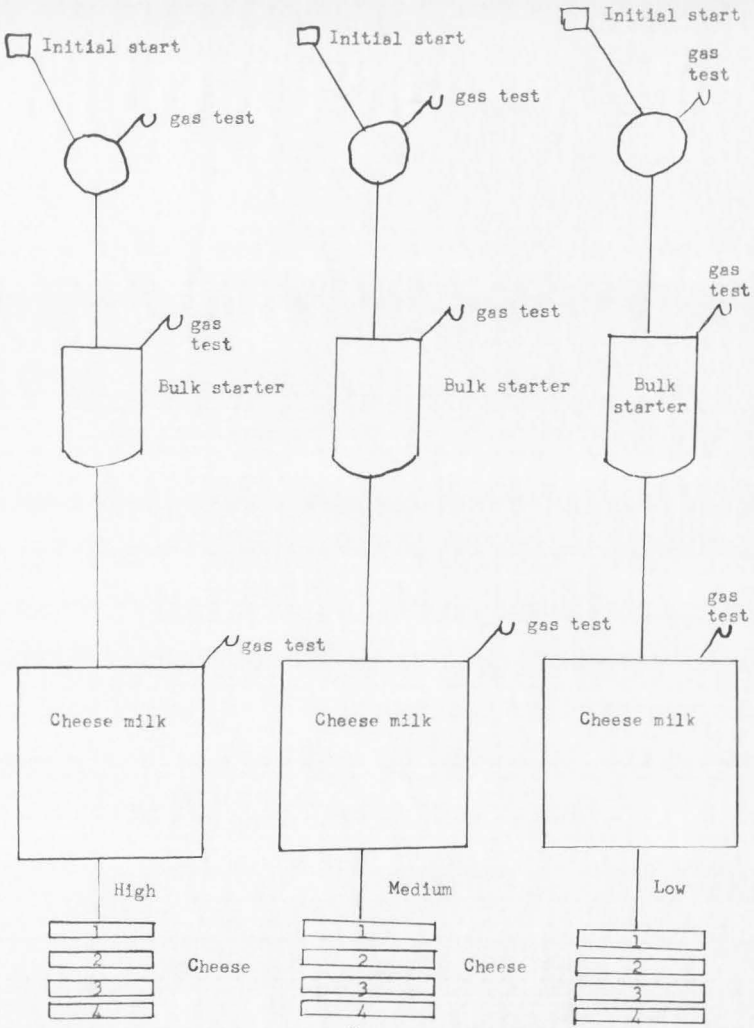
The milk used in these experiments was of good quality and was part of the regular supply of the Utah State University Dairy Plant. It was standardized to a 3.7 percent fat with freshly separated skim milk and pasteurized at 143°F for 30 minutes. The milk was then cooled to 40°F and held overnight in the pasteurizing vat. The following morning it was heated to 88°F and pumped into three cheese vats, each holding 600 pounds of milk.

Method of manufacture

Strict conformity was maintained as near as possible among all batches of cheese by the use of Wilson's (31) clock method of cheese manufacture as outlined in Figure 6.

Rennet was used in all batches at the rate of 3 oz. per 1000 pounds of milk. Salt was added to the curd at the rate of 2.5 pounds per 1000 pounds of milk, and starter was added at the rate of one percent.

The cheese manufacturing process took place in three vats using different starters in each vat. The first vat was inoculated with lactic culture which showed gas production of 200 microliters or less; the second with starter showing from 200-400 microliters; and the third was inoculated with starter exhibiting over 400 microliters of gas.



No. 1 Stored for 10 days at 40°F analyzed for pH, moisture and stored for body, texture and flavor.
No. 2 Stored 30 days at 40°F - repeat analysis.
No. 3 Stored 30 days at 50°F - repeat analysis.
No. 4 Stored 30 days at 60°F - repeat analysis.

