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Bacteriological studies on some defects of cream cheese spreads

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BACTERIOLOGICAL STUDIES ON SOME DEFECTS OF CREAM CHEESE SPREADS

by

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INTRODUCTION

The manufacture of cream cheese spreads is a relatively new branch of the dairy industry. As with various other dairy products, certain bacteriological defects have appeared in cream cheese spreads and these require study in order to develop control methods.

Cream cheese spreads consist of cream cheese to which various materials have been added to give a variety of flavors. The mixtures are heated to a relatively high temperature for a short time and packed in glass jars which are often vacuum sealed. Since the products are packed hot and commonly sealed under a vacuum, it would be expected that any bacteriological defects which developed were probably caused by anaerobic spore forming organisms.

Gas formation and liquefaction are the defects which have been most common in cream cheese spreads. In the past, neither of these defects has caused serious economic loss. However, the sale of one jar of defective spread may result in the loss of the customer. For this reason an investigation was made in an attempt to determine the nature of the organisms responsible for these defects and to develop methods for preventing the occasional appearance of a defective jar of spread.

STATEMENT OF PROBLEM

The purposes of this investigation were:

1. To isolate and study the organism or organisms causing gas formation in cream cheese spreads and to develop a method of preventing the defect commercially.

2. To isolate and study the organism or organisms causing liquefaction in cream cheese spreads and to develop a method of preventing the defect commercially.

REVIEW OF LITERATURE

Rodella (15) found anaerobic bacteria present in milk in numbers from 1 to 3 per 0.1 cc. (10 to 30 per cc.). Wolff (20) isolated anaerobic bacteria from 1 cc. portions of milk but only obtained them once from a 0.1 cc. sample.

Barthel (3), in discussing the occurrence of obligate anaerobic bacteria in milk, reported that they are almost always present in small numbers in milk handled in the ordinary manner, but that he was often unable to obtain them from 15 to 20 cc. samples. He stated that, in general, these anaerobes can be divided into two groups; the butyric acid bacteria of Schattenfroh and Grassberger and Bacillus putrificus (Bienstock). He concluded that no direct relationship existed between the hygienic nature of milk and the presence of obligate anaerobes.

Weinzirl and Veldee (18) suggested the use of Clostridium sporogenes as an index of manural pollution in milk since it can be employed with either raw or pasteurized milk. The method of determination advocated by them consisted of sealing 5 or 10 cc. of milk in a test tube with paraffine and heating to 80° C. for 10 minutes. After incubation, digestion with gas indicated the presence of Clostridium sporogenes, while digestion without gas indicated the presence of Bacillus vulgaris. Weinzirl and Veldee estimated by this method that from 33 to 35 Clostridium sporogenes cells are present per liter of average milk.

Wyant and Hormington (21) reported that the growth of the spores of Clostridium botulinum was not inhibited by salt concentrations from 1 to 10 per cent in an alkaline medium with a reaction of -0.5 per cent, but that the spores were not nearly so resistant in an acid medium.

Weiss (19) found that sodium chloride lowered the heat resistance of Clostridium botulinum spores and that as the salt concentration increased from 0.85 to 30 per cent the heat resistance decreased quite rapidly; however, he did not report any control culture in which no salt was added, so it is not evident how the lowest concentration compared with no salt.

Esty and Meyer (10), in a study on the heat resistance of the spores of Clostridium botulinum, showed that from 0.5 to 1 per cent sodium chloride increased the heat resistance of the spores, but that the protective action was lost at 2 per cent. From 2 to 8 per cent sodium chloride had little or no effect, but concentrations above 8 per cent greatly decreased the heat resistance. A phosphate buffer mixture also exhibited a striking protective action.

Viljoen (16) studied the effect of sodium chloride on the thermal death rate of bacterial spores in pea liquor and found that the maximum protective action of sodium chloride was in the range of 1 to 2.5 per cent. A 3 per cent solution had a slight protective action, but as the concentration was increased above this point there was a marked increase in the toxicity of the salt.

Matheson (14) stated before the World's Dairy Congress in 1923 that he had isolated a spore forming gas producing anaerobe from a Nissler

cheese and that it would reproduce the defect when it was inoculated into milk used to make Swiss cheese. He reported that the organism could not be checked by high heat or any other method which he tried. At the same meeting, Burri (4), in his paper on the relation of silage feeding to Swiss cheese made from the milk produced, stated that the butyric acid bacilli usually entered milk and cheese in the form of spores, and that the spores in cheese did no harm, but upon germination produced the very undesirable products, gas and butyric acid. He stated further:

> "Similar experiences are had, at least in Switzerland, with Bacillus putrificus. This organism possesses a very dangerous characteristic, inasmuch as it can, with complete exclusion of air, dissolve the casein and thus separate out the strong-smelling and strong-tasting by-products of metabolism. The spores of these bacilli are widely spread in nature; and inasmuch as they are contained in the feces of domestic animals, it is not surprising to find them in milk and cheese. In our own experiments with Emmental cheese they were found regularly, but in small numbers. It is important and fortunate that they are not able to grow and reproduce except under especially favorable conditions. Now and then, but only seldom, certain cheeses from a creamery show defects, namely, parts of the inside appear white and crumbly, with large irregular holes, and have a bad taste and smell which permeate the good parts. The bacteriological examination of such cheese, as a rule, shows the presence of spore-forming Bacillus putrificus and such cases were at once called putrificus cheese. Under what conditions the usually harmless putrificus bacilli ferment in the cheese and cause bad results is yet to be determined."

In studies on a strong acid and bitter flavor in pimento cheese, Warren (17) found that nine samples of the defective cheese contained Clostridium butyricum; the cheese was a soft, unripened cheese of the Neufchatel type. The acid and bitter flavor were always accompanied by an increase in the volatile acid of the cheese. Warren was able to re-

produce the defect experimentally by inoculating normal cheese with Clostridium butyricum. He noted that heat shocked spores produced a more active fermentation than unheated spores and that for this reason pasteurization merely acted as a stimulant to the growth of the organism. Warren also found that the action of Clostridium butyricum and Streptococcus lactis in combination was greater than the action of the anaerobe alone.

Albus (1) reported the isolation of a non-pathogenic strain of Clostridium welchii from a Swiss cheese which showed abnormal gas formation. Experimental cheese made from milk inoculated with this organism developed both "niszler" and "pressler" types of fermentation.

Albus and Ayers (2) reported an outbreak of gassiness in pimento cheese. They found that the gassiness could be prevented by removing the carbohydrates from the pimentos. They stated:

> "Certain anaerobic spore-forming bacteria are usually responsible for the gassy fermentation in reheated cheese products to which pimentos have been added to obtain variety. The source of these bacteria may be from the cheese from which the product is made. Spore-forming bacteria have been frequently isolated from cheese and in the spore state these bacteria survive the temperatures of processing. Because of the destruction by the heating process of practically all of the other types of organisms in the cheese, germination and subsequent growth of the anaerobes proceeds with little competition. Certain cocci which are known to survive the heating process multiply quite rapidly in the finished cheese product but these apparently do not interfere with the development of gas-producing bacteria."

They stated further:

> "It is possible, then, to protect reheated or processed cheese products from contamination with gas producing bacteria from every source except the cheese itself. Contamination

> from this source might also be controlled and gassy fermentation prevented if it were economically possible to use only the best quality of cheese. Inasmuch as a certain amount of poor quality cheese is always used, the possible presence of gas-producing bacteria must be accepted. Sterilization of the finished product is obviously impossible. It remains, therefore, to render the product less favorable as a media for the development of the gas-producing bacteria if the fermentation is to be prevented. This was attempted by removing the fermentable carbohydrate from the pimentos. In the absence of a fermentable sugar a gassy fermentation is not likely to take place. Certain anaerobic gas-producers can utilize lactates with the production of gas; but this rarely occurs unless very young cheeses are used."

Irwin and Harrison (13) studied the bacteriology of process cheese. They found Micrococcus varians to be the most common type of organism in this product. A very small portion of the organisms in the process cheese were spore producers. Bacillus subtilis, Bacillus cerus, Bacillus mycoides and Bacillus mesentericus were the four spore forming bacteria most commonly present. In a sample of pimento process cheese, 94.8 per cent of the organisms were found to be Streptococcus lactis, even though the cheese was supposed to have been heated to 68° C. for 10 minutes.

Hussong and Hammer (11 and 12) reported that they found anaerobes to be relatively common in dairy products, and suggested that the rapid development of anaerobes in milk in which conditions have been made favorable for their growth indicates that the restraining action of various factors is important in preventing growth under practical conditions.

Csiszar (6), in 1931, stated that the organisms most commonly found in process cheese are the same as those found in raw cheese, namely, the

streptococci and lactobacilli. Other types which he found to be common are the butyric acid organisms, the aerobic hay and potato bacilli, heat resistant cocci, rods, yeasts, and molds. He stated further that the numbers and kinds of organisms depend upon the raw cheese, the temperature and duration of processing, and the age of the process cheese. The counts obtained on process cheese varied from 0 to 310,400,000 per gram and averaged 19,500,000. Csiszar found that the bacterial counts were reduced from 99.9 to 100 per cent by heating at 65° to 80° C. for from 5 to 25 minutes. Immediately after process cheese was made there was a very rapid increase in the numbers of organisms in it.

In 1932, Csiszar (8) reported a study of the spore forming bacteria associated with swelling in process cheese. He found that Bacillus sporogenes, Bacillus putrificus and Bacillus saccharobutyricus are the organisms most commonly associated with the defect and that, in 94 per cent of the cheese examined, the defect was caused by Bacillus sporogenes. In the same year Csiszar (7), stated that in most cases of gas development in process cheese the causative organism was one of the three mentioned above, and that the organisms causing gas formation in cheese made from raw milk scarcely ever give any trouble in process cheese due to the high temperatures employed in the processing.

In 1933, Csiszar (9) reported data on the resistance of Bacillus sporogenes, Bacillus putrificus and Bacillus saccharobutyricus to heat, acid, and preservatives. He gave the following results on heat resistance:

Organism	Time of survival at				
	60° C.	70° C.	80° C.	90° C.	100° C.
Bacillus sporogenes	72 hrs.	24 hrs.	2.5 hrs.	50 min.	6 min.
Bacillus putrificus	70 min.	60 min.	50 min.	50 min.	6 min.
Bacillus saccharobutyricus	90 min.	80 min.	80 min.	50 min.	6 min.

Csiszar concluded that since the high exposures shown above cannot be used without influencing the quality of the cheese it is not possible to destroy the organisms in process cheese by heat alone. He found the minimum pH values permitting germination of the spores of the three species to be:

Bacillus sporogenes	4.83
Bacillus putrificus	4.88
Bacillus saccharobutyricus	4.88

Since cheese does not melt normally when the pH is as low as the values given, but becomes a granular pasty mass, Csiszar concluded that the regulation of the pH could not be used as a means of controlling the organisms. He included in this work a study of several preservatives, all of which failed to give satisfactory results, and only one of which, sodium chloride, could be used legally in the United States. He reported that it took from 7 to 8 per cent sodium chloride to inhibit the growth of spore bearing organisms, and since such a concentration is too high for commercial use, he concluded that sodium chloride could not be used to prevent gas production in process cheese.

Corbett, Frazier and Price (5) presented a paper on a gassy defect in cream cheese at the meeting of the American Dairy Science Association in 1935. They found the causative organism to be a sucrose fermenting yeast.

GENERAL METHODS

pH determinations

The pH determinations were made electrometrically, using a quinhydrone electrode.

Sealing the cultures to exclude air

Many of the cultures were sealed with a paraffine-vaseline plug about 1 in. thick to exclude air. The paraffine-vaseline mixture was made up of approximately 50 per cent paraffine and 50 per cent vaseline, and brought to a boil just before it was placed on the medium by means of a sterile pipette. In most cases the medium was heated just prior to inoculation or was heated after inoculating and sealing.

Preparation of peptone-litmus milk

Peptone-litmus milk was skim milk to which litmus and 0.5 per cent peptone were added. The peptone-litmus milk was sterilized in an autoclave at 15 lbs. pressure for 20 minutes.

Testing the action of an organism in cream cheese spread

In order to prove that an organism isolated from defective cheese spread was the causative agent, it was necessary to reproduce the defect in sterile cheese spread which was similar in general composition to the spread originally showing the abnormality. The spread used in testing the action of various organisms had the following composition:

600 gm. of unsalted cream cheese

350 gm. of grated imported Roquefort cheese

150 gm. of grated domestic blue cheese

30 gm. of sodium chloride

The ingredients were placed in a double boiler and heated and stirred until they were thoroughly mixed. About 20 gm. were then placed in each of a number of large test tubes, and the tubes plugged with cotton and sterilized in an autoclave at 15 lbs. pressure for 20 minutes. After the cheese spread had cooled each tube was inoculated with 5 cc. of a 48-hour peptone-litmus milk culture of the organism to be tested. The inoculum was mixed thoroughly with the cheese spread by means of a sterile pipette and the tube was sealed with a paraffine-vaseline plug. The sealed tubes were incubated at 37° C. for 8 to 10 days and observed frequently for changes.

Preparation of blue cheese emulsion

The blue cheese emulsion which was used to study the effect of pH in the heat resistance of the gas producing organisms was prepared by heating 1000 gm. of grated domestic blue cheese, 1000 cc. of water, and 40 gm. of sodium citrate. The mixture was heated and stirred in a double boiler until a smooth consistency was obtained. The emulsion was sterilized in 1000 cc. portions in an autoclave at 15 lbs. pressure for 20 minutes.

Determination of the heat resistance

All of the heat resistance studies were carried out in test tubes which had an outside diameter of about 18 mm. and walls about 1 mm. thick. Approximately 10 cc. of an inoculated medium were put in each test tube and covered with a paraffine-vaseline plug. Care was taken in placing the medium in each tube to avoid leaving any of it near the top of the tube where it would not be covered by the plug. The cotton plug that was originally present in the tube was returned to the tube after the paraffine-vaseline plug was placed over the medium. During the heating process, the tubes were held in an upright position in an agitated water bath, the water being deep enough to cover the medium and the paraffine-vaseline plug. The water bath was at the temperature to be used when the tubes were placed in it. The tempera-

ture of the medium was determined by a thermometer in an uninoculated tube which was covered with a paraffine-vaseline plug in the same manner as the inoculated tubes. As soon as the tubes had been held at the desired temperature for 20 minutes, they were placed in a cold water bath till cool. They were then incubated at 37° C. for at least a week to determine whether or not the organisms were able to reproduce the defect in the medium.

