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Bacteriological studies on Swiss-type cheese from pasteurized milk

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BACTERIOLOGICAL STUDIES ON SWISS-TYPE CHEESE FROM PASTEURIZED MILK

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INTRODUCTION

Swiss or Emmenthal cheese is a hard, rennet variety characterized by a sweetish flavor and by gas holes or eyes dispersed throughout the cheese. The normal holes of Swiss cheese vary considerably in size and the distances between them also vary. Several types of cheese bear a close resemblance to Swiss. Perhaps the best known is Gruyere, which is a Swiss-type cheese made in Switzerland, Austria and Italy. It is slightly softer than the regular Swiss cheese and is ripened in about 4 months.

Swiss-type cheese made in the United States has often lacked the characteristic sweetish flavor typical of good cheese, and the eye formation has frequently been irregular and undesirable. The inability of cheesemakers of the United States to make most of their Swiss cheese output meet the requirements for flavor and eye formation that are specified for good quality cheese has placed imported cheese at a premium over the domestic cheese.

Cheesemakers in the United States are not alone in experiencing difficulty in the manufacture of Swiss cheese. The literature shows that various defects have been encountered in other countries which have been making Swiss cheese for long periods. Those more frequently noted are poor eye formation and improper flavor development.

In the past, Swiss-type cheese has been manufactured largely from raw milk. Recently, pasteurized milk has been suggested for use since it should tend to eliminate certain defects due to objectionable organisms. Pasteurized milk for Swiss cheese has been investigated primarily in certain European countries, but a manufacturing procedure for Iowa Swiss-type cheese, which involves the use of pasteurized milk, has been published by Goss, Nielsen and Mortensen (37). The procedure for Iowa Swiss-type cheese involves the use of a vat instead of a kettle and does not make use of propionic acid cultures.

STATEMENT OF PROBLEM

The purpose of this investigation was to improve the general quality of Swiss-type cheese made from pasteurized milk. The points studied particularly were:

(1) The importance of the propionic acid bacteria in the ripening of the cheese, both from the flavor and eye formation standpoints.

(2) The influence of various factors on the development of these organisms.

(3) The changes produced in the cheese through the action of these organisms.

HISTORICAL

Isolation of Propionic Acid Bacteria.

von Freudenreich and Orla-Jensen (33) were the first investigators to isolate the causal organism of the regular propionic acid fermentation in Swiss cheese. They used an enrichment procedure which consisted of inoculating the test material into a peptone calcium lactate broth. Transfers were made to gelatine plates and shake cultures. The propionic acid organisms grew better in shake cultures made with whey agar than when calcium lactate agar was used.

Trioli-Pettersson (56) found it difficult to isolate propionic acid bacteria by using the whey agar of von Freudenreich and Orla-Jensen. An alkaline whey agar, using a long incubation period, was more satisfactory.

Sherman (52) criticized the medium used for enrichment by von Freudenreich and Orla-Jensen. He suggested that sodium lactate be used in place of calcium lactate because the latter forms a heavy precipitate of calcium phosphate upon sterilization. When calcium lactate was used the pH of the medium was 5.2 after sterilization while with sodium lactate it was 7.2. Sherman used a medium composed of peptone, yeast extract, lactic acid (as sodium lactate) and agar for the isolation of propionic acid bacteria.

Van Niel (58) obtained better development of propionic acid bacteria in enrichment liquids when yeast extract was used in the place of peptone. He neutralized the medium to pH 7.0 because the sodium lactate contained free lactic acid.

Bacterial Flora of Swiss-type Cheese.

The ripening of Emmenthal cheese in a reduced atmosphere was studied by von Freudenreich and Schaffer (34). Cheese ripened under these conditions was more or less swollen, soft and bitter. It was concluded that the ripening process goes on throughout the whole mass simultaneously and is not the result of the action of enzymes produced by bacteria growing at the surface.

von Freudenreich (29) conducted a series of experiments using *Thyrothrix* organisms which were believed by Duclaux to be the chief cause of ripening with Emmenthal cheese. Large numbers of these organisms were inoculated into the curd but soon disappeared. Then von Freudenreich attempted to prove that changes in the casein during ripening were due to lactic acid bacteria. By using various lactic acid cultures in skimmilk he found that these organisms caused a large increase in soluble protein. Lactic acid was also added to skimmilk but it failed to increase the soluble protein. The change in casein was therefore considered to be due to the organisms and not to the acid produced.

In another experiment von Freudenreich (30) inoculated skimmilk with lactic acid bacteria and after 4 weeks filtered the culture to obtain the serum. Freshly filtered skimmilk contained 0.033 per cent nitrogen while the filtrate from the culture contained 0.156 per cent, showing that casein had been changed to a soluble form. A chemical examination of the filtrate showed that the nitrogen of the soluble proteins was largely in the form of amides. Since practically only lactic acid producing organisms were found during the ripening, and other organisms occurred only in small numbers, it appeared that the lactic acid organisms were the cause of ripening.

Orla-Jensen (46) found that lactic acid bacteria exercised an indirect influence in cheese ripening by restraining gassy fermentation and putrefaction and a direct influence on the decalcification of the paracasein and on the decomposition of the albuminoid substances. In the early stages of acidification an unrecognized Streptococcus was the important organism. Later in the ripening process Bacillus casei ε predominated. This organism reached its maximum development soon after the cheese was made. The author stated this fact confirmed his previous view that the ripening of hard cheese is due to enzymes liberated by the disintegration of bacterial bodies which were present in the cheese during the first few days.

von Freudenreich and Orla-Jensen (32) succeeded in isolating the causal organism of the propionic acid fermentation. It was named Bacterium acidi propionici. The organism was able to ferment lactic acid with the production of propionic acid, acetic acid and carbon dioxide.

Three varieties of aerobic bacteria which decomposed glycerol were found in Swedish farm cheese by Trioli-Pettersson (56). Three other species were found which produced propionic and acetic acids from calcium lactate. The organisms were present in larger numbers in old than in young cheese. One variety grew only in the absence of oxygen and was variable in form.

Thöni (54) noted that cocci usually predominated in 1-day old cheese but after a time decreased rapidly. The decrease was followed by an increase in lactic acid rods which eventually made up 80 to 100 per cent of the flora. In small cheese the lactic acid rods predominated even when the fresh curd was examined.

Three domestic cheese of the Emmenthal-type were followed through most of a ripening period by Eldredge and Rogers (19). About 1,000 cultures were isolated and studied in detail. It was not possible to separate these cultures beyond three morphologic groups, one of which was a long rod, one a short rod and one a coccus. At the beginning the bacterial flora consisted almost entirely of short rods. The long rods appeared in the early stages of ripening and increased steadily. The short rods decreased in numbers and at 7 or 8 weeks, a period corresponding to the end of eye formation, made up about 50 per cent of the flora. Glycerol fermenting cocci appeared in small numbers after 5 or 6 weeks. At the end of 20 weeks the flora was composed almost exclusively of long rods.

Burri and Staub (10) examined 20 good quality Emmenthal cheese and found that during the ripening there were present from 10 to 100 million living bacteria per gram. These organisms were chiefly Bacterium casei α and Bacterium casei δ; the ratio of the two species was variable. Bacterium casei ε was isolated from a 3-month old cheese but not from cheese which was 5 to 6 months old.

Frazier and Wing (26) made bacterial counts on Swiss cheese at different stages during manufacture and ripening. Certain tetracocci and small streptobacilli usually predominated during the period of eye formation, and Bacterium acidi propionici types usually predominated after eye formation was complete.