Figure 5. Steps in starter preparation and cheese manufacturing

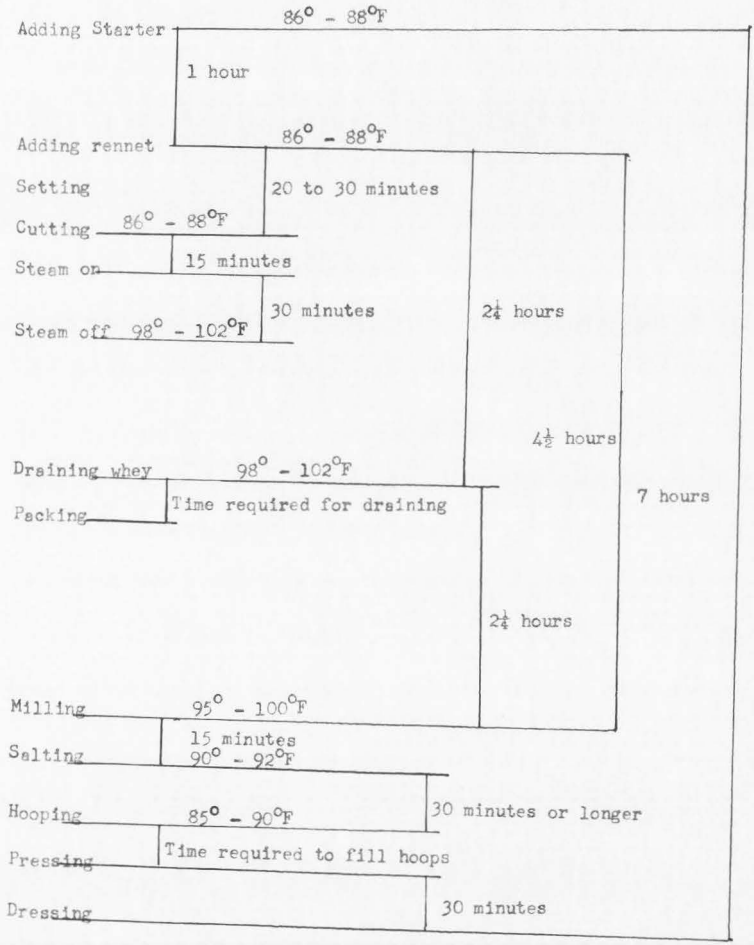


Figure 6. Time schedule for making Cheddar cheese

During the manufacture of the cheese, the rate of acid development in each of the three vats of cheese was carefully noted. The acid development was measured by determination of the titratable acidity expressed as percent lactic acid, and the pH as determined by the Beckman pH meter with a Calomel and glass electrode. The titratable acidity of the milk was measured before adding the starter, and at the time of adding the rennet. The titratable acidity and pH of the whey was taken at the time of cutting the curd, draining the whey, and milling the curd. Any noticeable differences between the appearance of the curd during cheddaring which may have been due to the different starters were observed and recorded.

The curd from each vat was placed in 20 pound square hoops. The curd was pressed for a minimum of 30 minutes before being removed from the press for dressing. One hoop from each vat was cut into four 5-pound loaves, which were then individually wrapped in rayon cheese clothes. The loaves were replaced in the hoops and pressed overnight.

Upon removal from the press, the loaves were wrapped in parakote wax wrapping, and again pressed for a minimum of 30 minutes. The loaves were then removed from the press and placed in a curing room at 40°F.

Nine separate trials consisting of three vats of cheese each were conducted in the manner described.

Method of analysis

The cheese was analyzed when ten days old. Samples were taken for analysis from the end of the loaves in such a manner that the plugs were evenly spaced, and the trier penetrating to the center of the cheese. Both ends of the loaves were used for sampling.

The pH of the cheese was determined by the Beckman pH meter. The sample was ground to a smooth paste with a motar and pestle and placed in contact with the electrodes of the meter.

The moisture content was determined in the manner described by the A O A C (3). Two to three grams of the prepared sample was weighed into a round flat-bottomed metal dish, provided with a close-fitting cover. The dish was placed in a vacuum oven and held at 100°C under a vacuum of 20" - 21" for four hours, weighed again and the loss of weight was expressed as percent of moisture.

The percent of fat in the cheese was determined by a modified Babcock method as recommended by Wilster (32), with the exception that acid having a specific gravity of 1.84 was used, and the test bottles were not placed in boiling water.

At the end of 10, 21, and 30 days the cheese was judged and scored for flavor, body, and texture. The standards on the cheese score card of the American Dairy Science Association were adhered to by the judges. The judging was done by two experienced dairy products judges from the staff of the Dairy Department at Utah State University.