EXPERIMENTAL

Studies on Gas Production in Cream Cheese Spreads

Description of the defect. The outbreak of gas formation in cream cheese spreads which was responsible for the investigation occurred in the spring and summer of 1954. Gassiness was observed to a limited extent in several varieties of spread, but the defect was particularly noticeable in Roquefort type spread. At the time the defect first appeared the particular plant was substituting domestic blue cheese for a part of the imported Roquefort cheese which they had previously used entirely and the gas production was attributed to organisms present in the domestic blue cheese that were not present in the Roquefort cheese.

Only a small percentage of the jars in a batch developed gas. Under ordinary marketing conditions the cheese spreads were held at room temperature and at times would remain normal for 2 or 3 months and then suddenly develop gas. Probably this irregularity was largely due to the temperature at which the cheese spread was held and to the fact that the extent of inoculation varied from jar to jar. When the cream cheese spread was held at 37° C. gassiness usually developed in 5 to 10 days if it developed at all.

The gassy cheese spreads had no off flavor or odor and appeared normal in every way except that gas was produced in the cheese. No typical gas holes were apparent but the spread, inside the glass jar, would break and the upper portion would be forced away from the lower portion with a clean break. In extreme cases the lid would be blown off and approximately half of the cheese would be pushed out of the container. Even in these instances, however, off flavor and odors were not present. In less extreme cases only enough gas would be produced to release the vacuum sealed lid and the gas would appear in the body of the cheese as small horizontal splits.

Microscopic examinations of the cream cheese spreads showed an abundance of various types of organisms, including bacteria, molds, and yeasts. This was to be expected since the product was a mixture of cream cheese and Roquefort type cheese, both of which contain large numbers of streptococci and the Roquefort type cheese would contribute mold mycelia and spores, bacteria and probably yeasts. No doubt most of the organisms seen in the microscopic preparations were dead, due to the fact that the spreads had been exposed to about 80° C. for 10 minutes and placed immediately in the glass jars.

Isolation of the gas producing organism. From the nature of the defect and the manner in which the spreads were prepared and packed, it seemed probable that the defect was the result of the activity of an anaerobic spore forming organism. Attempts were made to grow the organism in or on a variety of media and under various conditions. The media employed were: Standard beef extract agar, beef infusion agar, beef infusion agar plus 0.5 per cent dextrose, beef infusion agar plus 0.5 per cent lactose, tomato juice agar, standard broth, standard broth plus each of 15 carbohydrates and related materials, brain infusion medium, and an agar prepared from the extract of Roquefort cheese. Aerobic cultures failed to give growth so a number of different methods of obtaining anaerobic conditions were tried. These included: Agar shake cultures, an atmosphere of carbon dioxide, an atmosphere of hydrogen, removal of oxygen by alkaline pyrogallol, and the addition of reduced iron. Most of these methods were employed with a number of media but none of them resulted in growth.

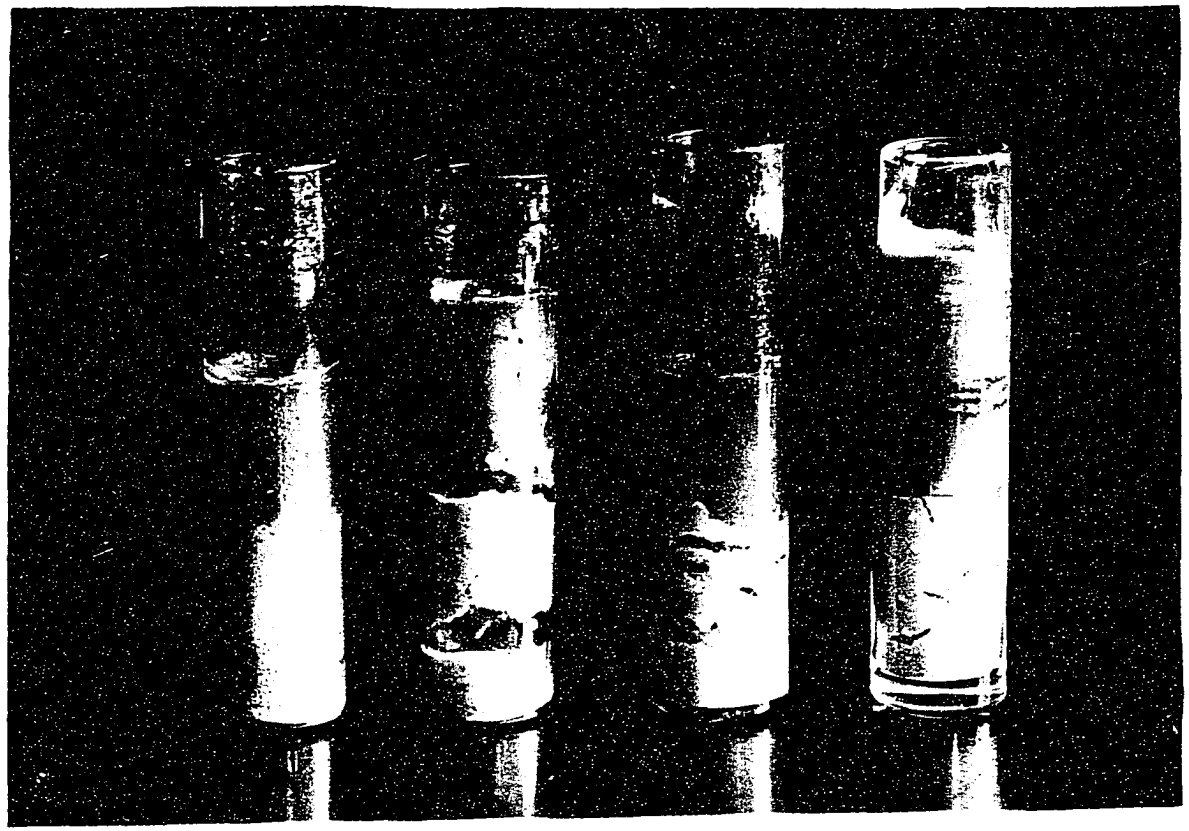
Approximately 0.5 gm. portions of the gassy cheese spreads were placed in tubes of peptone-litmus milk and sealed with paraffine-vaseline plugs. The tubes were then held in a water bath for 20 minutes at 85° C., cooled quickly and incubated at 37° C. for 3 or 4 days. Practically all of the tubes which showed any growth gave rapid reduction of the litmus and gas production without coagulation.

The organisms which developed in the peptone-litmus milk failed to grow on various solid media, so it was necessary to use the dilution method for purification. A few drops of a culture which was to be purified were removed by means of a capillary pipette. This was done by pushing the pipette through the paraffine-vaseline plug, breaking of the end, which was filled with the paraffine-vaseline mixture, against the bottom of the test tube and drawing the culture into the pipette. The material was placed in a tube containing about 9 cc. of peptone-litmus milk. The inoculum was thoroughly distributed and 1 cc. of this mixture transferred to the next tube of peptone-litmus milk. This process was repeated until a series of 10 tubes was obtained, each having about one-tenth as heavy an inoculation as the tubes just preceding it in the series. All of the tubes were sealed with paraffine-vaseline plugs and held at 85° C. for 20 minutes in a water bath, cooled rapidly, and incubated at 37° C. for 3 or 4 days. The highest dilution which showed gas production with rapid reduction of the litmus and without coagulation was again purified by the same process. The highest dilution which showed gas production, reduction of litmus, and no coagulation in the second series of tubes was considered a pure culture of the organism in question. Microscopic examinations showed the organisms in the culture were gram negative and rod shaped. Spores could not be detected even with special spore stains. The cells were approximately 0.7 by 2.0 to 2.5 microns, and occurred singly or in short chains.

The only media in which the organism could be grown successfully were peptone-litmus milk and litmus milk to which a small amount of Roquefort type cheese had been added. Peptone-litmus milk supported the growth of the organisms when anaerobic conditions were obtained in a number of different ways, but the simplest and most satisfactory method of obtaining anaerobic conditions was by the use of a paraffine-vaseline plug. Generally the medium used had been heated recently or heat was applied after inoculation.

When sterile cream cheese spread was inoculated with the organisms and incubated at 37° C., gas formation was noted in the cheese in from 5 to 14 days. The organisms isolated from the different lots of defective spread varied in the amount of gas produced in sterile cheese spread. In some cases the paraffine-vaseline plug was blown out of the tube in 3 or 4 days, while in other cases several weeks were required before gas production was noted. When the organisms were inoculated into sterile cream cheese and held at room temperature, gas production was much slower than when held at 37° C., but gas was eventually produced in all cases. Figure 1 illustrates the type of gas production obtained in sterile cheese spread.

Since the outbreak of gassiness in cream cheese spreads was attributed to the use of domestic blue cheese, an attempt was made to isolate the organism from domestic and foreign blue cheese and from Roquefort cheese. Essentially the same method of isolation was



Uninoculated control	Inoculated with Org. 1	Inoculated with Org. 14	Inoculated with Org. 3

Figure 1. Type of gas production obtained by inoculating sterile cream cheese spread with the organisms indicated.

employed with these cheeses as with the defective spreads. The organism was isolated from all five of the domestic blue cheese studied, from one out of five French bleu cheese and from one out of three Danish bleu cheeses. The organism could not be isolated from any of the three Roquefort cheeses studied. When the organisms which were isolated from the cheeses were inoculated into sterile cream cheese spread, essentially the same type of gas production resulted as with the organisms from the defective spreads.

Altogether 19 cultures of the organism in question were isolated; 12 came from 12 lots of defective cream cheese spread, 5 from domestic blue cheese and 1 each from French bleu and Danish bleu cheese. Table I gives a summary of the sources of the organisms isolated.

Effect of pH on the heat resistance of the gas producing organism. The pH of the normal Roquefort cream cheese spread was 5.8 to 6.0. It was thought that lowering the pH of the finished spread might prevent gas production and, accordingly, studies were carried out to determine what effect a low pH would have on the activity of the organism. The organism was found to produce gas at a pH far too low to be employed in a cheese spread.

Since lowering the pH of the finished product did not prevent gas production in the spread, a study was made to determine whether or not lowering the pH before the heating process would accomplish the desired result. Four trials were carried out, using Organism 1, 2,

TABLE I

SOURCES OF THE GAS PRODUCING ORGANISMS STUDIED

Organism number	Source of organism
1	Gassy Roquefort cream cheese spread containing domestic blue cheese.
2	Gassy Roquefort cream cheese spread containing domestic blue cheese.
3	Gassy Roquefort cream cheese spread containing Danish bleu cheese.
4	Gassy Roquefort cream cheese spread containing domestic blue cheese.
5	Gassy Roquefort cream cheese spread.
6	Gassy Roquefort cream cheese spread.
7	Gassy Roquefort cream cheese spread.
8	Gassy Roquefort cream cheese spread.
9	Gassy Roquefort cream cheese spread.
10	Gassy Roquefort cream cheese spread.
11	Gassy Roquefort cream cheese spread.
12	Gassy Roquefort cream cheese spread.
13	Domestic blue cheese.
14	Domestic blue cheese.
15	Domestic blue cheese.
16	Domestic blue cheese.
17	Domestic blue cheese.
18	Danish bleu cheese.
19	French bleu cheese.

and 3. In three of the trials blue cheese emulsion was used as the medium and in the fourth trial peptone-litmus milk was employed.

In the first trial, 25 cc. of a 48 hour peptone-litmus milk culture of Organism 2 were used to inoculate 1000 gm. of blue cheese emulsion. The inoculum was mixed with the emulsion by means of a sterile stirring rod and the pH determined. Eight 10 cc. samples were placed in sterile test tubes. A small amount of lactic acid was added to the remaining emulsion thoroughly mixed with it. After determining the pH, eight 10 cc. samples were placed in sterile test tubes. This general procedure was repeated until nine series of tubes (each series containing eight tubes) had been withdrawn. The principal difference between the various series was in the pH of the emulsion and the pH range covered was from 6.25 to 4.25. Each tube was sealed with a paraffine-vaseline plug. A tube from each of the nine series was held unheated as a control. Another tube from each series was heated at 60° C. for 20 minutes and cooled rapidly by placing in a cold water bath. One tube from each series was then heated at 65°, 70°, 75°, 80°, 85°, and 90° C. for 20 minutes and cooled rapidly. The tubes were incubated at 37° C. and observations were made daily. The data are given in Table II.

The results show that the organism was very resistant to a low pH when no heat was applied, producing gas at a pH as low as 4.25. With an exposure to 60° C. for 20 minutes, the lowest pH at which gas production occurred was 4.80 and with an exposure to 65° C. for 20

TABLE II

THE EFFECT OF THE pH OF THE MEDIUM ON THE HEAT RESISTANCE OF GAS PRODUCING ORGANISM 2

Cheese emulsion inoculated with 48 hour peptone-litmus milk culture, pH adjusted with lactic acid, samples covered with paraffino-vaseline plugs and heated.

Temperature of incubation 37° C.

pH of emulsion	Gas production in unheated controls	Gas production in samples heated 20 minutes at						
		60° C.	65° C.	70° C.	75° C.	80° C.	90° C.	95° C.
6.25	+	+	+	+	+	+	+	+
5.98	+	+	+	+	+	+	+	+
5.69	+	+	+	+	+	+	-	-
5.55	+	+	+	+	+	+	-	-
5.23	+	+	+	+	-	-	-	-
5.00	+	+	+	-	-	-	-	-
4.80	+	+	-	-	-	-	-	-
4.48	+	-	-	-	-	-	-	-
4.25	+	-	-	-	-	-	-	-

+ gas production - no gas production

minutes, the lowest pH permitting gas production was 5.00. As the temperature to which the inoculated medium was exposed increased, the pH permitting gas production became higher, until at a temperature of 90° C. for 20 minutes, no gas was produced at a pH of 5.69 or lower.

Similar series of tubes were prepared, using Organism 3. Eight different pH values were employed, ranging from 5.81 to 4.86; the same exposures were used as in the preceding trial. Table III gives the results obtained.

When no heat was applied, the organism produced gas at a pH of 4.86, while an exposure of 60° C. for 20 minutes was sufficient to prevent gas production at a pH of 5.02 and below. As the temperature of exposure was increased the pH required to prevent gas production increased until an exposure of 90° C. for 20 minutes prevented gas production at a pH of 5.60 and below.