Demeter and Schmid (14) obtained rather uniformly high counts of lactic acid bacteria from milk used for Emmenthal cheese. In the early stages of curdling the streptococci increased more than the lactobacilli. In the course of scalding the streptococci decreased and the numbers of long rods remained constant. The high temperature of the cheese during pressing caused an increase in thermophilic streptococci and lactobacilli. During ripening the rods decreased uniformly and the streptococci decreased for the first 3 weeks and then again increased. Streptococcus lactis types completely receded when the cheese was placed in the salt bath. At the same time Streptococcus thermophilus reached its high point. Streptococci appeared in the third week of ripening and increased until the seventh week; a decrease then occurred. Streptobacteria appeared to play an important role in the ripening after the seventh week.

Demeter and Schmid (15) isolated bacteria from Emmenthal cheese and classified them according to the system suggested by Orla-Jensen. The following organisms were found: Streptococcus thermophilus, a Streptococcus closely related to Streptococcus faecium, a Betacoccus, Tetracoccus liquefaciens, Tetracoccus casei, Thermobacterium helveticum, Thermobacterium lactis, Streptobacterium casei and Streptobacterium plantarum. Acid-proteolytic bacteria were found so rarely that their importance in the ripening of Emmenthal cheese was questioned.

According to Babel and Hammer (3) the correlation between the sweet flavor of Swiss-type cheese and the presence of large numbers of propionic acid organisms suggests that certain products formed by the organisms are of special significance as flavor contributors. Swiss-type cheese with considerable of the characteristic sweet flavor yielded a much higher volatile acidity on steam distillation than cheese lacking in flavor. Addition of calcium propionate to process Swiss-type cheese lacking in flavor gave the product a sweet flavor. The results suggest that propionates, of which calcium propionate is presumably the most significant, are important flavor contributors of Swiss-type cheese.

Eye Formation in Swiss-type Cheese.

Orla-Jensen (45) concluded from his studies on the formation of eyes in Emmenthal cheese that the normal eyes were not produced by the agents causing normal ripening, that is by lactic acid producing organisms and that the gas, which is the immediate cause of the eyes, is not produced from the milk sugar but from nitrogenous substances. The author believed that the lactic acid organisms of cheese under certain conditions formed traces of carbon dioxide from nitrogenous substances and that these traces were the cause of normal eye formation.

von Freudenreich and Orla-Jensen (32) found that lactic acid in Emmenthal cheese was fermented according to the following equation:

$$3\ CH_3.CHOH.COOH \rightarrow 2\ CH_3.CH_2.COOH + CH_3.COOH + CO_2 + H_2O$$

The carbon dioxide liberated by this fermentation was said to be the cause of normal eye formation.

Studies were made on the salt tolerance of propionic acid bacteria by Orla-Jensen (48); 0.5 per cent salt checked the development of the organisms and 10 per cent prevented their growth entirely. These results apparently explain why the outer layers of large Emmenthal cheese and also small cheese contain fewer eyes than the central part of large cheese.

Trioli-Pettersson (57) reported normal eye formation in cheese inoculated with lactic acid bacteria, liquefying cocci and *Bacterium glycerini*. In two cases normal eyes were obtained by adding propionic acid bacteria and liquefying cocci, however, when the cheese was well ripened the characteristic taste was less pronounced than normal cheese.

The large numbers of *Bacillus casei* ε found in Emmenthal cheese led Burri and Staub (10) to advance the question as to whether this organism plays the main role in the ripening process. Since propionic acid bacteria also occur in large numbers, the authors were undecided as to whether this gas forming group or *Bacillus casei* ε, or both cause the eye formation.

Clark (12) reviewed the literature on eye formation and found little or no evidence that the eyes of Emmenthal cheese are strictly localized at points of excessive bacterial growth. He believed that the gas produced in Emmenthal cheese separated in aggregates whose location had no necessary relation to the place where the gas was produced. Rapid gas production was considered to result in the formation of numerous small eyes and slow gas production in the formation of large eyes.

Sherman (52) examined 16 samples of American-made Swiss cheese and found the lactate fermenting organism in 0.000,001 gram, the smallest amount used. He stated that the organism responsible for the eye formation and the characteristic flavor was clearly established and gave a description of it. Cheese made in the laboratory from milk inoculated with this organism further demonstrated to him that it was the cause of eyes and the characteristic sweetish flavor.

Frazier and Wing (25) stated that in the past 5 ml. of a broth culture of propionic acid bacteria had been added to 100 pounds of milk intended for Swiss cheese, regardless of the age of the culture. Counts made on these broth cultures showed that the bacteria varied in numbers from 8 or 9 billion per ml. in 5-day cultures to about 6 million after 1 month and about 2 million after 2 months. The older cultures survived heat treatment better than young cultures. A study of the organisms in cheese at different stages during manufacture and ripening showed that they did not decrease markedly during storage of the cheese in the cold room (about 12°C.) for 14 days. After the cheese was removed to the warm room (about 22°C.) there was little increase in numbers until the beginning of eye formation, when the cheese was 3 or 4 weeks old. At the beginning of eye formation the increase was from 9,000 per gram to 50,000 to 150,000 in cheese to which propionic acid culture was added. In cheese to which no propionic acid culture was added, the number of propionic acid organisms 3 days previous to the first signs of eye formation was as low as 300 per gram. By the time the eyes had started this number increased to 190,000 per gram. The counts did not usually reach 1 billion per gram until the cheese was about 2 months old, or sometime after the eyes were completely formed. At this time the propionic acid bacteria usually completely outnumbered any other organisms in the cheese.

Frazier and Wing (26) found that in experimental cheese an excess of propionic acid culture usually produced an over-set cheese. Excessive growth of the propionic acid bacteria, especially in the later stages of ripening, tended to cause glass and checking. When moderate amounts of propionic acid culture were used, the number of eyes formed, the time of beginning eye formation, and the time for completion of eye formation were independent of the number of propionic acid bacteria added. Characteristic eye formation took place in some cheese when less than 100 propionic acid bacteria per gram were present at the beginning of eye formation and little or no increase in numbers had taken place 1 week later. Such cheese were usually not as sweet as cheese with more propionic acid bacteria.

Pregenzer (50) attempted to limit the size of eyes formed in Emmenthal cheese. Experiments were conducted on 244 cheese and of this number only 11 had the desired eye formation. The usual dairy culture was compared with a culture made from a good quality cheese. The regular dairy culture gave the better results. The author stated that when the long list of experiments is taken into account the inevitable conclusion is that the formation of eyes in Emmenthal cheese depends upon factors which are independent, or almost independent, of the method employed in manufacture. Certain natural bacteria were thought to be mainly responsible for good cheese.

Use of Pure Cultures in Manufacture of Swiss-type Cheese.

A series of trials was reported by von Freudenreich (28) in which pasteurized milk used for making Emmenthal cheese was inoculated with pure cultures of lactic acid bacteria, liquefying bacteria and anaerobic bacilli. Results indicated that lactic acid bacteria play a principal role in the ripening since normal ripening took place when these organisms were used alone or combined with other organisms. The cheese did not ripen properly when liquefying bacteria were used alone.

Adametz (1) described an experiment in which 10 cheese were made with the use of pure cultures of Bacillus nobilis and 10 cheese without. The cheese made with the pure cultures were superior to the control cheese in flavor and aroma.