RESULTS AND DISCUSSION

Starter classification

The results of the tests for the gas producing ability of several strains of commercial lactic acid cultures showed that certain strains have an inherent ability for high gas production. Others have varying ability for gas production. A group of twelve cultures were selected for the first phase of this study. Each of these cultures were tested for gas production and starter activity in six separate trials, each time in a different apparatus. The results of these trials are shown in Tables 5 and 6. The average amount of gas produced ranged from a low of three to a high of 925 microliters.

A second series of gas production and activity tests were run on some of these same starters along with some new cultures. These results are shown in Table 7. It was observed that upon reactivation following freezing, the gas production was lower, but upon more transfers, the gas production soon increased. Starter G was the only exception in that it produced considerably larger quantities of gas upon reactivation than before being frozen.

The third series of tests included some new cultures as well as a group of mixed strains made from combinations of single strains already being tested. These tests were run in duplicate and results are shown in Tables 8, 9, 10, and 11.

From the results of these trials, the cultures were classified as to their gas producing ability. Those cultures producing over 400 microliters of gas were classified as high gas producers, those

Table 5. Microliters of gas produced from selected commercial single strain lactic acid starter cultures

Culture	Trial Number						Ave.
	1	2	3	4	5	6	
F	10	30	50	30	30	50	33
J			950			900	925
A	70	50	70	10	100	70	62
G	10	50	20	0	0	0	13
I	0	0	10	0	10	0	3
E	500	360	540	510	500	570	496
K	310	340	326	300	340	335	325
L	15	10	8	10	0	0	7
M	0	10	0	0	15	0	4
N	208	215	210	240	200	210	212
O	650	700	675	710	670	680	680
P	475	480	460	490	480	490	480

Table 6. Acidity for selected commercial single strain lactic acid starters expressed as percent lactic acid[a]

Culture	Trial Number						Ave.
	1	2	3	4	5	6	
F	.49	.55	.40	.45	.30	.42	.43
J	.51	.47	.63	.56	.48	.52	.53
A	.56	.59	.61	.70	.66	.61	.62
G	.56	.62	.59	.69	.61	.63	.61
I	.50	.56	.63	.70	.56	.58	.59
E	.52	.62	.66	.65	.60	.61	.61
K	.48	.49	.42	.48	.47	.49	.44
L	.51	.47	.46	.52	.51	.49	.49
M	.46	.49	.51	.52	.47	.50	.49
N	.56	.53	.57	.54	.54	.55	.55
O	.61	.57	.59	.56	.57	.60	.58
P	.56	.51	.52	.53	.57	.60	.54

a Incubated four hours at 30°C.

Table 7. Gas production and acidity of single strain lactic acid starter cultures after three transfers

Culture	Trial Number 1	2	Acidity[a]	Classification
J	450	455	.49	high
I	10	0	.51	low
F	0	0	.50	low
E	210	205	.54	medium
G	190	190	.46	low
D	88	88	.56	low
C	375	375	.57	medium
B	390	380	.40	medium

a Incubated four hours at 30°C.

Table 8. Gas production and acidity of single strain lactic acid starter culture after four transfers

Culture	Trial Number 1	2	Acidity[a]	Classification
J	950	925	.44	high
I	0	0	.51	low
F	10	10	.44	low
E	300	300	.51	medium
G	210	210	.48	medium
D	75	70	.51	low

a Incubated four hours at 30°C.

Table 9. Gas production and acidity of selected commercial single strains of lactic acid starter cultures

Starter Number	Trial Number 1	Trial Number 2	Acidity[a]	Classification
Q	221	230	.49	medium
Q	288	310	.46	medium
R	0	10	.56	low
R	44	60	.56	low
S	0	0	.53	low
S	0	0	.50	low
I	22	30	.45	low
I	0	10	.42	low
J	600	615	.30	high
J	644	650	.30	high
G	233	250	.31	medium
G	200	190	.27	medium

a Incubated four hours at 30°C.