Organism 1 was used in a trial similar to the two preceding ones except that wider ranges of pH and temperatures were employed. Fourteen pH values were used, covering the range from 6.47 to 4.25, and the temperatures employed were from 55° to 95° C. inclusive in 5° C. intervals. The data are given in Table IV.

In the tubes to which no heat was applied gas production occurred with the pH as low as 4.25, while in the tubes exposed to 55° C. for 20 minutes gas production was prevented at a pH of 4.32. When heated to 60° C. for 20 minutes no gas production resulted at a pH below 5.00.

TABLE III

THE EFFECT OF THE pH OF THE MEDIUM ON THE HEAT RESISTANCE OF GAS PRODUCING ORGANISM 3

Cheese emulsion inoculated with 48 hour peptone-litmus milk culture, pH adjusted with lactic acid, samples covered with paraffine-vasoline plugs and heated.

Temperature of incubation 37° C.

pH of emulsion	Gas production in unheated controls	Gas production in samples heated 20 minutes at						
		60° C.	65° C.	70° C.	75° C.	80° C.	85° C.	95° C.
5.81	+	+	+	+	+	+	+	+
5.60	+	+	+	+	+	+	+	-
5.52	+	+	+	+	+	+	-	-
5.42	+	+	+	-	-	-	-	-
5.33	+	-	-	-	-	-	-	-
5.20	+	+	-	-	-	-	-	-
5.02	+	-	-	-	-	-	-	-
4.86	+	-	-	-	-	-	-	-

+ gas production - no gas production

TABLE IV

THE EFFECT OF THE pH OF THE MEDIUM ON THE HEAT RESISTANCE OF GAS PRODUCING ORGANISM 1

Cheese emulsion inoculated with 48 hour peptone-litmus milk culture, pH adjusted with lactic acid, samples covered with paraffine-vaseline plugs and heated.

Temperature of incubation 37° C.

pH of emulsion	Gas production in unheated controls	Gas production in samples heated 20 minutes at									
		55° C.	60° C.	65° C.	70° C.	75° C.	80° C.	85° C.	90° C.	95° C.	
6.47	+	+	+	+	+	+	+	+	+	+	
6.31	+	+	+	+	+	+	+	+	+	+	
6.24	+	+	+	+	+	+	+	+	+	-	
5.89	+	+	+	+	+	+	+	+	-	-	
5.74	+	+	+	+	+	+	+	-	-	-	
5.54	+	+	+	+	+	+	-	-	-	-	
5.40	+	+	+	+	-	-	-	-	-	-	
5.18	+	+	+	-	-	-	-	-	-	-	
5.00	+	+	+	-	-	-	-	-	-	-	
4.82	+	+	-	-	-	-	-	-	-	-	
4.65	+	+	-	-	-	-	-	-	-	-	
4.46	+	+	-	-	-	-	-	-	-	-	
4.32	+	-	-	-	-	-	-	-	-	-	
4.25	+	-	-	-	-	-	-	-	-	-	

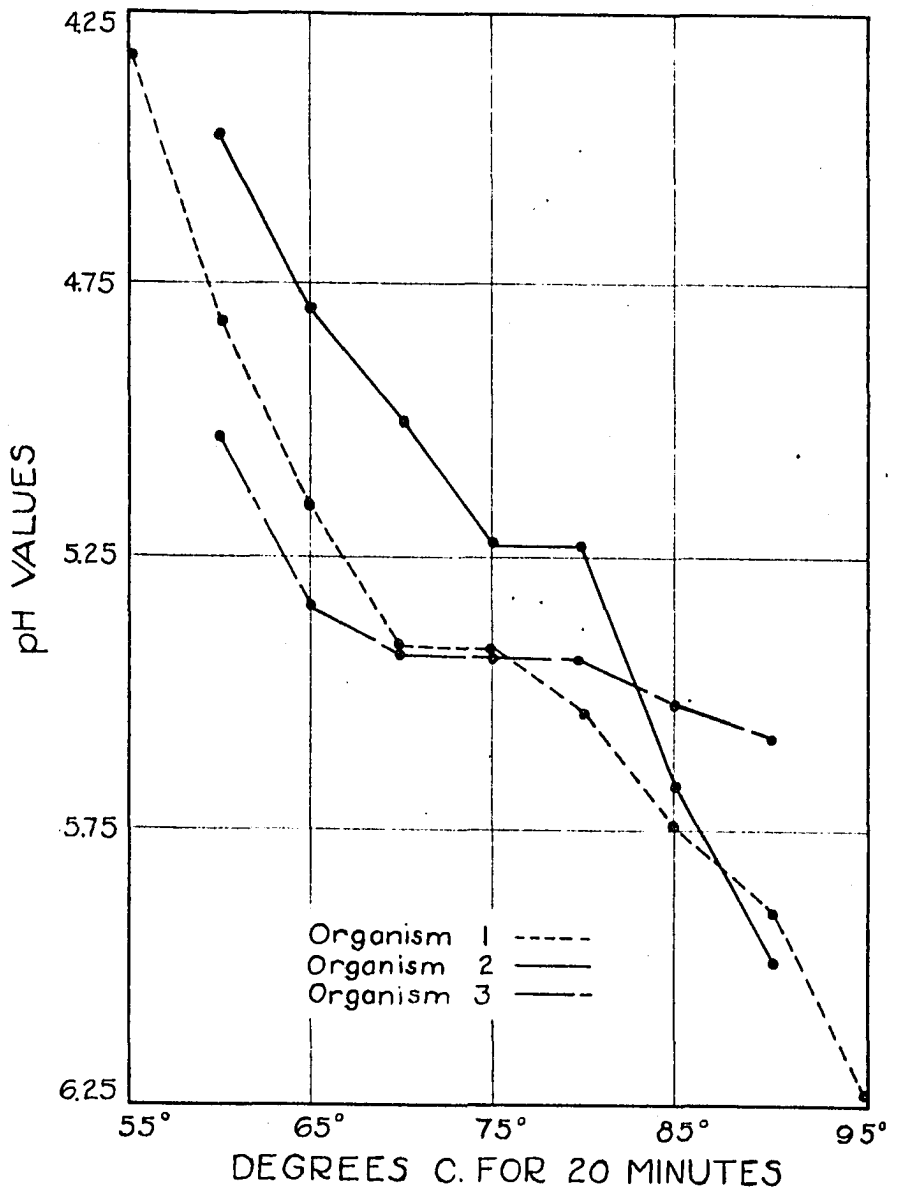
+ gas production - no gas production

As the temperature of exposure increased the pH required to prevent gas production became higher, until at 95° C. for 20 minutes the organism did not produce gas at a pH below 6.31.

The data presented in Tables II, III, and IV are plotted in Graph I. The graph shows clearly that there is a very distinct relationship between the pH of the medium at the time of heating and the temperature required to prevent gas production by the organism. A pH of 5.75 prevented gas production when heated to 90° C. for 20 minutes, while a pH of 5.00 was required to prevent gas production when an exposure of 60° C. for 20 minutes was used .

Since the blue cheese emulsion varied somewhat in composition and reaction from one batch to another, a trial was carried out using peptone-litmus milk as the medium instead of blue cheese emulsion in order to determine whether or not the peptone-litmus milk could be used in the future studies with satisfactory results. Organism 1 was employed in the trial. Twelve pH values were used, covering a pH range from 6.40 to 4.73, and the temperatures to which the tubes were exposed for 20 minutes ranged from 60° C. to 90° C. inclusive in 5° C. intervals. The results are given in Table V.

All the unheated tubes showed gas production, while with an exposure to 60° C. for 20 minutes no gas was produced in the tubes having a pH below 5.06. As the temperature of exposure increased, the pH required to prevent gas production increased, just as it did in the trials with cheese emulsion.



Graph I. pH values required to prevent gas production by Organisms 1, 2, and 3 in cheese emulsion heated 20 minutes at various temperatures.

TABLE V

THE EFFECT OF THE pH OF THE MEDIUM ON THE HEAT RESISTANCE OF GAS PRODUCING ORGANISM 1

Peptone-litmus milk inoculated with 48 hour peptone-litmus milk culture, pH adjusted with lactic acid, samples covered with paraffine-vaseline plugs and heated.

Temperature of incubation 37° C.

pH of peptone-litmus milk	Gas production in unheated controls	Gas production in samples heated 20 minutes at						
		60° C.	65° C.	70° C.	75° C.	80° C.	85° C.	90° C.
6.40	+	+	+	+	+	+	+	+
6.15	+	+	+	+	+	+	+	-
6.04	+	+	+	+	+	+	-	-
5.94	+	+	+	+	+	+	-	-
5.56	+	+	+	+	+	-	-	-
5.48	+	+	+	+	-	-	-	-
5.30	+	+	+	-	-	-	-	-
5.16	+	+	+	-	-	-	-	-
5.06	+	+	-	-	-	-	-	-
5.00	+	-	-	-	-	-	-	-
4.87	+	-	-	-	-	-	-	-
4.73	+	-	-	-	-	-	-	-

+ gas production - no gas production

Effect of salt (NaCl) concentration and the pH of the medium on the heat resistance of the gas producing organism. Esty and Meyer (10) and Viljoon (16) found that salt (NaCl) concentrations of 0.5 to 2.5 per cent increased the heat resistance of the bacterial spores which they studied, but that concentrations over 2.5 per cent lowered the heat resistance.

An attempt was made to determine the effect of salt concentration on the heat resistance of the gas producing organism when heated at various pH values. In the manufacture of Roquefort type cream cheese spread, the Roquefort type cheese is grated and heated before it is added to the cream cheese. Therefore it would be possible to add as much as 8 per cent salt to the Roquefort type cheese when it is heated and not have too much salt in the finished product.

In the first trial Organism 2 was used. Each of seven flasks, containing 1000 cc. of sterile peptone-litmus milk per flask, was inoculated with 25 cc. of a 48 hour peptone-litmus milk culture of the organism to be studied. The pH of one of the flasks was determined and five 10 cc. samples were placed in sterile test tubes. A small amount of lactic acid was added to the remaining milk and thoroughly distributed. The pH was determined and five more 10 cc. samples were taken. This same general procedure was repeated until eight series (each series having five tubes) were obtained. Each series differed from the others only in the pH. One, 2, 3, 4, 5, and 6 per cent sterile salt, respectively, was added to the other six flasks of inoculated

peptone-litmus milk. Samples were taken from each of these flasks in the same manner as from the flask to which no salt was added.

All of the tubes were covered with paraffine-vaseline plugs. The seven salt concentrations and eight pH series gave 56 combinations. One tube from each combination was held unheated and others were heated at 70°, 80°, 85°, and 90° C. respectively, for 20 minutes. The tubes were incubated at 37° C. Observations were made at the end of 18 days and those tubes which did not show gas production and reduction of litmus were cultured in peptone-litmus milk with no salt added and with a normal pH. These tubes were covered with paraffine-vaseline plugs and incubated at 37° C. At the end of 8 days observations were made. The results of the trial are given in Table VI.

The data show that salt concentrations of 2, 3, 4, 5, and 6 per cent in the unheated series inhibited the gas producing organisms at the lower pH values but, when the tubes in which inhibition occurred were cultured in normal peptone-litmus milk, gas was produced, indicating that the organism was not killed but only inhibited by the high salt concentration and low pH. When heated to 70° C. for 20 minutes the organism was killed at pH 5.29 when no salt was added, and at pH 5.25 when 1 per cent salt was added, while with 2 per cent salt, a pH of 5.47 inhibited the growth of the organism but did not kill it and a pH of 5.37 or lower killed the organism. As the amount of salt was increased and the pH was lowered the heat resistance of the organism decreased. When held at 85° C. for 20 minutes the organism did not show

TABLE VI

THE EFFECT OF THE SALT (NaCl) CONCENTRATION AND THE pH OF THE MEDIUM ON THE HEAT RESISTANCE OF GAS PRODUCING ORGANISM 2

Peptone-litmus milk, with salt added, inoculated with 48 hour peptone-litmus milk culture, pH adjusted with lactic acid, samples covered with paraffine-vaseline plugs and heated.

Temperature of incubation 37° C.

Heat treatment	No NaCl		1% NaCl		2% NaCl		3% NaCl		4% NaCl		5% NaCl		6% NaCl	
	pH	Growth	pH	Growth	pH	Growth	pH	Growth	pH	Growth	pH	Growth	pH	Growth
	6.42	+	6.38	+	6.33	+	6.31	+	6.27	+	6.26	+	6.24	+
No	6.21	+	6.18	+	6.17	+	6.17	+	6.18	+	6.15	+	6.16	+
	6.09	+	6.06	+	6.07	+	6.08	+	6.07	+	6.07	+	6.04	+
	5.84	+	5.85	+	5.83	+	5.86	+	5.85	+	5.83	+	5.81	+
Heat	5.61	+	5.66	+	5.64	+	5.63	+	5.65	+	5.61	+	5.60	o
	5.52	+	5.50	+	5.47	+	5.45	o	5.48	o	5.50	o	5.51	o
	5.40	+	5.38	+	5.37	+	5.35	+	5.34	o	5.41	o	5.42	o
	5.29	+	5.25	+	5.26	o	5.21	o	5.23	o	5.21	o	5.24	o
	6.42	+	6.38	+	6.33	+	6.31	+	6.27	+	6.26	+	6.24	+
70° C.	6.21	+	6.18	+	6.17	+	6.17	+	6.18	+	6.15	+	6.16	+
	6.09	+	6.06	+	6.07	+	6.08	+	6.07	+	6.07	o	6.04	o
	5.84	+	5.85	+	5.83	+	5.86	+	6.85	o	5.83	o	5.81	-
for 20	5.61	+	5.66	+	5.64	+	5.63	o	5.65	o	5.61	-	5.60	-
	5.52	+	5.50	+	5.47	o	5.45	-	5.48	-	5.50	-	5.51	-
	5.40	+	5.38	+	5.37	-	5.35	-	5.34	-	5.41	-	5.42	-
min.	5.29	-	5.25	-	5.26	-	5.21	-	5.23	-	5.21	-	5.24	-

\+ gas production in original sample tube.

o gas production only after transferring to a fresh tube of litmus milk, containing no salt and having a normal pH.

\- no gas production after culturing in a fresh tube of litmus milk containing no salt and having a normal pH.