Orla-Jensen (47) studied various cooking temperatures ranging from 48° to 63°C. with corresponding stirring periods ranging from 120 to 25 minutes. He found that a temperature lower than 55°C. was not satisfactory. The use of cultures of Bacterium acidi propionici produced desirable flavor and eye formation at all temperatures employed, even at 60°C.

Doane (16) reported that pure cultures of Bacillus bulgaricus could be used with perfect results in suppressing undesirable fermentations, principally gas. The efficiency of various strains in suppressing gas was not correlated with their acid producing ability. Bacillus bulgaricus cultures did not have any effect on eye formation or interfere with the flavor or texture. The author believed that such cultures would help make a more uniform cheese during the summer months.

Sherman (53) found that three different cultures could be used to control fermentations in Emmenthal cheese; Lactobacillus bulgaricus suppressed gassy fermentation produced by undesirable bacteria, *Bacterium acidi propionici* insured development of the characteristic flavor as well as formation of eyes, and *Lactobacillus casei* controlled over-swelling at times but was not always successful.

The benefits derived from the use of pure cultures are enumerated by Matheson (44) as follows: (1) pure cultures require that commercial rennet be used and this makes a more uniform cheese, (2) propionic acid culture and *Bacillus bulgaricus* culture open up the cheese without difficulty at all seasons of the year, (3) *Bacillus bulgaricus* culture reduces the number of "stinker cheese", and (4) an increase in the price of the cheese results.

Burkey and Sanders (6) studied the action of pure cultures of *Lactobacillus casei* and *Streptococcus thermophilus* with regard to their acid production and their effect on drainage of Swiss cheese in the press. *Lactobacillus casei* cultures which were active at high temperatures started to grow at the outside of the cheese 2 to 3 hours after it was dipped. Growth in the inside of the cheese was limited to *Streptococcus thermophilus* for the first 5 hours. A great difference in acid development between the outside and inside of a cheese was believed to check the flow of whey. They thought this trouble could be remedied by the use of starters which would provide an active growth of *Streptococcus thermophilus* in combination with the proper *Lactobacillus casei* culture.

Frazier, et al. (21) stated that results of field work indicated the use of raw whey or incubated kettle whey starters in Swiss cheese caused better draining of the cheese in the press and resulted in better cheese. From incubated kettle whey a pure culture of Streptococcus thermophilus was isolated. The use of pure cultures of Streptococcus thermophilus improved the quality of the cheese but seemed to cause more glass and checking than was found in the control cheese.

Frazier, et al. (24) used a series of pure cultures consisting of Streptococcus thermophilus, Lactobacillus casei, Lactobacillus bulgaricus and Propionibacterium shermanii and studied their growth and activity in the Swiss cheese kettle. Streptococcus thermophilus increased in numbers during the kettle process and the increase was most rapid when the temperature of the kettle contents was high. Lactobacillus casei, Lactobacillus bulgaricus and Propionibacterium shermanii did not increase in numbers during the kettle process and usually decreased. Streptococcus lactis usually increased in numbers during the first part of the kettle process but was stopped by the cooking temperature.

Frazier, et al. (20) also studied the growth of various organisms during the time the cheese was in the press. Streptococcus thermophilus usually decreased during the first 2 or 3 hours and then increased rapidly until the seventh hour;

after this there was either a slight increase or decrease. Lactobacillus helveticus usually decreased slowly until the sixth or seventh hour in the press, after which a fairly rapid increase took place. Lactobacillus bulgaricus usually decreased for the first 5 hours after dipping and then a rapid increase in numbers took place. Lactobacillus bulgaricus did not grow until the temperature was below 44°C. Propionibacterium shermanii did not grow in the press.

Burkey, Sanders and Matheson (7) studied initiation of growth of certain organisms in the interior of Swiss cheese. Streptococcus thermophilus began to grow within 2 or 3 hours after dipping, Lactobacillus bulgaricus within 5 or 6 hours after dipping and Lactobacillus helveticus within 9 or 10 hours after dipping.

Frazier, Long and Johnson (23) determined the suitability of Streptococcus thermophilus for the ripening of milk for Swiss cheese. These investigators thought that if this organism could be used it might be grown at such a high temperature that other organisms could not grow and ripening would be accomplished in a short time. Milk ripened with Streptococcus thermophilus for a short period improved the quality of the cheese but ripening for 75 minutes either did not improve or else harmed the quality of the cheese. Streptococcus thermophilus was active in the kettle and grew to a limited extent in the press. Ripening part of the milk with Streptococcus thermophilus improved the quality of the cheese when the kettle milk acted "dead".

Burri (8) stated that although the propionic acid bacteria were discovered in 1904 it has taken a comparatively long time for practical use of the discovery to be made in Switzerland. In 1925 cheesemakers were supplied with pure cultures of propionic acid bacteria and have since recognized the advantage of using such cultures. By 1936 there were 342 factories voluntarily employing cultures.

Use of Pasteurized Milk in the Manufacture of Swiss-type Cheese.

von Freudenreich and Orla-Jensen (31) made small experimental cheese from pasteurized milk inoculated with different lactic acid bacteria, Duclaux's *Thyrothrix* bacilli, etc. The results led them to conclude that the pasteurization of milk for Emmenthal cheese unfavorably affected the quality.

Winkler (61) reported that all the pasteurized milk cheese made by him in 1927 was third grade. In 1928 some second grade cheese was made and additional progress followed. The pure cultures used in the trials were obtained from the Austrian Experiment Station.

Fruhwald (35) stated that some defects in Emmenthal cheese originate from manufacturing methods but most originate from harmful bacteria. Defects caused by organisms can be eliminated by using good milk or by pasteurization. The author recommends a simple pasteurization process, such as heating in the kettle at 62° to 63°C. for at least 15 minutes. He also recommends the use of pure cultures. Pasteurized milk cheese was rarely found to swell in the press and when it did there was defective pasteurization. Short-time, high-temperature pasteurization was suitable when the milk was of good quality, but when the milk was less satisfactory the holder method was more suitable.

Zeiler, Demeter and Christiansen (62) were unsuccessful in their attempts to make Emmenthal cheese from raw milk. Various cultures were used. The first successful cheese was made from pasteurized milk to which a culture of *Bacillus casei* ε had been added. A bacteriological examination of the milk did not forecast the quality of cheese that would result.

Klang (41) found that Emmenthal cheese could be made from heated milk when pure cultures were added. Pasteurization favored the production of characteristic eyes, but the flavor and texture of the cheese were unfavorably influenced. To overcome the disadvantages of pasteurization, the evening milk was heated and then mixed with raw morning milk.

Burtscher (11) believed that pasteurizing the milk for Emmenthal cheese would be a means of facilitating and simplifying production of milk for cheese. He found pasteurization had a favorable effect on formation of eyes, but an unfavorable influence on flavor, texture and keeping quality of the cheese. Customers preferred cheese of average quality made from raw milk. Burtscher noted that the heating of milk originated in the necessities and emergencies of industrial conditions and has been useful; however, there is no reason why the heating process should be described as the modern method or be recommended for general use as long as it is impossible to produce as good cheese with it as with raw milk supplied under normal conditions.

Fruhwald (36) stated that pasteurization by the holding method may be regarded as especially well suited to the making of Emmenthal cheese. In the field of short-time pasteurization the Todt pasteurizer was exceedingly well adapted to Emmenthal cheese production.