Table 10. Gas production and acidity of selected commercial single strains of lactic acid starter cultures

Starter Number	Trial Number 1	Trial Number 2	Acidity[a]	Classification
F	22	10	.31	low
R	22	32	.33	low
I	22	20	.40	low
J	688	700	.32	high
V	88	90	.29	low
Q	222	210	.37	medium
U	422	450	.30	high
T	222	225	.31	medium
V	22	10	.42	low
G	150	180	.26	low

a Incubated four hours at 30°C.

Table 11. Gas production and acidity on selected mixed strains of lactic acid starter cultures

Starter Number	Trial Number 1	2	Acidity[a]	Classification
1	33	25	.52	low
2	40	50	.54	low
3	4	0	.47	low
4	18	5	.16	low
5	44	50	.38	low
6	0	10	.43	low
7	40	50	.45	low
8	0	0	.41	low
9	150	190	.52	low
10	30	20	.48	low
11	0	0	.49	low

a Incubated four hours at 30°C.

producing 200 to 400 microliters were classified as medium gas producers, and those producing less than 200 microliters were classified as low. Table 12 shows the classification of all the cultures tested. From this information a culture from each class was chosen to use in the manufacture of Cheddar cheese. Culture J was selected for high, culture G was selected for medium, and culture F was selected for low.

The results of the gas tests taken during the steps of starter preparation and cheese manufacture are shown in Tables 16 to 21, which are included in the appendix. It is to be noted that the cultures produced similar amounts of gas at each step as was exhibited in actual trials, demonstrating a consistency of gas production for each strain once it has been classified.

Manufacture of Cheddar cheese

The results of the preliminary work concerning the use of starters of varied gas production showed a definite relationship between the gas produced by the starter and the degree of openness exhibited in the cheese at ten days.

The cheese made from low gas producing cultures was of fine flavor, and generally good body and texture. The cheese made from the medium gas producing organisms were all of good flavor but were criticized for gas in the mechanical openings. All cheese made from the high gas producing cultures were noted to have a definite fruity flavor, and criticized for gas holes and gassiness in the mechanical openings. The scoring of the cheese is recorded in Table 13.

Further trials demonstrated that cheese made from low gas producing cultures was definitely A Grade and that made from

medium gas producing strains was generally B Grade. Cheese made from high gas producing strains were B Grade or lower due to the late gas defect. The results of these trials are shown in Table 14.

Vat Y of Lot D was criticized for high acid due to an excess of acid at milling. All other vats were of the proper acidity at milling.

Analysis of cheese

The amount of moisture, fat and pH seemed to have no effect upon the defect studied. The results of analysis are shown in Table 15.

It may be observed from the attached photograph, the difference in the body of the cheese made with cultures of low gas production and that made with cultures producing high gas. Sample 1 was made with low gas producing cultures, while sample 2 was made from the high gas producing strain.

Table 12. Classification of cultures according to gas production key to cultures

Original Culture Identity	Experimental Culture No.	Rating Based on Gas Production
11	A	low
15	B	medium
18	C	medium
20	D	low
22	G	high
23	F	low
25	G	medium
32	I	low
33	J	high
M L	K	medium
H P	L	low
K	M	low
F-8	N	medium
1	O	high
2	P	high
D L-4	Q	medium
D L-7	R	low
D L-9	S	low
M D-2	T	medium
M D-4	U	high
M D-9	V	low
Mixtures	1	low
	2	low
	3	low
	4	low
	5	
	6	
	7	
	8	
	9	
	10	
	11	

Table 13. Flavor, body and texture scores for cheese made for first series

Lot No.	Vat X		Vat Y		Vat Z	
	Flavor	Body and Texture	Flavor	Body and Texture	Flavor	Body and Texture
A	40	29.5	40	28 Mech. openings with gas	38 Slight fruity	27 Mech. openings with gas Gas holes
B	40	29.5	40	28 Mech. openings with gas	38 Slight fruity	26.5 Mech. openings with gas Gas holes
C	39 High acid	27 Mealy	40	28 Mech. openings with gas	37 Fruity	27 Mech. openings with gas Gas holes