TABLE VI. (CONTINUED)

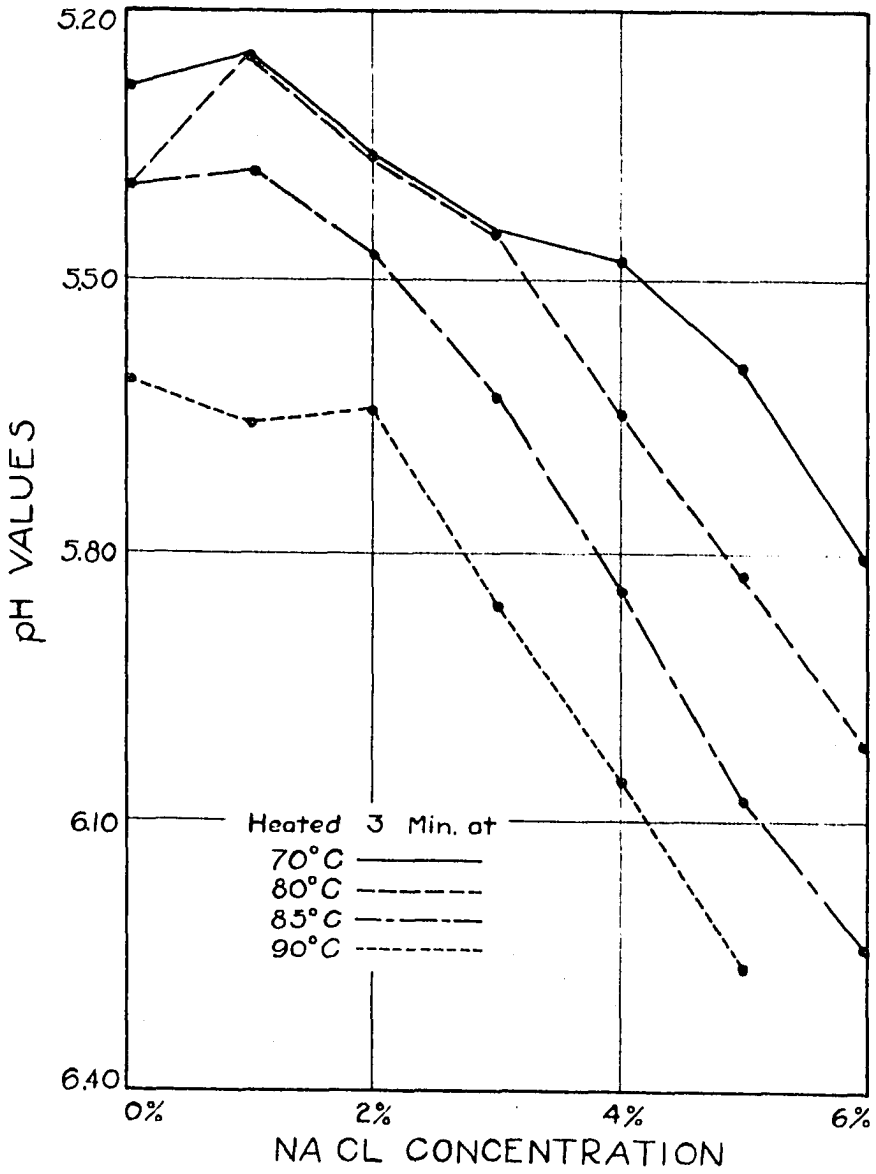
Heat treatment	No NaCl		1% NaCl		2% NaCl		3% NaCl		4% NaCl		5% NaCl		6% NaCl	
	pH	Growth	pH	Growth	pH	Growth	pH	Growth	pH	Growth	pH	Growth	pH	Growth
	6.42	+	6.38	+	6.33	+	6.31	+	6.27	+	6.26	+	6.24	o
80° C.	6.21	+	6.18	+	6.17	+	6.17	+	6.18	+	6.15	+	6.16	o
	6.09	+	6.06	+	6.07	+	6.08	+	6.07	+	6.07	o	6.04	-
	5.84	+	5.85	+	5.83	+	5.86	+	5.85	o	5.83	-	5.81	-
for 20	5.61	+	5.66	+	5.64	+	5.63	+	5.65	-	5.61	-	6.60	-
	5.52	+	5.50	+	5.47	o	5.45	-	5.48	-	5.50	-	5.51	-
	5.40	-	5.38	+	5.37	-	5.35	-	5.34	-	5.41	-	5.42	-
min.	5.29	-	5.25	-	5.26	-	5.21	-	5.23	-	5.21	-	5.24	-
	6.42	+	6.38	+	6.33	+	6.31	+	6.27	+	6.26	+	6.24	-
85° C.	6.21	+	6.18	+	6.17	+	6.17	+	6.18	+	6.15	o	6.16	-
	6.09	+	6.06	+	6.07	+	6.08	+	6.07	o	6.07	-	6.04	-
	5.84	+	5.85	+	5.83	+	5.86	o	5.85	-	5.83	-	5.81	-
for 20	5.61	+	5.66	+	5.64	+	5.63	-	5.65	-	5.61	-	5.60	-
	5.52	+	5.50	+	5.47	-	5.45	-	5.48	-	5.50	-	5.51	-
	5.40	-	5.38	-	5.37	-	5.35	-	5.34	-	5.41	-	5.42	-
min.	5.29	-	5.25	-	5.26	-	5.21	-	5.23	-	5.21	-	5.24	-
	6.42	+	6.38	+	6.33	+	6.31	+	6.27	o	6.26	-	6.24	-
90° C.	6.21	+	6.18	+	6.17	+	6.17	+	6.18	o	6.15	-	6.16	-
	6.09	+	6.06	+	6.07	o	6.08	o	6.07	-	6.07	-	6.04	-
	5.84	+	5.85	+	5.83	o	5.86	-	5.85	-	5.83	-	5.81	-
for 20	5.61	-	5.66	-	5.64	-	5.63	-	5.65	-	5.61	-	5.60	-
	5.52	-	5.50	-	5.47	-	5.45	-	5.48	-	5.50	-	5.51	-
	5.40	-	5.38	-	5.37	-	5.35	-	5.34	-	5.41	-	5.42	-
min.	5.29	-	5.25	-	5.26	-	5.21	-	5.23	-	5.21	-	5.24	-

any growth at all in 6 per cent salt even after being cultured, indicating that a combination of 85° C. per 20 minutes and 6 per cent salt killed the organism even at a pH of 6.24. When no salt was added, 85° C. for 20 minutes killed the organism only when the pH was 5.40 or lower.

The data in Table VI are plotted in Graph II. This graph shows clearly that salt concentrations of 2 per cent or below did not have much effect on the heat resistance of the Organism 2, but as the salt concentration increased about 2 per cent the pH required to prevent gas production at a given temperature was lowered.

A second trial was carried out using Organism 1. The pH range used was from 6.38 to 5.10 and the salt concentrations from no salt to 6 per cent. The heat exposures employed ranged from 60° C. to 90° C., inclusive, in 5° C. intervals for 20 minutes. All of the tubes were prepared in duplicate, one series being incubated at 37° C. and the other series at room temperature. The results are given in Table VII. The tubes which did not show gas were not cultured as in first trial; therefore Table VII shows only the salt concentration and the pH required to prevent gas production when exposed to various temperatures for 20 minutes.

In the unheated samples incubated at 37° C. gas production was prevented with 2, 3, 4, 5, and 6 per cent salt at pH values of 5.18, 5.20, 5.21, 5.59, and 6.00, respectively. When heated to 60° C. for 20 minutes 3, 4, 5, and 6 per cent salt prevented gas production at



Graph II. Combinations of pH values and salt concentrations required to prevent gas production by organism in peptone-litmus milk heated 20 minutes at 70° C., 80° C., 85° C., or 90° C.

THE EFFECT OF THE SALT (NaCl) CONCENTRATION AND T

Peptono-litmus milk, with salt adde
pH adjusted with lactic acid, samp

Heat treatment	Incubated at 37° C.																	
	No NaCl		1% NaCl		2% NaCl		3% NaCl		4% NaCl		5% NaCl							
	pH	Growth	pH	Growth	pH	Growth	pH	Growth	pH	Growth	pH	Growth						
No heat	6.38	x	6.31	x	6.28	x	6.19	x	6.20	x	6.20	x	6					
	6.30	x	6.17	x	6.12	x	6.07	x	6.12	x	6.12	x	6					
	6.12	x	6.08	x	6.06	x	6.04	x	6.04	x	6.03	x	6					
	5.90	x	5.83	x	5.80	x	5.80	x	5.82	x	5.85	-	5					
	5.69	x	5.75	x	5.72	x	5.72	x	5.73	x	5.75	x	5					
	5.57	x	5.66	x	5.62	x	5.63	x	5.65	x	5.59	-	5					
	5.46	x	5.50	x	5.52	x	5.55	x	5.55	x	5.52	-	5					
	5.37	x	5.40	x	5.44	x	5.41	x	5.42	x	5.45	-	5					
	5.27	x	5.27	x	5.27	x	5.30	x	5.32	x	5.30	-	5					
	5.16	x	5.18	x	5.18	-	5.20	-	5.21	-	5.11	-	5					
60° C. for 20 min.	6.38	x	6.31	x	6.28	x	6.19	x	6.20	x	6.20	x	6					
	6.30	x	6.17	x	6.12	x	6.07	x	6.12	x	6.12	x	6					
	6.12	x	6.08	x	6.06	x	6.04	x	6.04	x	6.03	x	6					
	5.90	x	5.83	x	5.80	x	5.80	x	5.82	x	5.85	x	5					
	5.69	x	5.75	x	5.72	x	5.72	x	5.73	x	5.75	x	5					
	5.57	x	5.66	x	5.62	x	5.63	x	5.65	x	5.59	-	5					
	5.46	x	5.50	x	5.52	x	5.55	x	5.55	x	5.52	-	5					
	5.37	x	5.40	x	5.44	x	5.41	x	5.42	x	5.45	-	5					
	5.27	x	5.27	x	5.27	x	5.30	x	5.32	x	5.30	-	5					
	5.16	x	5.18	x	5.18	x	5.20	-	5.21	-	5.11	-	5					

x reduction of litmus and gas production
- no reduction of litmus or gas production

TABLE VII

) CONCENTRATION AND THE pH OF TH E MEDIUM ON THE HEAT RESISTANCE OF GAS PRODUCING ORGANISM 1

milk, with salt added, inoculat ed with 48 hour peptone-litmus milk culture, .th lactic acid, samples covered with paraffine-vaseline plugs and heated.

Cl	5% NaCl		6% NaCl	
owth	pH	Growth	pH	Growth
x	6.20	x	6.20	x
x	6.12	x	6.13	x
x	6.03	x	6.00	-
x	5.85	-	5.86	-
x	5.75	x	5.74	-
x	5.59	-	5.62	-
x	5.52	-	5.52	-
x	5.45	-	5.43	-
x	5.30	-	5.31	-
-	5.11	-	5.16	-
x	6.20	x	6.20	x
x	6.12	x	6.13	-
x	6.03	x	6.00	-
x	5.85	x	5.86	-
x	5.75	x	5.74	-
x	5.59	-	5.62	-
x	5.52	-	5.52	-
x	5.45	-	5.43	-
x	5.30	-	5.31	-
-	5.11	-	5.16	-

Incubated at room temperature									
No NaCl		1% NaCl		2% NaCl		3% NaCl		4% NaCl	
pH	Growth	pH	Growth	pH	Growth	pH	Growth	pH	Growth
6.38	x	6.31	x	6.28	x	6.19	x	6.20	x
6.30	x	6.17	x	6.12	x	6.07	x	6.12	x
6.12	x	6.08	x	6.06	x	6.04	x	6.04	x
5.90	x	5.83	x	5.80	x	5.80	x	5.82	-
5.69	x	5.75	x	5.72	x	5.72	x	5.73	-
5.57	x	5.66	x	5.62	x	5.63	x	5.65	-
5.46	x	5.50	x	5.52	x	5.55	x	5.55	-
5.37	x	5.40	x	5.44	x	5.41	x	5.42	-
5.27	x	5.27	x	5.27	x	5.30	x	5.32	-
5.16	x	5.18	x	5.18	x	5.20	x	5.21	-
6.38	x	6.31	x	6.28	x	6.19	x	6.20	x
6.30	x	6.17	x	6.12	x	6.07	x	6.12	x
6.12	x	6.08	x	6.06	x	6.04	x	6.04	x
5.90	x	5.83	x	5.80	x	5.80	x	5.82	x
5.69	x	5.75	x	5.72	x	5.72	x	5.73	-
5.57	x	5.66	x	5.62	x	5.63	x	5.65	-
5.46	x	5.50	x	5.52	x	5.55	x	5.55	-
5.37	x	5.40	x	5.44	x	5.41	x	5.42	-
5.27	x	5.27	x	5.27	x	5.30	x	5.32	-
5.16	x	5.18	x	5.18	x	5.20	-	5.21	-

:I

MEDIUM ON THE HEAT RESISTANCE OF GAS PRODUCING ORGANISM 1

l with 48 hour peptone-litmus milk culture,
th paraffine-vaseline plugs and heated.