Gratz (38) concluded from his experiences that it is hopeless to endeavor to improve bacteriologically, kettle milk used for the manufacture of hard cheese by any kind of pasteurization method if the milk is strongly infected with butyric acid bacteria. In his work cheese swelled when they contained only 3 or 4 butyric acid bacteria per kilo. Making hard cheese from pasteurized milk was not recommended if the milk was obtained from cows which were being fed materials containing large numbers of butyric acid bacteria. Ensiled feeds, peanut cake and feeds (such as beets, beet heads and beet leaves) soiled with earth were found to contain large numbers of such organisms. Gratz believed that with pasteurization one could not expect to obtain hard cheese of the quality obtained when the required precautionary measures are followed in the production of the milk.

Hanusch (39) made cheese from both raw and pasteurized milk produced during the autumn grazing season. The cheese made from pasteurized milk were of much better quality than those made from raw milk. These results and the fact that the two best quality cheese were made from pasteurized milk is opposed to the frequent prejudice that the addition of pasteurized milk inhibits the development of a raw milk cheese aroma.

Defects of Swiss-type Cheese.

von Freudenreich (27) made cheese from milk which had been inoculated with <u>Bacillus schafferi</u> and noted that this organism could produce nissler cheese and also blown cheese. He therefore considered these faults were not necessarily due to different bacteria.

Russell and Hastings (51) stated that Swiss cheese more commonly shows abnormal fermentations than Cheddar cheese because the milk is worked up in a rather sweet condition. The authors described a gassy defect caused by a yeast capable of fermenting milk sugar and producing alcohol, carbon dioxide and other products. Old whey was ideal for its growth.

Since whole rennets were soaked in whey to extract the active curdling principal, the organism was carried from one batch of cheese to another. Whey taken back to farms was also infected and the organism was found in each patron's milk except one.

Cultures of an organism taken from abnormal red spots on Emmenthal cheese were studied by Thoni and Allemann (55) and their characteristics found to be similar to the propionic acid bacteria isolated by von Freudenreich and Orla-Jensen. Since the new species had the power to produce a red pigment as well as propionic acid, the authors named it _Bacterium acidi propionici_ var. _rubrum_.

Burri and Staub (9) noted black spots of irregular size and shape on the rind of Emmenthal cheese. The portion of cheese on which the spots were located was dry and crumbly and this condition extended for some distance into the cheese. A microscopic examination of material taken from these dark spots revealed the presence of bacteria, yeasts and an unknown species of fungus with yeast-like cells 10 to 16 microns long and with hyphae containing a brownish-black coloring material. In pure culture, the fungus formed yeast-like buds which developed into organisms with branching hyphae; no conidia or other spores were observed. The formation of the black color was not considered to be due to enzymes but to a purely chemical process. The authors named the causative organism _Monilia nigra_.

Hastings and Frazier (40) found on analysis of certain defective cheese that the odor increased as the cheese aged and was characterized by hydrogen sulphide. Large numbers of butyric acid bacteria were present in the spoiled areas while very few of them were found in the unaffected cheese. The butyric acid organisms were able to grow in the presence of considerable acid.

An investigation by Albus (2) to determine the cause of an unusually large number of nissler and pressler cheese in one factory led to the isolation of Clostridium welchii. Repeated efforts to isolate a gas forming aerobic organism from defective cheese were unsuccessful. Experimental cheese were made from milk to which cultures of Clostridium welchii and Lactobacillus bulgaricus had been added. Large amounts of Lactobacillus bulgaricus culture together with an increased cooking temperature had little or no effect on the development of Clostridium welchii in the cheese. It was found that Clostridium welchii could produce gassy cheese of both the nissler and pressler types. On a few occasions the cheese made from milk inoculated with Clostridium welchii showed little or no gas, although there was no variation in the method of manufacture used. An undetermined factor was believed to play a part in the occurrence of gassy fermentation in cheese, especially in cooked cheese.

Demeter (13) studied the frequency of butyric acid bacteria in kettle milk during 1931 and found an increase in

March, then a drop which was followed by a maximum during the second half of May and all of June. The presence of these organisms was determined by the Burri method (shake cultures in glucose agar) and the Weinzirl method (sporogenes test). In instances where both the Burri and Weinzirl tests of kettle milk showed a low content of anaerobic sporeformers (September to November), the cheese were almost blind. By proper use of good Emmenthal cheese starters, even the strongest contamination by butyric acid organisms could be held in check.

Koestler (43) stated that slow eye formation, bicolored curd and brown colonies of bacteria visible to the naked eye are defects which frequently occur together in Emmenthal cheese. The distribution of water, fat, salt and acid (pH) did not explain the formation of the two kinds of curd. Most bicolored cheese had an accumulation of calcium under the rind; the non-bicolored cheese had a higher percentage of calcium in the interior. A marked irregular distribution of nitrogen was noted in the bicolored cheese. The author believed that the origin of the two kinds of curd was in certain conditions found in the fresh cheese and arising from an irregularity in the fermentation.

Dorner and Thoni (18) found odoriferous fermentation or gray decay of cheese to be a defect which appeared 4 or 5 months after the cheese was made. It was first evident when the pH of the cheese increased. This change in pH was accompanied by a change in flavor and odor of the cheese. The cause of the abnormal fermentation was found to be *Bacterium proteolyticum* n. sp. When the milk used for making cheese contained 10 or more cells of this organism per ml., a secondary odoriferous fermentation resulted. Cheese badly infected with this organism had a high pH, a bad odor, a gray color or dark spots and was gassy.

Hydrogen Ion Concentration in Swiss-type Cheese.

Watson (59) noted that the changes in hydrogen ion concentration in cheese have been given very little attention. He described a rapid and accurate method for following these changes. The pH values of cheese plugs were found to be slightly lower than those of the whey from the same cheese. When the same amount of culture Ga (*Lactobacillus* plus mycoderm) was added, a cheese cooked to 52°C. developed acid faster and to a higher point than a cheese cooked to 58°C. Culture Ga developed greater acidity in a shorter time than culture 39a (*Lactobacillus bulgaricus*). Cheese made with 2 per cent of culture Ga developed more acid than when 0.125 per cent was used.

Watson (60) found the use of culture Ga gave a greater development of acid in Swiss cheese during the first day than the use of culture 39a. The difference in acid resulted in a more thorough proteolysis and harder texture.

Production of a desirable soft texture was not necessarily coincident with the highest degree of proteolysis; quite the opposite was true. Experiments indicated that the choice of the culture was an important factor in the control of hydrogen ion concentration, ripening and texture of Swiss cheese.

Koestler (42) determined the importance of hydrogen ion concentration on certain properties of hard cheeses. The pH of Emmenthal cheese 3 months old, according to numerous determinations, was 5.45 to 5.75. Gruyere cheese was higher by 0.1 pH. By taking into consideration the fact that normal ripening causes an increase in pH, Koestler believed Emmenthal cheese 4 days old should have a pH of 5.10 to 5.40. Cheese that was too hard was thought to be due to many factors and not necessarily to an abnormal acidity.

Frazier, _et al_. (20) noted that Swiss cheese showed a fairly rapid drop in pH during the first 7 hours in the press and a more gradual decrease thereafter. The increase in acid was due to _Streptococcus thermophilus_. The pH of the cheese after 21 hours in the press served as an indication of the activity of lactobacilli. Results indicated that the pH after 3 hours in the press should be 5.80 or less, and after 21 hours 5.15 to 5.30. Acid production in the press that was too rapid resulted in rapid drainage. Slow acid production favored insufficient drainage and delayed eye formation.