Table 14. Flavor, body and texture scores for cheese made for second series

Lot No.	Vat X		Vat Y		Vat Z	
	Flavor	Body and Texture	Flavor	Body and Texture	Flavor	Body and Texture
D	39 High acid	29.5	38 High acid	29.5	40	28 Mech. openings with gas Gas holes
E	40	29.5	40	29 Mech. openings with gas	39.5 Slight fruity	28 Mech. openings with gas Gas holes
F	38 Slight acid	29 Slightly open Slightly mealy	40	29 Mech. openings with gas	39 Slight ferm. Acid	28 Mech. openings with gas Gas holes

Table 15. Moisture, pH and fat values of 18 vats of Cheddar cheese cured at 45°F for ten days

Vat No.	Percent Moisture		pH Value		Percent Fat	
	1	2	1	2	1	2
AX	36.2	36.3	5.14	5.13	31.5	31.6
AY	36.8	36.8	5.03	5.03	31.2	21.1
AZ	36.4	36.5	5.10	5.12	31.0	31.0
BX	37.1	37.1	5.08	5.08	31.5	31.6
BY	36.9	36.9	5.11	5.11	32.0	31.9
BZ	37.0	37.0	5.16	5.17	31.0	31.0
CX	38.0	37.9	5.09	5.09	32.0	32.0
CY	36.8	36.8	5.10	5.09	31.8	31.7
CZ	36.6	36.6	5.14	5.13	31.6	31.5
DX	36.3	36.4	5.06	5.05	31.4	31.4
DY	37.7	37.8	5.09	5.09	32.1	32.2
DZ	37.0	37.0	5.08	5.07	31.4	31.4
EX	36.9	36.9	5.12	5.11	31.5	31.6
EY	36.8	36.8	5.07	5.07	31.7	31.7
EZ	37.1	37.1	5.10	5.10	32.0	31.9
FX	36.2	36.2	5.09	5.09	31.8	31.8
FY	37.1	37.1	5.01	5.01	31.4	31.4
FZ	37.7	37.8	5.04	5.04	31.2	31.2

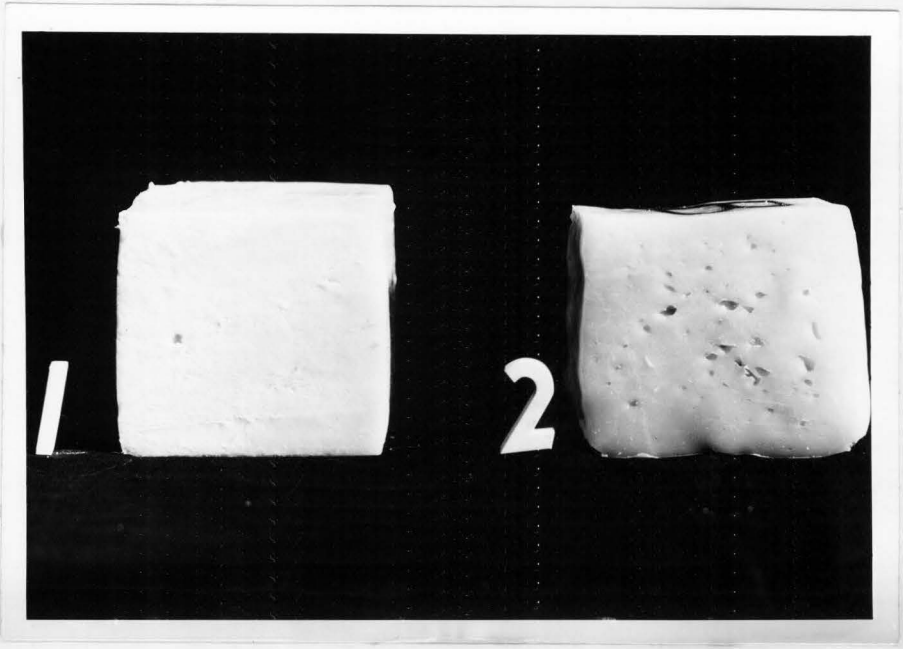


Figure 7. Samples of Cheddar Cheese made from High and Low Gas Producing Cultures
1. Low gas culture 2. High gas culture

SUMMARY

Twenty-one single strain lactic acid cultures and eleven cultures of mixed strains were tested and classified according to the volume of gas produced. Those producing 0 to 200 microliters were classified as low gas producers, those evolving 200 to 400 microliters were classified as medium, and those cultures producing over 400 microliters were classified as high.