Incubated at room temperature													
No NaCl		1% NaCl		2% NaCl		3% NaCl		4% NaCl		5% NaCl		6% NaCl	
pH	Growth	pH	Growth	pH	Growth	pH	Growth	pH	Growth	pH	Growth	pH	Growth
6.38	x	6.31	x	6.28	x	6.19	x	6.20	x	6.20	-	6.20	-
6.30	x	6.17	x	6.12	x	6.07	x	6.12	x	6.12	-	6.13	-
6.12	x	6.08	x	6.06	x	6.04	x	6.04	x	6.03	-	6.00	-
5.90	x	5.85	x	5.80	x	5.80	x	5.82	-	5.85	-	5.86	-
5.69	x	5.75	x	5.72	x	5.72	x	5.75	-	5.76	-	5.74	-
5.57	x	5.66	x	5.62	x	5.63	x	5.65	-	5.59	-	5.62	-
5.46	x	5.50	x	5.52	x	5.55	x	5.55	-	5.52	-	5.52	-
5.37	x	5.40	x	5.44	x	5.41	x	5.42	-	5.45	-	5.43	-
5.27	x	5.27	x	5.27	x	5.30	x	5.32	-	5.30	-	5.31	-
5.16	x	5.18	x	5.18	x	5.20	x	5.21	-	5.11	-	5.16	-
6.38	x	6.31	x	6.28	x	6.19	x	6.20	x	6.20	-	6.20	-
6.30	x	6.17	x	6.12	x	6.07	x	6.12	x	6.12	-	6.13	-
6.12	x	6.08	x	6.06	x	6.04	x	6.04	x	6.03	-	6.00	-
5.90	x	5.85	x	5.80	x	5.80	x	5.82	x	5.85	-	5.86	-
5.69	x	5.75	x	5.72	x	5.72	x	5.75	-	5.76	-	5.74	-
5.57	x	5.66	x	5.62	x	5.63	x	5.65	-	5.59	-	5.62	-
5.46	x	5.50	x	5.52	x	5.55	x	5.55	-	5.52	-	5.52	-
5.37	x	5.40	x	5.44	x	5.41	x	5.42	-	5.45	-	5.43	-
5.27	x	5.27	x	5.27	x	5.30	x	5.32	-	5.30	-	5.31	-
5.16	x	5.18	x	5.18	x	5.20	-	5.21	-	5.11	-	5.16	-

	Incubated at 37° C.												
	No NaCl		1% NaCl		2% NaCl		3% NaCl		4% NaCl		5% NaCl		
	pH	Growth	pH	Growth	pH	Growth	pH	Growth	pH	Growth	pH	Growth	
65° C. for 20 min.	6.38	x	6.31	x	6.28	x	6.19	x	6.20	x	6.20	x	
	6.30	x	6.17	x	6.12	x	6.07	x	6.12	x	6.12	x	
	6.12	x	6.08	x	6.06	x	6.07	x	6.04	x	6.03	x	
	5.90	x	5.83	x	5.80	x	5.80	x	5.82	x	5.85	x	
	5.69	x	5.75	x	5.72	x	5.72	x	5.73	x	5.75	x	
	5.57	x	5.66	x	5.62	x	5.63	x	5.65	x	5.59	-	
	5.46	x	5.50	x	5.52	-	5.55	x	5.55	x	5.52	-	
	5.37	x	5.40	x	5.44	x	5.41	x	5.42	x	5.45	-	
	5.27	x	5.27	x	5.27	x	5.30	x	5.32	-	5.30	-	
	5.16	x	5.18	x	5.18	x	6.20	-	5.21	-	5.11	-	
70° C. for 20 min.	6.38	x	6.31	x	6.28	x	6.19	x	6.20	x	6.20	x	
	6.30	x	6.17	x	6.12	x	6.07	x	6.12	x	6.12	x	
	6.12	x	6.08	x	6.06	x	6.04	x	6.04	x	6.03	x	
	5.90	x	5.83	x	5.80	x	5.80	x	5.82	x	5.85	x	
	5.69	x	5.75	x	5.72	x	5.72	x	5.73	x	5.75	-	
	5.57	x	5.66	x	5.62	x	5.63	x	5.65	x	5.59	-	
	5.46	x	5.50	x	5.52	x	5.55	x	5.55	x	5.52	-	
	5.37	x	5.40	x	5.44	x	5.41	x	5.42	x	5.45	-	
	5.27	x	5.27	x	5.27	x	5.30	x	5.32	-	5.30	-	
	5.16	-	5.18	x	5.18	x	5.20	-	5.21	-	5.11	-	
75° C. for 20 min.	6.38	x	6.31	x	6.28	x	6.19	x	6.20	x	6.20	x	
	6.30	x	6.17	x	6.12	x	6.07	x	6.12	x	6.12	-	
	6.12	x	6.08	x	6.06	x	6.04	x	6.04	x	6.03	-	
	5.90	x	5.83	x	5.80	x	5.80	x	5.82	x	5.85	-	
	5.69	x	5.75	x	5.72	x	5.72	x	5.73	x	5.75	-	
	5.57	x	5.66	x	5.62	x	5.63	x	5.65	x	5.59	-	
	5.46	x	5.50	x	5.52	x	5.55	x	5.55	-	5.52	-	
	5.37	x	5.40	x	5.44	x	5.41	x	5.42	-	5.45	-	
	5.27	x	5.27	x	5.27	x	5.30	x	5.32	-	5.30	-	
	5.16	-	5.18	-	5.18	x	5.20	-	5.21	-	5.11	-	

TABLE VII (CONTINUED)

5% NaCl		6% NaCl	
pH	Growth	pH	Growth
6.20	x	6.20	-
6.12	x	6.13	-
6.03	x	6.00	-
5.85	x	5.86	-
5.75	x	5.74	-
5.59	-	5.62	-
5.52	-	5.52	-
5.45	-	5.43	-
5.30	-	5.31	-
5.11	-	5.16	-
6.20	x	6.20	x
6.12	x	6.13	-
6.03	x	6.00	-
5.85	x	5.86	-
5.75	-	5.74	-
5.59	-	5.62	-
5.52	-	5.52	-
5.45	-	5.43	-
5.30	-	5.31	-
5.11	-	5.16	-
6.20	x	6.20	x
6.12	-	6.13	-
6.03	-	6.00	-
5.85	-	5.86	-
5.75	-	5.74	-
5.59	-	5.62	-
5.52	-	5.52	-
5.45	-	5.43	-
5.30	-	5.31	-
5.11	-	5.16	-

Incubated at room temperature

No NaCl		1% NaCl		2% NaCl		3% NaCl		4% NaCl		5
pH	Growth	pH	Growth	pH	Growth	pH	Growth	pH	Growth	pH
6.38	x	6.31	x	6.28	x	6.19	x	6.20	x	6.2
6.30	x	6.17	x	6.12	x	6.07	x	6.12	-	6.1
6.12	x	6.08	x	6.06	x	6.04	x	6.04	-	6.0
5.90	x	5.83	x	5.80	x	5.80	x	5.82	-	5.8
6.69	x	5.75	x	5.72	x	5.72	x	5.73	-	5.7
5.57	x	5.66	x	5.62	x	5.63	x	5.65	-	5.5
5.46	x	5.50	x	5.52	x	5.55	x	5.55	-	5.5
5.37	x	5.40	x	5.44	x	5.41	x	5.42	-	5.4
5.27	x	5.27	x	5.27	x	5.30	x	5.32	-	5.3
5.16	x	5.18	x	5.18	x	5.20	-	5.21	-	5.1
6.38	x	6.31	x	6.28	x	6.19	x	6.20	x	6.2
6.30	x	6.17	x	6.12	x	6.07	x	6.12	-	6.1
6.12	x	6.08	x	6.06	x	6.04	x	6.04	-	6.0
5.90	x	5.83	x	5.80	x	5.80	x	5.82	-	5.8
5.69	x	5.75	x	5.72	x	5.72	x	5.72	-	5.7
5.57	x	5.66	x	5.62	x	5.63	x	5.65	-	5.5
5.46	x	5.50	x	5.52	x	5.55	x	5.55	-	5.5
5.37	x	5.40	x	5.44	x	5.41	x	5.42	-	5.4
5.27	x	5.27	x	5.27	x	5.30	-	5.32	-	5.3
5.16	x	5.18	x	5.18	x	5.20	-	5.21	-	5.1
6.38	x	6.31	x	6.28	x	6.19	x	6.20	x	6.2
6.30	x	6.17	x	6.12	x	6.07	x	6.12	-	6.1
6.12	x	6.08	x	6.06	x	6.04	x	6.04	-	6.0
5.90	x	5.83	x	5.80	x	5.80	x	5.82	-	5.8
5.69	x	5.75	x	5.72	x	5.72	x	5.73	-	5.7
5.57	x	5.66	x	5.62	x	5.63	x	5.65	-	5.6
5.46	x	5.50	x	5.52	x	5.55	x	5.56	-	5.5
5.37	x	5.40	x	5.44	x	5.41	-	5.42	-	5.4
5.27	x	5.27	x	5.27	x	5.30	-	5.32	-	5.3
5.16	x	5.18	x	5.18	x	5.20	-	5.21	-	5.2

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Incubated at room temperature													
No NaCl		1% NaCl		2% NaCl		3% NaCl		4% NaCl		5% NaCl		6% NaCl	
pH	Growth	pH	Growth	pH	Growth	pH	Growth	pH	Growth	pH	Growth	pH	Growth
6.38	x	6.31	x	6.28	x	6.19	x	6.20	x	6.20	-	6.20	-
6.30	x	6.17	x	6.12	x	6.07	x	6.12	-	6.12	-	6.13	-
6.12	x	6.08	x	6.06	x	6.04	x	6.04	-	6.03	-	6.00	-
5.90	x	5.83	x	5.80	x	5.80	x	5.82	-	5.85	-	5.86	-
5.69	x	5.75	x	5.72	x	5.72	x	5.73	-	5.75	-	5.74	-
5.57	x	5.66	x	5.62	x	5.63	x	5.65	-	5.59	-	5.62	-
5.46	x	5.50	x	5.52	x	5.55	x	5.55	-	5.52	-	5.52	-
5.37	x	5.40	x	5.44	x	5.41	x	5.42	-	5.45	-	5.43	-
5.27	x	5.27	x	5.27	x	5.30	x	5.32	-	5.30	-	5.31	-
5.16	x	5.18	x	5.18	x	5.20	-	5.21	-	5.11	-	5.16	-
6.38	x	6.31	x	6.28	x	6.19	x	6.20	x	6.20	-	6.20	-
6.30	x	6.17	x	6.12	x	6.07	x	6.12	-	6.12	-	6.13	-
6.12	x	6.08	x	6.06	x	6.04	x	6.04	-	6.03	-	6.00	-
5.90	x	5.83	x	5.80	x	5.80	x	5.82	-	5.85	-	5.82	-
5.69	x	5.75	x	5.72	x	5.72	x	5.72	-	5.75	-	5.74	-
5.57	x	5.66	x	5.62	x	5.63	x	5.65	-	5.59	-	5.62	-
5.46	x	5.50	x	5.52	x	5.55	x	5.55	-	5.52	-	5.52	-
5.37	x	5.40	x	5.44	x	5.41	x	5.42	-	5.45	-	5.43	-
5.27	x	5.27	x	5.27	x	5.30	-	5.32	-	5.30	-	5.31	-
5.16	x	5.18	x	5.18	x	5.20	-	5.21	-	5.11	-	5.16	-
6.38	x	6.31	x	6.28	x	6.19	x	6.20	x	6.20	-	6.20	-
6.30	x	6.17	x	6.12	x	6.07	x	6.12	-	6.12	-	6.13	-
6.12	x	6.08	x	6.06	x	6.04	x	6.04	-	6.03	-	6.00	-
5.90	x	5.83	x	5.80	x	5.80	x	5.82	-	5.85	-	5.86	-
5.69	x	5.75	x	5.72	x	5.72	x	5.73	-	5.75	-	5.74	-
5.57	x	5.66	x	5.62	x	5.63	x	5.65	-	5.69	-	5.62	-
5.46	x	5.50	x	5.52	x	5.55	x	5.55	-	5.52	-	5.52	-
5.37	x	5.40	x	5.44	x	5.41	-	5.42	-	5.45	-	5.43	-
5.27	x	5.27	x	5.27	x	5.30	-	5.32	-	5.30	-	5.31	-
5.16	x	5.18	x	5.18	x	5.20	-	5.21	-	5.11	-	5.16	-

TAB

	Incubated at 37° C.															
	No NaCl		1% NaCl		2% NaCl		3% NaCl		4% NaCl		5% NaCl					
	pH	Growth	pH	Growth	pH	Growth	pH	Growth	pH	Growth	pH	Growth				
	6.38	x	6.31	x	6.28	x	6.19	x	6.20	-	6.20	x				
	6.30	x	6.17	x	6.12	x	6.07	x	6.12	x	6.12	-				
80° C.	6.12	x	6.08	x	6.06	x	6.04	x	6.04	x	6.03	-				
	5.90	x	5.83	x	5.80	x	5.80	x	5.82	x	5.85	-				
	5.69	x	5.75	x	5.72	x	5.72	x	5.73	x	5.75	-				
for 20	5.57	x	5.66	x	5.62	x	5.63	x	5.65	x	5.59	-				
	5.46	x	5.50	x	5.52	x	5.55	x	5.55	-	5.52	-				
	5.37	x	5.40	x	5.44	x	5.41	-	5.42	-	5.45	-				
min.	5.27	x	5.27	x	5.27	-	5.30	-	5.32	-	5.30	-				
	5.16	x	5.18	x	5.18	-	5.20	-	5.21	-	5.11	-				
	6.38	x	6.31	x	6.28	x	6.19	x	6.20	-	6.20	x				
	6.30	x	6.17	x	6.12	x	6.07	x	6.12	-	6.12	-				
85° C.	6.12	x	6.08	-	6.06	x	6.04	-	6.04	x	6.03	-				
	5.90	x	5.83	x	5.80	x	5.80	x	5.82	-	5.85	-				
for 20	5.69	-	5.75	-	5.72	-	5.72	-	5.73	-	5.75	-				
	5.57	-	5.66	x	5.62	x	5.63	-	5.65	-	5.59	-				
min.	5.46	-	5.50	-	5.52	-	5.55	-	5.55	-	5.52	-				
	5.37	-	5.40	-	5.44	-	5.41	-	5.42	-	5.45	-				
	5.27	-	5.27	-	5.27	-	5.30	-	5.32	-	5.30	-				
	5.16	-	5.18	-	5.18	-	5.20	-	5.21	-	5.11	-				
	6.38	x	6.31	x	6.28	x	6.19	-	6.20	-	6.20	-				
	6.30	x	6.17	x	6.12	x	6.07	x	6.12	-	6.12	-				
90° C.	6.12	-	6.08	-	6.06	-	6.04	-	6.04	-	6.03	-				
	5.90	-	5.83	-	5.80	-	5.80	-	5.82	-	5.85	-				
	5.69	-	5.75	-	5.72	-	5.72	-	5.73	-	5.75	-				
for 20	5.57	-	5.66	-	5.62	-	5.63	-	5.65	-	5.59	-				
	5.46	-	5.50	-	5.52	-	5.55	-	5.55	-	5.52	-				
	5.37	-	5.40	-	5.44	-	5.41	-	5.42	-	5.45	-				
min.	5.27	-	5.27	-	5.27	-	5.30	-	5.32	-	5.30	-				
	5.16	-	5.18	-	5.18	-	5.20	-	5.21	-	5.11	-				

TABLE VII (CONTINUED)