Frazier, *et al.* (22) noted that *Lactobacillus helveticus* was most effective as a milk culture when it had an acidity of 1.00 to 1.09. A 12 hour culture of *Streptococcus thermophilus* had an acidity of about 0.70 to 0.75 per cent when grown in milk and 0.30 to 0.33 per cent when grown in whey. The pH of the cheese in the press, which served as an indication of the activity of the culture, was 6.0 to 6.09 after 3 hours when made from milk of pH 6.5 to 6.6. The pH of the cheese in the press was found to be most desirable when it was less than 5.2 after 21 hours and preferably between 5.15 and 5.0. With good milk, a slow development of acid throughout the first 21 hours usually gave a good cheese. The pH of such cheese could be as high as 6.1 to 6.25 after 3 hours in the press and over 5.2 after 21 hours.

Burkey, Sanders and Matheson (7) found large differences in pH between the interior of the cheese and the portion just beneath the rind. This condition resulted in insufficient drainage and high moisture. The difference in pH was thought to cause such defects as checking near the rind or glass.

Dorner and Ritter (17) carried out controlled tests in the manufacture of a large number of Emmenthal and Gruyere cheeses. These tests were used as the basis of a statistical study designed to indicate correlations with the grade of cheese. It was found that the pH value of a cheese 24 hours old may be used as the basis for predicting the quality of the finished cheese and for adjusting details of manufacture that may improve the quality of cheese subsequently made. A pH of 5.2 in a cheese 24 hours old was most desirable.

METHODS

Method of Enumeration and Isolation of Propionic Acid Bacteria.

The method used for enumeration and isolation of propionic acid bacteria was essentially that employed by Sherman (52). It consisted of inoculating the test material into shake cultures of sodium lactate agar having the following composition:

Peptone	2.0 per cent
Sodium lactate	1.0 per cent
Yeast extract	1.0 per cent
Washed agar	1.5 per cent
Water to make	100.0 per cent
pH 7.0	

The cheese used for inoculation of the shake cultures was weighed (1 gram) on a sterile paper, ground in a mortar to a smooth mass, 9 ml. of sterile aqueous 2 per cent sodium citrate added, and the grinding continued until a homogeneous suspension resulted. The suspension was used in preparing further dilutions in water. The various dilutions were added to the tubes of melted agar after it had cooled to 45° to 50° C., and were distributed by rolling the tubes between the palms of the hands. When the agar had solidified about 1 inch of sterile 3 per cent agar was poured over the top of each tube to limit the entrance of air and to prevent the cultures from drying too rapidly. The shake cultures were incubated at 30°C. for 2 weeks. Colonies which had a smooth or winged surface and which were yellow to light brown in color by transmitted light were considered to be propionic acid bacteria and were enumerated as such when making counts. They were also the type picked when isolations were made.

Method of Identification of Propionic Acid Bacteria.

Colonies which showed the characteristics of propionic acid bacteria in shake cultures were picked into tomato bouillon of the following composition:

Tomato juice	40.0 per cent
Peptone	1.0 per cent
Peptonized milk	1.0 per cent
Water to make	100.0 per cent
pH 7.0	

When sufficient growth had occurred, the cultures were purified by making loop dilutions in sodium lactate agar shake cultures. After incubation for 2 weeks at 30°C. a typical, isolated colony was picked.

After a culture had been purified, it was checked for morphology using the Gram stain. Members of the genus Propionibacterium are rather short rods when grown under anaerobic conditions, Gram positive in young cultures and do not form spores.

Cultures were also tested for catalase since all but one of the known species of the genus Propionibacterium are catalase positive. The catalase test was made by placing a few drops of an actively growing culture in a depression of a spot plate and adding one or two drops of hydrogen peroxide (3 per cent). Cultures showing an evolution of gas were designated as catalase positive.

The final step in identification of propionic acid bacteria was to determine their ability to ferment lactic acid or sodium lactate under anaerobic conditions. A purified culture was inoculated into one flask containing 0.5 per cent yeast extract and 1.0 per cent sodium lactate in water and into another flask containing only 0.5 per cent yeast extract in water. These flasks were incubated at 30°C. for 2 weeks under an atmosphere of carbon dioxide and then distilled for volatile acids. Ten times the original volume was collected. The distillate containing the volatile acids was used for identification of the acids by the partition method of Osburn, Wood and Werkman (49). All members of the genus Propionibacterium ferment lactic acid under anaerobic conditions with the formation of propionic and acetic acids.

Method of Determining Volatile Acidity of Cheese.

The volatile acidity of cheese was determined by steam distillation. Four hundred grams of shredded cheese was placed in a 3 liter round bottom flask and 25 ml. of N/1 sulfuric acid added to free the volatile acids. The round bottom flask was partially immersed in a boiling water bath to keep the increase in volume at a minimum and connected so that the steam entered at the bottom of the flask through a steam distributor and escaped from the flask through a trap connected to a condenser. The steam distributor was made so that the steam entered the cheese through four small openings and gave the contents of the flask more agitation than if a single opening were used. Usually 1 liter of distillate was collected. The time required to collect this was approximately 2 hours. The distillate was titrated with N/20 sodium hydroxide to the phenolphthalein endpoint. The volatile acidity was expressed as the number of milliliters of N/20 sodium hydroxide required to neutralize the volatile acids in 1000 ml. distillate from 400 grams of cheese.

Method of Determining Acid Number of Cheese-fat.

The method used in extracting the fat from the cheese was that suggested by Barthel, Sandberg and Haglund (4). It consisted of mixing 400 grams of shredded cheese with 400 grams of sand and subjecting the mixture to 1,000 to 2,000 pounds pressure by means of a hydraulic press.

The acid number of the cheese-fat was determined by the method of Breazeale and Bird (5). It consisted of weighing 10 grams of filtered fat into a 125 ml. Erlenmeyer flask, dissolving the fat in 25 ml. of acid-free petroleum ether, adding 10 ml. of absolute alcohol (previously distilled over solid potassium hydroxide) and titrating to the phenolphthalein endpoint with N/20 potassium hydroxide in absolute alcohol. The acid number was expressed as the number of milliliters of N/20 potassium hydroxide required to neutralize the free fatty acids in 10 grams of fat.

Method of Determining Hydrogen Ion Concentration of Cheese.

Two grams of cheese was placed in a mortar and ground to a thick paste. Ten ml. of boiled and cooled distilled water was added, and the mixture ground to a homogeneous suspension. The sample was again mixed prior to use.

Measurements were made with a potentiometer using a quinhydrone electrode and saturated calomel cell.

RESULTS OBTAINED

Numbers of Propionic Acid Bacteria in Various Cheeses.

Iowa Swiss-type cheese.

The Swiss-type cheese made on a semi-commercial scale in the cheese laboratory of the Iowa State College Dairy Industry Department with the method suggested by Goss, Nielson and Mortensen (37) was examined for the numbers of propionic acid bacteria present. Forty samples of cheese classed as having good, fair and poor flavor and eye formation were selected. This cheese ranged in age from 2 to 14 months, with the majority of the samples being 3 to 4 months old.

Table 1 shows the numbers of propionic acid bacteria in Iowa Swiss-type cheese of various qualities. Although cheese having a characteristic sweet flavor had a wide range of counts of propionic acid bacteria, over 80 per cent of the samples classed as having a good flavor contained more than 100,000 propionic acid bacteria per gram. All the cheese having a fair or poor flavor contained less than 100,000 per gram. The majority of samples classed as having a poor flavor contained less than 1,000 per gram. In general, good flavored cheese contained large numbers of propionic acid bacteria while poor flavored cheese contained relatively small numbers.