The activity of starters were expressed in terms of percent lactic acid produced with five percent inoculation in four hours of incubation at 30°C. A nine milliliter sample was used for all tests. It was found that all the starters tested were quite uniform in activity.

Six lots of milk with three vats in each lot were made into Cheddar cheese. One vat of each lot was made using cultures of low gas production, a second vat was made using cultures of medium gas production, and the third vat contained starters of high gas producing ability. The clock method of manufacture was followed in all vats.

The cheese was scored for flavor, body and texture, moisture, fat, pH and color.

This report indicates that with the milk used it is possible to produce cheese of uniform high grade by testing and selecting cultures that are low gas producers. This selection program includes the use of a gasometer along with tests for the activity of the cultures.

CONCLUSIONS

The study and analysis of this problem leads to the following conclusions:

1. The use of the gasometer is a satisfactory method of establishing the gas producing ability of lactic acid starter cultures.

2. The gasometer as designed by Elliker (26) was modified in order to increase its rigidity and make it more adaptable for plant as well as laboratory use.

3. Lactic acid cultures can be classified according to their gas producing ability. Those producing under 200 microliters were considered to be low gas production, those producing 200 to 400 as medium, and over 400 as high.

4. The activity of all starters tested showed very close uniformity.

5. Freezing the cultures had a tendency to lower their gas producing ability for the first two or three transfers following reactivation, after which the gas production was as high or higher than before freezing. The gas analysis of a culture is an important step in selecting starters for freezing and cheese-making.

6. Cheddar cheese manufactured from cultures classified as low gas producers was judged to be A Grade and free from late gas.

7. Cheese manufactured from cultures from the medium gas producers exhibited slight evidence of gas in mechanical openings

with no off flavor, but was generally given a B Grade.

8. Cheese manufactured from high gas producing cultures demonstrated definite gas production in mechanical openings and formation of some gas holes. This was accompanied by some off flavors such as fruity or fermented. The cheese was definitely B Grade or lower.

9. This system of starter classification can be adapted to a general selection program for cheese manufacturing plants.

10. Two commercial factories, after inaugurating this program increased the vats of A Grade cheese produced in one month from ten percent A Grade to 90 percent A Grade.

11. The pH, moisture, and fat content of the cheese of this study seemed to have no effect upon the late gas defect.

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APPENDIX

Table 16. Gas production of starters during steps of manufacture. Lot A

Classification	First Transfer	Second Transfer	Bulk Starter	Cheese Milk
Low	20	0	40	20
	20	20	50	20
Medium	270	220	200	230
	290	230	210	220
High	440	500	410	410
	450	510	410	400

Table 17. Gas production of starters during steps of manufacture. Lot B

Classification	First Transfer	Second Transfer	Bulk Starter	Cheese Milk
Low	20	0	10	0
	20	10	20	10
Medium	250	310	310	300
	250	300	320	310
High	600	590	570	560
	590	590	565	560

Table 18. Gas production of starters during steps of manufacture. Lot C

Classification	First Transfer	Second Transfer	Bulk Starter	Cheese Milk
Low	150	160	190	180
	155	155	180	180
Medium	230	220	230	240
	250	225	235	235
High	410	450	450	470
	415	460	460	465

Table 19. Gas production of starters during steps of manufacture. Lot D

Classification	First Transfer	Second Transfer	Bulk Starter	Cheese Milk
Low	0	0	20	10
	15	0	30	30
Medium	285	310	290	320
	300	300	310	315
High	725	750	690	710
	700	740	710	695

Table 20. Gas production of starters during steps of manufacture. Lot E

Classification	First Transfer	Second Transfer	Bulk Starter	Cheese Milk
Low	25	0	0	25
	35	20	10	40
Medium	200	240	290	210
	220	210	260	200
High	650	690	610	625
	680	645	630	650

Table 21. Gas production of starters during steps of manufacture. Lot F

Classification	First Transfer	Second Transfer	Bulk Starter	Cheese Milk
Low	0	10	15	30
	10	40	0	10
Medium	310	300	210	250
	290	280	250	275
High	710	685	790	780
	750	710	765	765