5% NaCl		6% NaCl		Incubated at room temperature											
				No NaCl		1% NaCl		2% NaCl		3% NaCl		4% NaCl		5%	
pH	Growth	pH	Growth	pH	Growth	pH	Growth	pH	Growth	pH	Growth	pH	Growth	pH	
6.20	x	6.20	-	6.38	x	6.31	x	6.28	x	6.19	x	6.20	-	6.20	
6.12	-	6.13	-	6.30	x	6.17	x	6.12	x	6.07	x	6.12	-	6.12	
6.03	-	6.00	-	6.12	x	6.08	x	6.06	x	6.04	x	6.04	-	6.03	
5.85	-	5.86	-	5.90	x	5.83	x	5.80	x	5.80	-	5.82	-	5.85	
5.75	-	5.74	-	5.69	x	5.75	x	5.72	x	5.72	-	5.73	-	5.75	
5.59	-	5.62	-	5.57	x	5.66	x	5.62	x	5.63	-	5.65	-	5.59	
5.52	-	5.52	-	5.46	x	5.50	x	5.52	x	5.55	-	5.55	-	5.52	
5.45	-	5.43	-	5.37	-	5.40	x	5.44	x	5.41	-	5.42	-	5.45	
5.30	-	5.31	-	5.27	-	5.27	x	5.27	x	5.30	-	5.32	-	5.30	
5.11	-	5.16	-	5.16	-	5.18	x	5.18	-	5.20	-	5.21	-	5.11	
6.20	x	6.20	-	6.38	x	6.31	x	6.28	x	6.19	-	6.20	-	6.20	
6.12	-	6.13	-	6.30	x	6.17	-	6.12	x	6.07	-	6.12	-	6.12	
6.03	-	6.00	-	6.12	x	6.08	x	6.06	-	6.04	-	6.04	-	6.03	
5.85	-	5.86	-	5.90	-	5.83	x	5.80	-	5.80	-	5.82	-	5.85	
5.75	-	5.74	-	5.69	-	5.75	x	5.72	-	5.72	-	5.73	-	5.75	
5.59	-	5.62	-	5.57	-	5.66	-	5.62	-	5.63	-	5.65	-	5.59	
5.52	-	5.52	-	5.46	-	5.50	-	5.52	-	5.55	-	5.55	-	5.52	
5.45	-	5.43	-	5.37	-	5.40	-	5.44	-	5.41	-	5.42	-	5.45	
5.30	-	5.31	-	5.27	-	5.27	-	5.27	-	5.30	-	5.32	-	5.30	
5.11	-	5.16	-	5.16	-	5.18	-	5.18	-	5.20	-	5.21	-	5.11	
6.20	-	6.20	-	6.38	x	6.31	-	6.28	x	6.19	-	6.20	-	6.20	
6.12	-	6.13	-	6.30	-	6.17	x	6.12	-	6.07	x	6.12	-	6.12	
6.03	-	6.00	-	6.12	-	6.08	-	6.06	-	6.04	-	6.04	-	6.03	
5.85	-	5.86	-	5.90	-	5.83	-	5.80	-	5.80	-	5.82	-	5.85	
5.75	-	5.74	-	5.69	-	5.75	-	5.72	-	5.72	-	5.73	-	5.75	
5.59	-	5.62	-	5.57	-	5.66	-	5.62	-	5.63	-	5.65	-	5.59	
5.52	-	5.52	-	5.46	-	5.50	-	5.52	-	5.55	-	5.55	-	5.52	
5.45	-	5.43	-	5.37	-	5.40	-	5.44	-	5.41	-	5.42	-	5.45	
5.30	-	5.31	-	5.27	-	5.27	-	5.27	-	5.30	-	5.32	-	5.30	
5.11	-	5.16	-	5.16	-	5.18	-	5.18	-	5.20	-	5.21	-	5.11	

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Incubated at room temperature

No NaCl		1% NaCl		2% NaCl		3% NaCl		4% NaCl		5% NaCl		6% NaCl	
pH	Growth	pH	Growth	pH	Growth	pH	Growth	pH	Growth	pH	Growth	pH	Growth
.38	x	6.31	x	6.28	x	6.19	x	6.20	-	6.20	-	6.20	-
.30	x	6.17	x	6.12	x	6.07	x	6.12	-	6.12	-	6.15	-
.12	x	6.08	x	6.06	x	6.04	x	6.04	-	6.03	-	6.00	-
.90	x	5.83	x	5.80	x	5.80	-	5.82	-	5.85	-	5.86	-
.69	x	5.75	x	5.72	x	5.72	-	5.73	-	5.75	-	5.74	-
.57	x	5.66	x	5.62	x	5.63	-	5.65	-	5.59	-	5.62	-
.46	x	5.50	x	5.52	x	5.55	-	5.55	-	5.52	-	5.52	-
.37	-	5.40	x	5.44	x	5.41	-	5.42	-	5.45	-	5.43	-
.27	-	5.27	x	5.27	x	5.30	-	5.32	-	5.30	-	5.31	-
.16	-	5.18	x	5.18	-	5.20	-	5.21	-	5.11	-	5.16	-
.38	x	6.31	x	6.28	x	6.19	-	6.20	-	6.20	-	6.20	-
.30	x	6.17	-	6.12	x	6.07	-	6.12	-	6.12	-	6.15	-
.12	x	6.08	x	6.06	-	6.04	-	6.04	-	6.03	-	6.00	-
.90	-	5.83	x	5.80	-	5.80	-	5.82	-	5.85	-	5.86	-
.69	-	5.75	x	5.72	-	5.72	-	5.73	-	5.75	-	5.74	-
.57	-	5.66	-	5.62	-	5.63	-	5.65	-	5.59	-	5.62	-
.46	-	5.50	-	5.52	-	5.55	-	5.55	-	5.52	-	5.52	-
.37	-	5.40	-	5.44	-	5.41	-	5.42	-	5.45	-	5.43	-
.27	-	5.27	-	5.27	-	5.30	-	5.32	-	5.30	-	5.31	-
.16	-	5.18	-	5.18	-	5.20	-	5.21	-	5.11	-	5.16	-
.38	x	6.31	-	6.28	x	6.19	-	6.20	-	6.20	-	6.20	-
.30	-	6.17	x	6.12	-	6.07	x	6.12	-	6.12	-	6.12	-
.12	-	6.08	-	6.06	-	6.04	-	6.04	-	6.03	-	6.00	-
.90	-	5.83	-	5.80	-	5.80	-	5.82	-	5.85	-	5.86	-
.69	-	5.75	-	5.72	-	5.72	-	5.73	-	5.75	-	5.74	-
.57	-	5.66	-	5.62	-	5.63	-	5.65	-	5.59	-	5.62	-
.46	-	5.50	-	5.52	-	5.55	-	5.55	-	5.52	-	5.52	-
.37	-	5.40	-	5.44	-	5.41	-	5.42	-	5.45	-	5.43	-
.27	-	5.27	-	5.27	-	5.30	-	5.32	-	5.30	-	5.31	-
.16	-	5.18	-	5.18	-	5.20	-	5.21	-	5.11	-	5.16	-

pH values of 5.20, 5.21, 5.59, and 6.13, respectively. As the salt concentration increased and the pH decreased, the heat resistance of the organism decreased. In the samples incubated at room temperature no growth was obtained with 5 or 6 per cent salt at any of the exposures. The results obtained with 1, 2, 3, and 4 per cent salt were very similar to those obtained at 37° C., except that the time required for gas production was greater with the samples incubated at room temperature.

Description of the organism. The organism causing gas production in cream cheese spreads could be grown only in peptone-litmus milk, in litmus milk to which a small amount of Roquefort type cheese had been added and in Roquefort type cheese emulsion so that many of the tests commonly used in the preparation of a description of an organism could not be made. Since the organism was very resistant to heat, it is tentatively placed in the genus Clostridium, even though spores were not observed. The name Clostridium peptophilum is proposed. The description of the organism is as follows:

Morphology:

Shape: Rod

Size: 0.7 by 2.0 to 2.5 microns

Spores: Spores not observed even with special spore stains

Staining reactions: Organism stained readily with ordinary stains; gram negative

Cultural characteristics:

Peptone-litmus milk: In tubes sealed with paraffine-vaseline plugs there was rapid reduction of the litmus and gas production.

Roquefort type cheese emulsion: In tubes sealed with paraffine-vaseline plugs gas was produced.

Growth requirements:

Oxygen relationship: Obligate anaerobe.

Growth temperature: Organism grew best at 37° C., but also grew at 21° C. and at 55° C.

Heat resistance: In peptone-litmus milk or Roquefort type cheese emulsion, at pH 6.50 the organism withstood 95° C. for 20 minutes but at pH 5.40 and below it did not withstand 80° C. for 20 minutes.

Studies on Liquefaction in Cream Cheese Spreads

Description of the defect. About the time that the outbreak of gassiness occurred, the same plant began having trouble with liquefaction in a few varieties of cream cheese spread, especially the Roquefort and pineapple spreads. This defect developed in about 3 to 4 weeks at room temperature and in about 5 to 10 days at 37° C. Only a small percentage of the jars in a batch showed liquefaction. The defect occurred in all degrees. At the one extreme only a small amount of liquid collected on the surface of the spread while at the other approximately one-half of the spread in a jar was liquefied. When liquefaction was extensive the liquid collected around the outside of the cheese spread and was translucent. In some instances enough gas was produced to release the vacuum seal, but in most cases the lid remained in place. When the lid was removed a very pronounced putrid odor was noted.

Microscopic examination of the liquefied spreads showed an abundance of organisms of various kinds, especially streptococci, molds, and gram positive spore bearing rods. The streptococci and molds were to be expected since the cheese spread contained cream cheese and Roquefort type cheese, but the large numbers of spore bearing rods suggested that the flora had been modified by the growth of organisms in the spread.

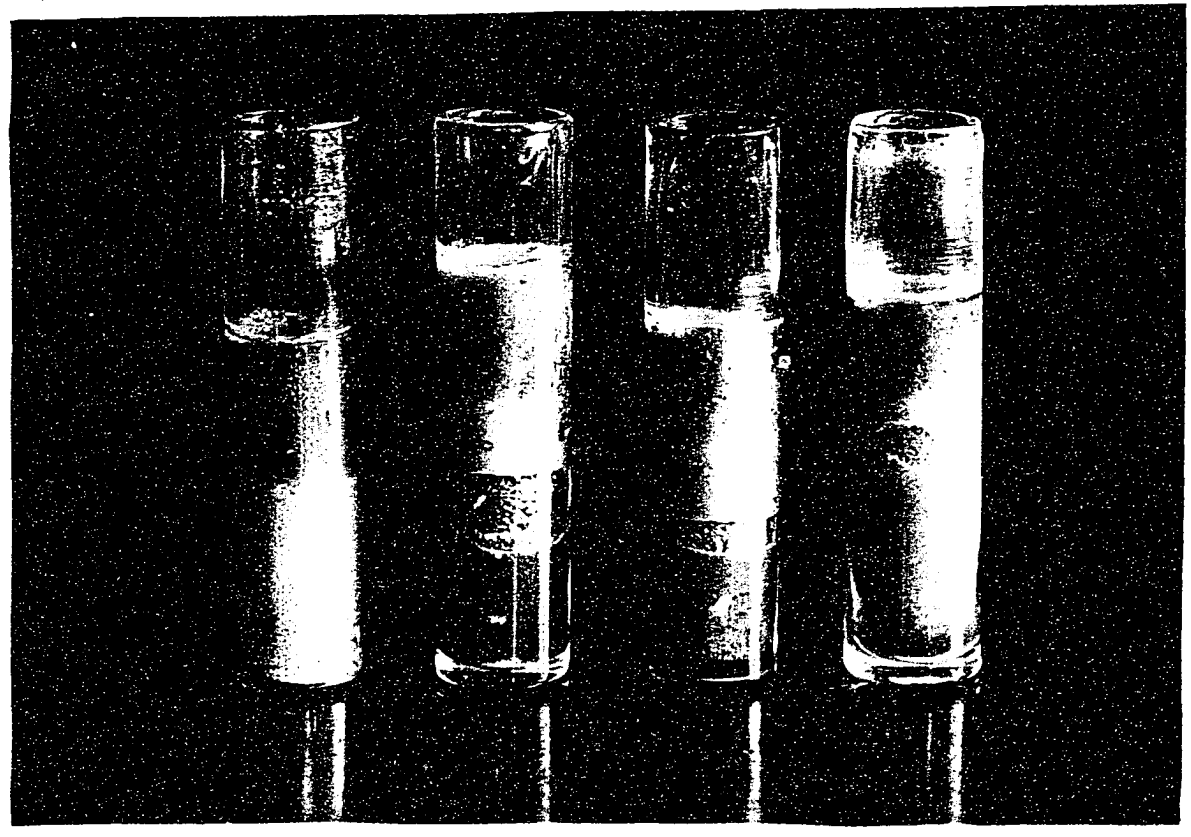
Isolation of the causative organism. From the manner in which the cream cheese spread was manufactured and packed it would be assumed that the liquefaction was the result of the action of an anaerobic spore forming organism.

Loopfulls of the liquid from a jar of liquefied cheese spread were placed in test tubes of litmus milk and covered with paraffine-vaseline plugs. The tubes were then heated at 85° C. for 20 minutes, cooled and incubated at 37° C. After 4 to 5 days the milk in most of the tubes was completely digested, leaving a dark brown liquid, while the remainder of the tubes were unchanged. The organisms in the tubes showing proteolysis were purified by removing a few drops from each with capillary pipettes and placing them in tubes containing approximately 9 cc. of litmus milk. One cc. of this was transferred to another tube of litmus milk. This general procedure was continued until a series of ten tubes was obtained, each containing approximately one-tenth as heavy an inoculation as the tube preceding it in the series. All of the tubes were covered with paraffine-vaseline plugs and held at 85° C. for 20 minutes, cooled and incubated at 37° C. The highest dilution that showed reduction of the litmus and then proteolysis was purified farther by the agar shake method. Beef infusion plus 1 per cent dextrose was used as the medium with this method. Tubes containing approximately 9 cc. of melted agar were cooled to about 45° C. A few drops of the culture to be purified were placed in one tube of the agar and thoroughly mixed with it. One cc. of this mixture was

transferred to the next tube and the procedure continued until a series of 10 tubes was obtained, each containing about one-tenth as heavy an inoculation as the tube just preceding it in the series. The medium was allowed to harden and the tubes incubated at 37° C. for 3 to 4 days. The bottom was broken from a tube having a few well defined colonies and colonies were picked into tubes of sterile litmus milk. The tubes were covered with paraffine-vaseline plugs and incubated at 37° C. Those tubes which showed reduction with subsequent digestion of the milk were considered to be pure cultures of the organism in question. Microscopic examinations showed gram positive spore bearing organisms which were about 0.7 by 4 to 5 microns in size. The spores were located in the center of the cells giving the organism a spindle shape.