Table 1. General Numbers of Propionic Acid Bacteria in Forty Swiss-type Cheese of Various Qualities.

No propionic acid bacteria added

Propionic acid bacteria per gram	Number of cheese with flavor			Number of cheese with eye formation		
	Good	Fair	Poor	Good	Fair	Poor
>1,000,000	4	0	0	4	0	0
100,000 - 1,000,000	5	0	0	5	0	0
10,000 - 100,000	0	3	3	4	3	0
1,000 - 10,000	1	4	4	6	2	1
<1,000	1	6	9	9	5	1

Swiss-type cheese with good eye formation showed a wide range of counts of propionic acid bacteria. The counts on cheese with well formed eyes ranged from less than 1,000 (actually less than 10) to over 1,000,000 propionic acid bacteria per gram. Cheese having fair eye formation contained less than 100,000 and cheese having poor eye formation less than 10,000 per gram. The results indicated that a cheese may have good, fair, or poor eye formation and yet contain essentially the same numbers of propionic acid bacteria. Cheese containing over 100,000 propionic acid bacteria per gram, however, regularly had good eye formation. Since 11 of the 40 cheese examined had a good flavor and 28 of the 40 had good eye formation, it appears that with this method of manufacture it is easier to obtain good eye formation than good flavor.

Domestic Swiss cheese.

Twelve samples of domestic Swiss cheese of average quality as regards flavor and eye formation were examined for numbers of propionic acid bacteria. The samples were from cheese about 1 year old. All of them contained rather large numbers of propionic acid bacteria, the counts ranging from 400,000 to 1,100,000 per gram.

Cheddar cheese.

Fifty-four samples of Cheddar cheese of good and poor quality, and varying in age, were examined for numbers of propionic acid bacteria. Most of the samples contained less than 5,000 per gram. With approximately 15 per cent of the samples examined, no propionic acid bacteria could be detected in 0.1 gram. Occasionally samples were encountered which contained over 1,000,000 of these organisms per gram. All of the samples containing very large numbers of propionic acid bacteria came from one locality. There was no correlation between the number of propionic acid bacteria and quality or age of the cheese.

Canned Cheddar cheese.

Two samples of canned Cheddar cheese were examined for propionic acid bacteria. One of the samples had eyes similar to those in Swiss cheese; it contained 510,000 propionic acid organisms per gram, and from it three species of propionic acid bacteria were isolated. With the other sample of canned Cheddar cheese, no propionic acid bacteria were detected in 0.1 gram.

Manufacture of Swiss-type Cheese with the Addition of Propionic Acid Bacteria.

Manufacturing procedure.

The milk used in all experiments was mixed herd milk of average quality which had been pasteurized at 61.7°C. (143°F.) for 30 minutes. The titratable acidity of the milk prior to the addition of cheese starter ranged from 0.11 to 0.15 per cent, calculated as lactic acid.

The propionic acid cultures were carried as stab cultures in sodium lactate agar. The culture to be used for cheesemaking was inoculated into tomato bouillon and incubated for

48 to 72 hours at 30°C. Various amounts of propionic acid culture were used, ranging from 5 to 100 ml. per 100 pounds of milk. It will be noted in the experimental data that 25 ml. of culture per 100 pounds of milk gave good results.

After the milk was placed in the cheese vat it was brought to a temperature of 29.4°C. (85°F.) and 1 per cent of regular cheese starter and the propionic acid culture added. The milk was allowed to ripen at this temperature until an increase in titratable acidity of 0.01 to 0.015 per cent over that present immediately after adding the cultures was obtained. The period required to obtain this increase ranged from 30 to 45 minutes. At the end of the ripening period 3 ounces of rennet per 1000 pounds of milk was added. Usually from 30 to 40 minutes were required for the curd to reach the correct cutting consistency. The curd was cut with 1/4 inch knives into cubes and further cut with the vertical knife until the curd particles were about the size of wheat kernels. It was then stirred with a fork for approximately 20 minutes in order to expel the whey. At the end of this time one-third of the whey was removed from the vat. Cooking was accomplished by adding water at 65.5°C. (150°F.) to the curd-whey mixture. The water was added in three or four portions with constant stirring until the temperature reached 38.8° to 41.1°C. (102° to 106°F.). In the first experiments a temperature of 38.8°C. (102°F.) was used; in later experiments the cooking temperature was raised to 41.1°C. (106°F.) in order to obtain cheese with less moisture and a firmer body. The curd was stirred at the cooking temperature for about 20 minutes. When the curd was suitably firm it was collected at the upper end of the vat by means of a perforated retainer and allowed to mat. The whey was drained at this point. Matting was assisted by placing thin metal plates on top of the curd. When the curd had matted sufficiently it was cut into blocks and placed in wooden hoops. The curd was turned in the hoops several times at 10 minute intervals to make the curd mass conform to the shape of the hoop. The cheese were wrapped in muslin cloths, placed in the hoops and pressure gradually applied in the press. The cheese were turned in the press after a 2 hour interval and allowed to remain in the press for about 2 hours more. After removal from the press, the cheese were allowed to cool slowly in a room at about 12.7°C. (55°F.). On the following day the cheese were placed in a 20 to 22 per cent salt solution. They were salted for 24 hours, turned in the salting tank, and allowed to remain 24 hours more. After removal from the salting tank the cheese were placed in a curing room maintained at 10.0° to 12.7°C. (50° to 55°F.). The green weight of a single experimental cheese was about 16 pounds. Cheese which showed excessive swelling were removed from the curing room to a room maintained at about 4.4°C. (40°F.). The cheese were examined at 30 day intervals and cut at the end of 90 days.

Comparison of various cultures of organisms.

Various cultures of propionic acid organisms were used in making Swiss-type cheese from pasteurized milk for the purpose of determining differences between cultures and with the hope of obtaining a culture which would produce good quality cheese consistently. A total of eighteen cultures were employed, thirteen of which were recently isolated. The cultures came from the following sources: cultures 46, 47, 57, 58 and 62 were isolated from Cheddar cheese; cultures 50, 51 and 52 were isolated from canned Cheddar cheese; cultures 37, 43, 44 and 45 were isolated from domestic Swiss cheese; culture 106 was isolated from Swiss-type cheese; and cultures 31a, 31c, 49w, 62d and 4866 were obtained from various laboratories.

The propionic acid cultures which had been grown in tomato bouillon for 48 to 72 hours at 30°C., were added to the milk after it was pasteurized. Five ml. of culture per 100 pounds of milk was used in the trials for comparison of cultures. The milk used for each series of cheese was pasteurized in one lot (at 61.7°C. (143°F.) for 30 minutes) and then divided into four parts, each part being placed in a separate vat. Different cultures were added to each of three vats and the fourth vat was used as a control. All the cultures except 37 and 57 were used at least twice.

The cheese were examined at 30-day intervals for flavor and eye formation and also for the number of propionic acid organisms per gram of cheese. The flavor designations used most frequently in comparing the cheese were excellent, good, fair, flat and poor. Cheese having an excellent flavor was conspicuously sweet. A good flavored cheese was one which had some sweet flavor, but not as much as those designated excellent. Cheese with a fair flavor had very little sweet flavor, and a flat flavored cheese had no sweet flavor. A poor flavored cheese was one which had no sweet flavor and had some undesirable flavor.