Sixteen cultures of the liquefying organisms were isolated from as many different lots of defective cream cheese spread; 14 of the lots were Roquefort type spread and the other 2 were pineapple spread.

When sterile cream cheese spread was inoculated with one of the organisms and incubated at 37° C., proteolysis was apparent after 6 to 8 days, and the proteolysis continued until the cheese spread was completely surrounded by clear liquid just as it was in the original defective jars. In most cases a small amount of gas was produced. Figure II shows some tubes of sterile cheese spread which were inoculated with pure cultures of the liquefying organism and incubated at 37° C.



Uninoculated control	Inoculated with Org. C1-6	Inoculated with Org. C1-11	Inoculated with Org. C1-9

Figure II. Type of liquefaction obtained by inoculating sterile cream cheese spread with the organisms indicated.

Effect of salt (NaCl) concentration and the pH of the medium on the heat resistance of the liquefying organism. Preliminary studies showed that the pH alone or the pH plus the usual temperature used in preparing cheese spreads would not prevent liquefaction of sterile cream cheese inoculated with the organism isolated unless the pH was so low that it gave the spread an objectionable flavor. Two trials were carried out to determine whether or not a lowered pH in the presence of salt (NaCl) would reduce the heat resistance of the organism to the point where it would not liquefy inoculated cheese spread after exposure to heat.

In the first trial, Organism Cl-9 was used as the test organism. Each of seven flasks containing 1000 cc. of sterile litmus milk per flask were inoculated with 25 cc. of a 48 hour litmus milk culture of the organism to be studied. The pH of one of the flasks was determined and twelve 10 cc. samples were placed in sterile test tubes. A small amount of lactic acid was added to the remaining milk and thoroughly distributed, the pH was determined and twelve more 10 cc. samples were taken. This same general procedure was repeated until six series (each series having twelve tubes) were obtained. Each series differed from the other only in the pH. One, two, three, four, five, and six per cent sterile salt, respectively, was added to the other six inoculated flasks of litmus milk. Samples were taken from each of these flasks in the same manner as from the flask to which no salt was added. All of the tubes were covered with paraffine-vaseline plugs.

The seven salt concentrations and six pH series gave 42 combinations. Two tubes from each of these combinations were held unheated and two tubes were heated at 60°, 70°, 80°, 90° C. and in boiling water, respectively, for 20 minutes. One tube from each pair was incubated at 37° C. and the other held at room temperature. Observations were made at the end of 18 days on the tubes incubated at 37° C. and at the end of 35 days on the tubes incubated at room temperature. Reduction of the litmus with proteolysis was considered evidence of growth. The results are given in Table VIII.

The table shows that at 37° C. all of the tubes containing 3 per cent salt or less gave growth regardless of the pH or of the temperature to which they were exposed, while with 4 per cent or more salt the growth was irregular. With 4 per cent salt no growth occurred at pH 5.26 when the cultures were exposed to 60°, 80° C. or in boiling water for 20 minutes, while without heating and when exposed to 70° or 90° C. growth occurred at this same pH. With 5 per cent salt no growth took place at pH 5.40 or lower without heating or when exposed to 60° or 90° C., but did occur when heated at 70°, 80° C. or in boiling water for 20 minutes. With 6 per cent salt no growth occurred at pH 5.56 or lower when heated at 70° C. or in boiling water for 20 minutes and no growth resulted at a pH of 5.40 or lower when exposed to the other temperature used in the trial. When room temperature was used for incubation proteolysis was much slower in developing, and the results were even more irregular than they were in the samples incubated

THE EFFECT OF THE SALT (NaCl) CONCENTRATION AND T

Litmus milk, with salt added, inoculated
samples covered wi

Heat treatment	Incubated at 37° C.															
	No NaCl			1% NaCl			2% NaCl			3% NaCl			4% NaCl			5% NaCl		
	pH	Growth		pH	Growth		pH	Growth		pH	Growth		pH	Growth		pH	Growth	
	6.55	+		6.42	+		6.37	+		6.37	+		6.37	+		6.34	+	6
No	6.27	+		6.25	+		6.10	+		6.10	+		6.10	+		6.07	+	6
	5.95	+		5.95	+		5.87	+		5.85	+		5.85	+		5.82	+	5
heat	5.64	+		5.70	+		5.62	+		5.62	+		5.65	+		5.60	+	5
	5.40	+		5.45	+		5.42	+		5.42	+		5.41	+		5.40	-	5
	5.22	+		5.30	+		5.30	+		5.28	+		5.26	+		5.25	-	5
60° C.	6.55	+		6.42	+		6.37	+		6.37	+		6.37	+		6.34	+	6
	6.27	+		6.25	+		6.10	+		6.10	+		6.10	+		6.07	+	6
for 20	5.95	+		5.95	+		5.87	+		5.85	+		5.85	+		5.82	+	5
	5.64	+		5.70	+		5.62	+		5.62	+		5.65	+		5.60	+	5
min.	5.40	+		5.45	+		5.42	+		5.42	+		5.41	+		5.40	-	5
	5.22	+		5.30	+		5.30	+		5.28	+		5.26	-		5.25	-	5
70° C.	6.55	+		6.42	+		6.37	+		6.37	+		6.37	+		6.34	+	6
	6.27	+		6.25	+		6.10	+		6.10	+		6.10	+		6.07	+	6
for 20	5.95	+		5.95	+		5.87	+		5.85	+		5.85	+		5.82	+	5
	5.64	+		5.70	+		5.62	+		5.62	+		5.65	+		5.60	+	5
min.	5.40	+		5.45	+		5.42	+		5.42	+		5.41	+		5.40	+	5
	5.22	+		5.30	+		5.30	+		5.28	+		5.26	+		5.25	-	5

+ gas production and proteolysis. - no gas production or proteolysi

TABLE VIII

NCENTRATION AND THE pH OF TH E MEDIUM ON THE HEAT RESISTANCE OF LIQUEFYING ORGANISM C1-9

dded, inoculated with 48 hou r litmus milk culture, pH adjusted with lactic acid, amples covered with paraffin e-vaseline plugs and heated.

								Incubated at room temperature														
5% NaCl			6% NaCl			No NaCl			1% NaCl			2% NaCl			3% NaCl			4% NaCl			
h	pH	Growth	pH	Growth		pH	Growth		pH	Growth		pH	Growth		pH	Growth		pH	Growth		pH	
	6.34	+	6.36	+		6.55	+		6.42	+		6.37	+		6.37	+		6.37	+		6.3	
	6.07	+	6.05	+		6.27	+		6.25	+		6.10	+		6.10	+		6.10	+		6.0	
	5.82	+	5.81	+		5.95	+		5.95	+		5.87	+		5.85	+		5.85	+		5.8	
	5.60	+	5.56	+		5.64	+		5.70	+		5.62	+		5.62	+		5.65	-		5.0	
	5.40	-	5.40	-		5.40	+		5.45	+		5.42	+		5.42	+		5.41	+		5.4	
	5.25	-	5.27	-		5.22	+		5.30	+		5.30	+		5.28	+		5.26	+		5.2	
	6.34	+	6.36	+		6.55	+		6.42	+		6.37	+		6.37	+		6.37	+		6.3	
	6.07	+	6.50	+		6.27	+		6.25	+		6.10	+		6.10	+		6.10	+		6.0	
	5.82	+	5.81	+		5.95	+		5.95	+		5.87	-		5.85	+		5.85	+		5.8	
	5.60	+	5.56	+		5.64	+		5.70	+		5.62	-		5.62	-		5.65	-		5.0	
	5.40	-	5.40	-		5.40	+		5.45	+		5.42	+		5.42	-		5.41	-		5.4	
	5.25	-	5.27	-		5.22	+		5.30	+		5.30	-		5.28	+		5.26	-		5.2	
	6.34	+	6.36	+		6.55	+		6.42	+		6.37	+		6.37	+		6.37	+		6.3	
	6.07	+	6.05	+		6.27	+		6.25	+		6.10	+		6.10	+		6.10	+		6.0	
	5.82	+	5.81	+		5.95	+		5.95	+		5.87	+		5.85	+		5.85	+		5.8	
	5.60	+	5.56	-		5.64	+		5.70	+		5.62	+		5.62	-		5.65	+		5.0	
	5.40	+	5.40	-		5.40	+		5.45	+		5.42	-		5.42	+		5.41	+		5.4	
	5.25	-	5.27	-		5.22	+		5.30	+		5.30	-		5.28	+		5.26	+		5.2	

tion or proteolysis.

:I

EDIUM ON THE HEAT RESISTANCE OF LIQUEFYING ORGANISM C1-9

.itmus milk culture, pH adjusted with lactic acid,
aseline plugs and heated.

Incubated at room temperature															
No NaCl		1% NaCl		2% NaCl		3% NaCl		4% NaCl		5% NaCl		6% NaCl			
pH	Growth	pH	Growth	pH	Growth	pH	Growth	pH	Growth	pH	Growth	pH	Growth		
6.55	+	6.42	+	6.37	+	6.37	+	6.37	+	6.34	+	6.36	+		
6.27	+	6.25	+	6.10	+	6.10	+	6.10	+	6.07	+	6.05	+		
5.95	+	5.95	+	5.87	+	5.85	+	5.85	+	5.82	+	5.81	+		
5.64	+	5.70	+	5.62	+	5.62	+	5.65	-	5.60	+	5.56	-		
5.40	+	5.45	+	5.42	+	5.42	+	5.41	+	5.40	-	5.40	-		
5.22	+	5.30	+	5.30	+	5.28	+	5.26	+	5.25	-	5.27	-		
6.55	+	6.42	+	6.37	+	6.37	+	6.37	+	6.34	+	6.36	+		
6.27	+	6.25	+	6.10	+	6.10	+	6.10	+	6.07	+	6.05	+		
5.95	+	5.95	+	5.87	-	5.85	+	5.85	+	5.82	+	5.81	+		
5.64	+	5.70	+	5.62	-	5.62	-	5.65	-	5.60	+	5.56	+		
5.40	+	5.45	+	5.42	+	5.42	-	5.41	-	5.40	-	5.40	-		
5.22	+	5.30	+	5.30	-	5.28	+	5.26	-	5.25	-	5.27	-		
6.55	+	6.42	+	6.37	+	6.37	+	6.37	+	6.34	+	6.36	+		
6.27	+	6.25	+	6.10	+	6.10	+	6.10	+	6.07	+	6.05	-		
5.95	+	5.95	+	5.87	+	5.85	+	5.85	+	5.82	-	5.81	+		
5.64	+	5.70	+	5.62	+	5.62	-	5.65	+	5.60	+	5.56	-		
5.40	+	5.45	+	5.42	-	5.42	+	5.41	+	5.40	-	5.40	-		
5.22	+	5.30	+	5.30	-	5.28	+	5.26	+	5.25	-	5.27	-		

TABLE V

Heat treatment	Incubated at 37° C.															
	No NaCl		1% NaCl		2% NaCl		3% NaCl		4% NaCl		5% NaCl					
	pH	Growth	pH	Growth	pH	Growth	pH	Growth	pH	Growth	pH	Growth				
80° C.	6.55	+	6.42	+	6.37	+	6.37	+	6.37	+	6.34	+	6			
	6.27	+	6.25	+	6.10	+	6.10	+	6.10	+	6.07	+	6			
for 20	5.95	+	5.95	+	5.87	+	5.85	+	5.85	+	5.82	+	5			
	5.64	+	5.70	+	5.62	+	5.62	+	5.65	+	5.60	+	5			
min.	5.40	+	5.45	+	5.42	+	5.42	+	5.41	+	5.40	+	5			
	5.22	+	5.30	+	5.30	+	5.28	+	5.26	-	5.25	-	5			
90° C.	6.55	+	6.42	+	6.37	+	6.37	+	6.37	+	6.34	+	6			
	6.27	+	6.25	+	6.10	+	6.10	+	6.10	+	6.07	+	6			
for 20	5.95	+	5.95	+	5.87	+	5.85	+	5.85	+	5.82	+	5			
	5.64	+	5.70	+	5.62	+	5.62	+	5.65	+	5.60	+	5			
min.	5.40	+	5.45	+	5.42	+	5.42	+	5.41	+	5.40	-	5			
	5.22	+	5.30	+	5.30	+	5.28	+	5.26	+	5.25	-	5			
Boiling	6.55	+	6.42	+	6.37	+	6.37	+	6.37	+	6.34	+	6			
water	6.27	+	6.25	+	6.10	+	6.10	+	6.10	+	6.07	+	6			
for 20	5.95	+	5.95	+	5.87	+	5.85	+	5.85	+	5.82	+	5			
min.	5.64	+	5.70	+	5.62	+	5.62	+	5.65	+	5.60	+	5			
	5.40	+	5.45	+	5.42	+	5.42	+	5.41	+	5.40	+	5			
	5.22	+	5.30	+	5.30	+	5.28	+	5.26	-	5.25	-	5			

TABLE VIII (CONTINUED)

5% NaCl		6% NaCl		Incubated at room temperature													
				No NaCl		1% NaCl		2% NaCl		3% NaCl		4% NaCl		5			
pH	Growth	pH	Growth	pH	Growth	pH	Growth	pH	Growth	pH	Growth	pH	Growth	pH	Growth	pH	Growth
6.34	+	6.36	+	6.55	+	6.42	+	6.37	+	6.37	+	6.37	+	6.3			
6.07	+	6.05	+	6.27	+	6.25	+	6.10	+	6.10	+	6.10	+	6.0			
5.82	+	5.81	+	5.95	+	5.95	+	5.87	+	5.86	+	5.85	+	5.8			
5.60	+	5.56	+	5.64	+	5.70	+	5.62	+	5.62	+	5.65	+	5.6			
5.40	+	5.40	-	5.40	+	5.45	+	5.42	+	5.42	-	5.41	+	5.4			
5.25	-	5.27	-	5.22	+	5.30	+	5.30	+	5.28	+	5.26	-	5.2			
6.34	+	6.36	+	6.55	+	6.42	+	6.37	+	6.37	+	6.37	+	6.3			
6.07	+	6.05	+	6.27	+	6.25	+	6.10	+	6.10	+	6.10	+	6.0			
5.82	+	5.81	+	5.95	+	5.95	+	5.87	+	5.85	+	5.86	+	5.8			
5.60	+	5.56	+	5.64	+	5.70	+	5.62	+	5.62	+	5.65	+	5.6			
5.40	-	5.40	-	5.40	+	5.45	+	5.42	-	5.42	+	5.41	-	5.4			
5.25	-	5.27	-	5.22	+	5.30	+	5.30	+	5.28	+	5.26	-	5.2			
6.34	+	6.36	+	6.55	+	6.42	+	6.37	+	6.37	+	6.37	+	6.3			
6.07	+	6.05	+	6.27	+	6.25	+	6.10	+	6.10	+	6.10	+	6.0			
5.82	+	5.81	+	5.95	+	5.95	+	5.87	+	5.85	+	5.85	+	5.8			
5.60	+	5.56	-	5.64	+	5.70	+	5.62	+	5.62	+	5.65	+	5.6			
5.40	+	5.40	-	5.40	+	5.45	+	5.42	+	5.42	+	5.41	+	5.4			
5.25	-	5.27	-	5.22	+	5.30	+	5.30	+	5.28	+	5.26	-	5.2			

Incubated at room temperature

aCl	1% NaCl		2% NaCl		3% NaCl		4% NaCl		5% NaCl		6% NaCl	
rowth	pH	Growth	pH	Growth	pH	Growth	pH	Growth	pH	Growth	pH	Growth
+	6.42	+	6.37	+	6.37	+	6.37	+	6.34	+	6.36	+
+	6.25	+	6.10	+	6.10	+	6.10	+	6.07	+	6.05	+
+	5.95	+	5.87	+	5.85	+	5.85	+	5.82	+	5.81	-
+	5.70	+	6.62	+	5.62	+	5.65	+	5.60	-	5.56	-
+	5.45	+	5.42	+	5.42	-	5.41	+	5.40	-	5.40	-
+	5.30	+	5.30	+	5.28	+	5.26	-	5.25	-	5.27	-
+	6.42	+	6.37	+	6.37	+	6.37	+	6.34	+	6.36	+
+	6.25	+	6.10	+	6.10	+	6.10	+	6.07	+	6.05	-
+	5.95	+	5.87	+	5.85	+	5.85	+	5.82	+	5.81	-
+	5.70	+	5.62	+	5.62	+	5.65	+	5.60	-	5.56	-
+	5.45	+	5.42	-	5.42	+	5.41	-	5.40	-	5.40	-
+	5.30	+	5.30	+	5.28	+	5.26	-	5.25	-	5.27	-
+	6.42	+	6.37	+	6.37	+	6.37	+	6.34	+	6.36	+
+	6.25	+	6.10	+	6.10	+	6.10	+	6.07	+	6.05	-
+	5.95	+	5.87	+	5.85	+	5.85	+	5.82	+	5.81	+
+	5.70	+	5.62	+	5.62	+	5.65	+	5.60	+	5.56	-
+	5.45	+	5.42	+	5.42	+	5.41	+	5.40	-	5.40	-
+	5.30	+	5.30	+	5.28	+	5.26	-	5.25	-	5.27	-

at 37° C. All of the tubes containing no salt or 1 per cent salt showed proteolysis but several tubes in the higher salt concentrations did not, and with some of these the duplicate tubes at 37° C. did show proteolysis. All of the exposures which permitted proteolysis at room temperature also permitted it at 37° C.

Another trial in which Organism C1-C was used, was made in the same manner as the first, except that tubes were not incubated at room temperature. No salt, 2, 4, 6, 8, and 10 per cent salt, respectively, were added to the 6 flasks of inoculated litmus milk. Seven pH series were employed, ranging from pH 6.60 to 4.92. The exposures employed were: no heat, 60°, 70°, 80°, and 90° C. for 20 minutes. After incubation at 37° C. for 16 days the tubes which showed proteolysis were recorded and those which did not show proteolysis were cultured in fresh litmus milk. These transfers were covered with paraffine-vaseline plugs and incubated at 37° C. for 8 days. The results of the trial are given in Table IX and Graph III.

When no salt was added, proteolysis occurred at a pH as low as 4.92, but with 2 per cent salt the lowest pH at which proteolysis occurred was 5.39. As the salt concentration increased the pH required to prevent proteolysis increased until with 10 per cent salt proteolysis did not occur at pH 6.06 or lower. Exactly the same results were obtained at all of the temperatures studied, ranging from no heat to 90° C. for 20 minutes. When those tubes which did not show proteolysis were cultured in normal litmus milk all of them produced proteolysis, indicat-

TABLE IX

THE EFFECT OF THE SALT (NaCl) CONCENTRATION AND THE pH OF THE MEDIUM ON THE HEAT RESISTANCE OF LIQUEFYING ORGANISM C1-6

Litmus milk, with salt added, inoculated with 48 hour litmus milk culture, pH adjusted with lactic acid, samples covered with paraffine-vaseline plugs and heated.

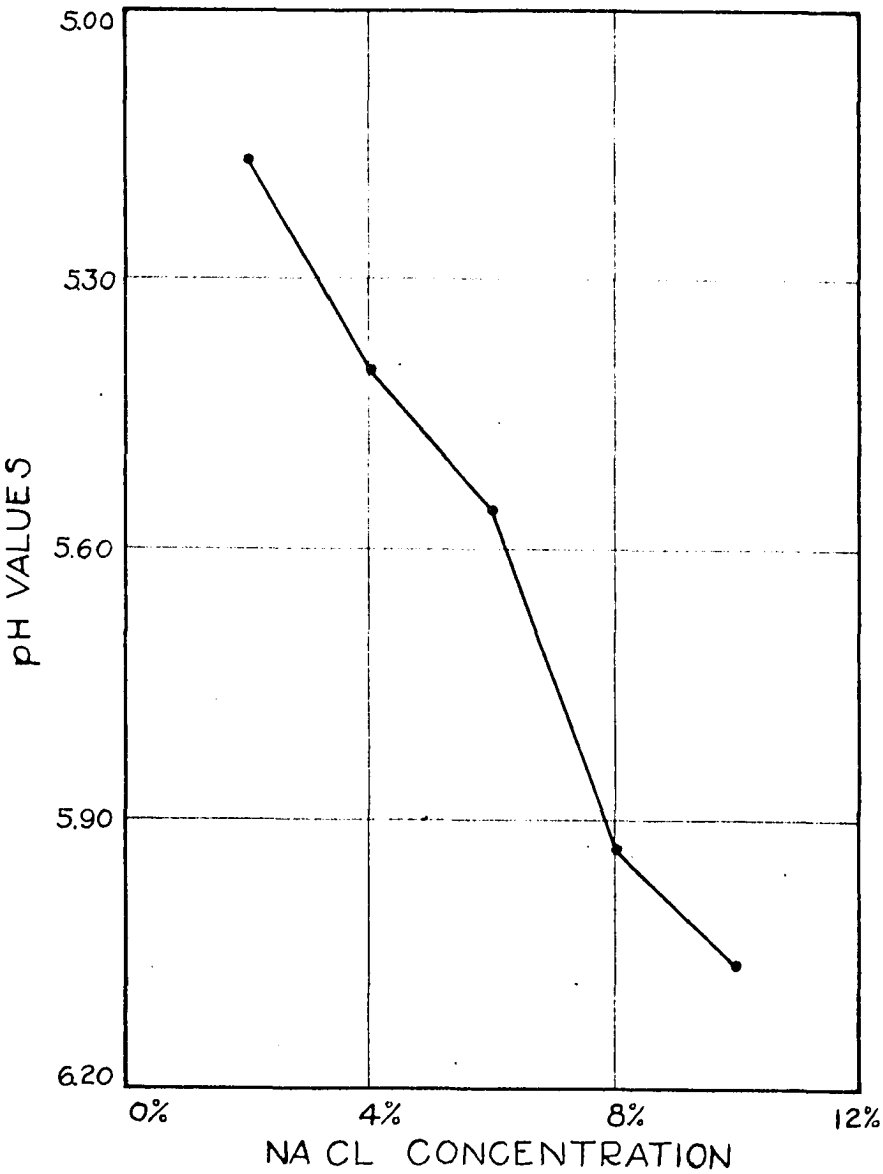
Temperature of incubation 37° C.

Heat treatment	No NaCl		2% NaCl		4% NaCl		6% NaCl		8% NaCl		10% NaCl	
	pH	Growth	pH	Growth	pH	Growth	pH	Growth	pH	Growth	pH	Growth
No heat	6.60	x	6.38	x	6.31	x	6.30	x	6.30	x	6.24	x
	6.42	x	6.22	x	6.17	x	6.07	x	6.07	x	6.06	o
	6.05	x	5.96	x	5.85	x	5.93	x	5.92	o	5.90	o
	5.75	x	5.80	x	5.66	x	5.68	x	5.68	o	5.65	o
	5.49	x	5.58	x	5.57	x	5.55	o	5.57	o	5.55	o
	5.23	x	5.39	x	5.40	o	5.35	o	5.39	o	5.36	o
	4.92	x	5.18	o	5.10	o	5.18	o	5.18	o	5.21	o
60° C. for 20 min.	6.60	x	6.38	x	6.31	x	6.30	x	6.30	x	6.24	x
	6.42	x	6.22	x	6.17	x	6.07	x	6.07	x	6.06	o
	6.05	x	5.96	x	5.85	x	5.93	x	5.92	o	5.90	o
	5.75	x	5.80	x	5.66	x	5.68	x	5.68	o	5.65	o
	5.49	x	5.58	x	5.57	x	5.55	o	5.57	o	5.55	o
	5.23	x	5.39	x	5.40	o	5.35	o	5.39	o	5.36	o
	4.92	x	5.18	o	5.10	o	5.18	o	5.18	o	5.21	o

x gas production and proteolysis in the original sample tube.
o gas production and proteolysis only after transferring to a fresh tube of litmus milk containing no salt and having a normal pH.

TABLE IX (CONTINUED)

Heat treatment	No NaCl		2% NaCl		4% NaCl		6% NaCl		8% NaCl		10 NaCl	
	pH	Growth	pH	Growth	pH	Growth	pH	Growth	pH	Growth	pH	Growth
70° C. for 20 min.	6.60	x	6.38	x	6.31	x	6.30	x	6.30	x	6.34	x
	6.42	x	6.22	x	6.17	x	6.07	x	6.07	x	6.06	o
	6.05	x	5.96	x	5.85	x	5.93	x	5.92	o	5.90	o
	5.75	x	5.80	x	5.66	x	5.68	x	5.68	o	5.65	o
	5.49	x	5.58	x	5.57	x	5.55	o	5.57	o	5.55	o
	5.23	x	5.39	x	5.40	o	5.35	o	5.39	o	5.36	o
	4.92	x	5.18	o	5.10	o	5.18	o	5.18	o	5.21	o
80° C. for 20 min.	6.60	x	6.38	x	6.31	x	6.30	x	6.30	x	6.34	x
	6.42	x	6.22	x	6.17	x	6.07	x	6.07	x	6.06	o
	6.05	x	5.96	x	5.85	x	5.93	x	5.92	o	5.90	o
	5.75	x	5.80	x	5.66	x	5.68	x	5.68	o	5.65	o
	5.49	x	5.58	x	5.57	x	5.55	o	5.57	o	5.55	o
	5.23	x	5.39	x	5.40	o	5.35	o	5.39	o	5.36	o
	4.92	x	5.18	o	5.10	o	5.18	o	5.18	o	5.21	o
90° C. for 20 min.	6.60	x	6.38	x	6.31	x	6.30	x	6.30	x	6.34	x
	6.42	x	6.22	x	6.17	x	6.07	x	6.07	x	6.06	o
	6.05	x	5.96	x	5.85	x	5.93	x	5.92	o	5.90	o
	5.75	x	5.80	x	5.66	x	5.68	x	5.68	o	5.65	o
	5.49	x	5.58	x	5.57	x	5.55	o	5.57	o	5.55	o
	5.23	x	5.39	x	5.40	o	5.35	o	5.39	o	5.36	o
	4.92	x	5.18	o	5.10	o	5.18	o	5.18	o	5.21	o



Graph III. Combinations of pH values and salt concentration required to prevent proteolysis by the liquefying Organism C1-6.

ing that the organism had not been killed by the exposure, but was only inhibited by the high salt and low pH.

Description of the organism. The organism that was found to be responsible for liquefaction in cream cheese spreads was studied morphologically, culturally, and biochemically. It was an anaerobic, spore forming organism which was 0.7 by 4 or 5 microns in size and motile by means of peritrichous flagella. Most of the strains formed acid and gas from levulose, galactose, dextrose, maltose, mannitol, dextrin, and glycerol; and a few strains also formed acid and gas from arabinose, salicin and starch. From the results of the study, the organism was identified as Clostridium sporogenes. It should be noted that Csiszar (7 and 8) found Clostridium sporogenes to be the organism most commonly responsible for spoilage in process cheese.

SUMMARY AND CONCLUSIONS

As was to be expected, the outbreak of gas formation in cream cheese spreads was caused by a heat resistant anaerobic organism. The organism was very unusual in its growth requirements, since it could be grown only in peptone-litmus milk, in litmus milk to which a small amount of Roquefort type cheese had been added and in Roquefort type cheese emulsion.

Nineteen cultures of the gas producing organism were isolated; 12 came from 12 different lots of defective Roquefort type cream cheese spread, 5 from domestic blue cheese, and 1 each from Danish bleu and French bleu. The fact that the organism was isolated from French bleu and Danish bleu cheese disproved the theory that the gassiness in cream cheese spreads was caused by an organism present in domestic blue but not present in the imported cheeses.

The gas producing organism was found to be very heat resistant in peptone-litmus milk at high pH values, but as the pH was lowered the heat resistance was also lowered. The addition of salt in concentrations over 2 per cent decreased the heat resistance of the organism at a given pH. Since Roquefort type cheese contains 4 to 5 per cent salt, the addition of enough acid to lower the pH to about 5.40, together with the holding of the cheese at 85° C. for 20 minutes, should enable the manufacturers of Roquefort type cheese spreads to make spread in which

gas production does not occur, even though the organism is known to be present in the cheese.

Since the organism was so heat resistant, it was placed in the genus Clostridium, even though spores were never observed. The name Clostridium peptophilum is proposed for it. The description of the organism is very meager because it could be grown in only a very few media.

Liquefaction in cream cheese spreads was also found to be due to the action of a heat resistant anaerobic organism. The organism was identified as Clostridium sporogenes.

Clostridium sporogenes was not killed by heating at 95° C. for 20 minutes in litmus milk with a pH of 5.21 and a salt concentration of 10 per cent. It was possible to inhibit the digestion of the milk by combinations of salt concentration and pH, but in all cases the salt concentration and pH values which would inhibit digestion were too low to be used commercially in cream cheese spreads.

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