The eye formation obtained with various cultures was classed as good, fair or poor. Cheese classed as having good eye formation contained eyes about 0.5 inch in diameter and spaced 1 to 1.5 inches apart. Figure 1 illustrates a cheese having good eye formation. Cheese having fair eye formation were those which deviated slightly from the cheese having good eye formation, the eyes varying either in size or number. Figure 2 illustrates a cheese having fair eye formation. Cheese having poor eye formation had many small eyes, few small eyes, many large eyes or few large eyes.

The results obtained in comparing the various propionic acid cultures with respect to their ability to produce desirable flavor and eye formation are given in Table 2. The data pertain to the cheese ripened 90 days. Several of the cultures were rather consistent in producing cheese with an excellent or good flavor, while others were very variable in the type of flavor produced. Cultures 43, 44, 51, 31a, 62d and 4866 did not

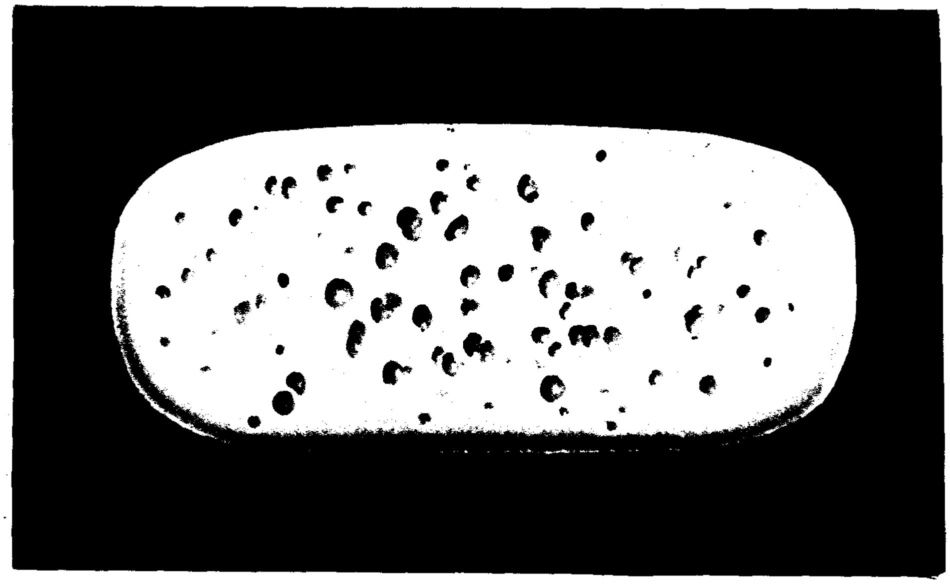


Figure 1.
Swiss-type cheese classed as having good eye formation.

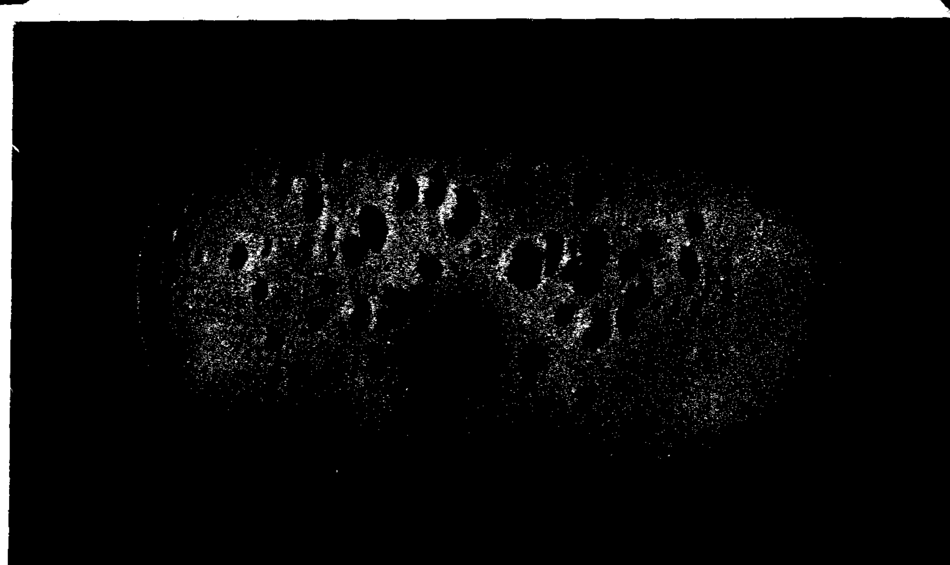


Figure 2.
Swiss-type cheese classed as having fair eye formation.

Table 2. Flavor and Eye Formation of Swiss-type Cheese Made With Various Cultures of Propionic Acid Bacteria.

Series no.	Cheese no.	Culture no.	Flavor	Eye Formation	Growth of culture in cheese
1	1	50	Flat	Fair	Fair
	2	47	Poor	Fair	Poor
	3	4866	Excellent	Good	Good
	4	Control	Fair	Good	----
2	1	43	Excellent	Fair	Good
	2	52	Flat	Poor	Poor
	3	31a	Fair	Good	Good
	4	Control	Fair	Good	----
3	1	44	Flat	Good	Fair
	2	51	Excellent	Good	Good
	3	31c	Fair	Poor	Fair
	4	Control	Poor	Good	----
4	1	46	Good	Good	Good
	2	45	Good	Poor	Fair
	3	62d	Good	Poor	Good
	4	Control	Poor	Fair	----
5	1	50	Good	Good	Good
	2	47	Flat	Fair	Fair
	3	4866	Excellent	Good	Good
	4	Control	Flat	Fair	----
6	1	43	Good	Good	Good
	2	52	Poor	Fair	Poor
	3	31a	Good	Good	Good
	4	Control	Flat	Good	----
7	1	44	Excellent	Good	Good
	2	51	Good	Fair	Fair
	3	31c	Fair	Fair	Fair
	4	Control	Flat	Good	----
8	1	45	Poor	Good	Poor
	2	46	Good	Good	Good
	3	62d	Good	Good	Good
	4	Control	Flat	Good	----

Table 2. Continued

Series no.	Cheese no.	Culture no.	Flavor	Eye Formation	Growth of culture in cheese
9	1	57	Poor	Poor	Poor
	2	37	Good	Poor	Fair
	3	4866	Good	Poor	Good
	4	Control	Fair	Poor	----
10	1	58	Poor	Poor	Poor
	2	62	Fair	Poor	Fair
	3	31a	Good	Poor	Good
	4	Control	Poor	Poor	----
11	1	58	Fair	Fair	Poor
	2	62	Poor	Fair	Fair
	3	31a	Good	Fair	Good
	4	Control	Fair	Good	----
12	1	4866	Fair	Poor	Good
	2	62d	Good	Poor	Good
	3	46	Poor	Poor	Good
	4	Control	Fair	Poor	----
13	1	44	Good	Good	Good
	2	4866	Fair	Good	Fair
	3	49w	Fair	Good	Poor
	4	Control	Poor	Good	----
14	1	44	Good	Good	Good
	2	4866	Good	Good	Good
	3	49w	Fair	Good	Poor
	4	Control	Poor	Good	----
15	1	31a	Good	Poor	Good
	2	31a	Good	Poor	Fair
	3	106	Fair	Poor	Poor
	4	Control	Poor	Poor	----
16	1	44	Good	Good	Good
	2	106	Fair	Fair	Poor
	3	106	Fair	Fair	Poor
	4	Control	Fair	Fair	----

produce any cheese having a poor flavor. Culture 44 was used in five trials; one trial resulted in excellent flavored cheese, three in good flavored cheese and one in fair flavored cheese. Cultures 43, 51 and 72d produced cheese in all trials which had either an excellent or good flavor. Culture 37 was used once and the cheese had a good flavor. This culture suddenly failed to grow on various laboratory media and was lost. Cultures 31c, 49w and 106 consistently produced cheese with a fair flavor. Cultures 47, 52, 57, 58 and 62 did not produce any good flavored cheese, and usually produced cheese having a fair or poor flavor.

The data indicate that none of the cultures were consistent in producing good eye formation. In the majority of cases, the cheese made in a single series and ripened under the same conditions tended to have rather similar eye formation, whether different cultures or no culture was used in the various cheese of the series. In thirteen of the sixteen series, the control cheese had as good or better eye formation than the cheese made with propionic acid culture.

In most instances cultures which increased appreciably in number during the ripening period gave favorable results with regard to flavor. Since the characteristic sweet flavor is due largely to the formation of propionates by the propionic acid organisms (3), it would be expected that cultures which did not increase in number, and which were present in the cheese only in small numbers, would not be satisfactory.

Table 3 presents, in detail, data showing the effect of

Table 3. Flavor Development and Growth of Propionic Acid Bacteria in Swiss-type Cheese.

Culture no.	Flavor	Propionic acid bacteria per gram at various stages in the ripening			
		Fresh curd	30 days	60 days	90 days
4866	Excellent	250,000	120,000,000	370,000,000	340,000,000
4866	Excellent	110,000	120,000,000	130,000,000	135,000,000
4866	Excellent	1,100,000	3,100,000	50,000,000	75,000,000
44	Excellent	300,000	270,000,000	210,000,000	192,000,000
51	Excellent	160,000	51,000,000	170,000,000	126,000,000
43	Excellent	400,000	77,000,000	62,000,000	81,000,000
31a	Excellent	3,500,000	55,000,000	55,000,000	80,000,000
62d	Good	800,000	65,000,000	26,000,000	30,000,000
37	Good	2,000	900,000	2,900,000	4,000,000
51	Fair	400,000	50,000	70,000	110,000
57	Fair	22,000	71,000	190,000	300,000
58	Fair	80,000	130,000	110,000	90,000
58	Flat	12,000	32,000	21,000	4,000
106	Flat	11,000	36,000	76,000	112,000
106	Flat	78,000	130,000	620,000	420,000
45	Poor	6,000	3,000	23,000	10,000
45	Poor	350,000	700,000	610,000	410,000
47	Poor	1,050,000	300,000	100,000	400,000
52	Poor	600,000	140,000	120,000	80,000
52	Poor	1,500,000	80,000	370,000	90,000

growth of propionic acid bacteria in Swiss-type cheese on the production of sweet flavor. Cultures 4866, 44, 51, 45 and 31a produced cheese having an excellent flavor. These cultures increased appreciably during the first 30 days of ripening and maintained a high count of propionic acid organisms throughout the entire ripening period. Cultures 62d and 37 produced cheese having a good flavor; culture 62d grew appreciably during the first 30-day period but did not maintain the high count for the remainder of the ripening period, and culture 37 showed a slow but steady growth during the entire ripening period. Cultures 51, 57 and 58 produced cheese with a fair flavor; the counts on the cheese show a corresponding decrease from those on cheese made with cultures 62d and 37 which produced a good flavor. Cultures 58, 106, 45, 47 and 52 produced cheese having a flat or poor flavor; these cultures grew very poorly or showed a decrease in numbers throughout the ripening period.

Flavor development by selected cultures of propionic acid bacteria.

In Table 4 are presented the results of trials that were made to determine the constancy of various cultures in producing excellent or good flavored cheese. The cultures selected were those which gave satisfactory results in the c omparisons reported in Table 2.

The data indicate that certain cultures were very consistent in producing an excellent or good flavor. Culture 44 was selected as the best culture because out of 17 trials, 16 re-

Table 4. Ability of Various Cultures of Propionic Acid Bacteria to Consistently produce Swiss-type Cheese of Good Flavor.

Culture no.	No. of trials	Number of cheese with flavor		
		Excellent or good	Fair	Poor
31a	8	7	1	0
43	4	4	0	0
44	17	16	1	0
62d	5	3	2	0
4866	7	4	3	0

sulted in excellent or good flavored cheese. None of the cultures produced cheese with a poor flavor.

Comparison of various amounts of culture.

In an attempt to produce cheese having more sweet flavor, various amounts of the same culture were added to three vats of the same milk; a fourth vat served as a control. The lots of cheese made in this manner were examined at 30-day intervals for flavor, eye formation and numbers of propionic acid bacteria.

Results obtained in representative trials are presented in Table 5. The data show that by increasing the number of propionic acid bacteria in the cheese, a corresponding increase was obtained in the degree of sweet flavor produced. The cheese receiving an inoculation of 100 ml. of culture per 100 pounds of milk were occasionally criticized as being too sweet. The cheese receiving an inoculation of 25 ml. of culture per 100 pounds of milk were considered to have about the desired sweet flavor. The difference in sweet flavor resulting from inoculations of 25 ml. and 50 ml. of culture per 100 pounds of milk was not as great as that resulting from inoculations of 50 ml. and 100 ml. of culture. Cheese receiving an inoculation of 5 ml. of culture per 100 pounds of milk were usually slightly lacking in sweet flavor, apparently more so than with the comparisons reported in Table 2.

The cheese which received an inoculation of 100 ml. of culture per 100 pounds of milk had a tendency to develop eyes which were a little larger than was desirable, although this

Table 5. Flavor and Eye Formation of Swiss-type Cheese Made with Various Amounts of Propionic Acid Bacteria.

Cheese no.	Amount of culture* per 100 pounds of milk	Flavor	Eye formation	Propionic acid bacteria per gram
18-1	100 ml.	Very sweet	Good	5,700,000,000
18-2	25 ml.	Sweet	Good	620,000,000
18-3	5 ml.	Slightly sweet	Very good	80,000,000
18-4	None	No sweet flavor	Good	3,000
19-1	50 ml.	Very sweet	Good	820,000,000
19-2	25 ml.	Sweet	Good	180,000,000
19-3	5 ml.	Slightly sweet	Good	27,000,000
19-4	None	No sweet flavor	Good	3,500
20-1	50 ml.	Very sweet	Poor	21,000,000
20-2	25 ml.	Sweet	Good	9,700,000
20-3	5 ml.	Slightly sweet	Fair	1,200,000
20-4	None	No sweet flavor	Good	34,000

* Culture 44 was used in these trials.

was not true in all instances. The cheese made with 25 or 50 ml. of culture per 100 pounds of milk usually had good eye formation as far as size of the eyes was concerned but at times there were too many eyes. Five ml. of culture per 100 pounds of milk gave the best eyes in the majority of instances.

Flavor development in control cheese.

In several series of cheese, the control cheese to which no culture was added developed as good a flavor as the cheese made with added propionic acid bacteria. When this was the case, propionic acid bacteria were always found in the control cheese in large numbers. In approximately 60 per cent of the cheese made without the addition of propionic acid bacteria, these organisms were detected in the ripened cheese or during the course of ripening. Generally, even though propionic acid organisms were present in the control cheese, they were not present in large enough numbers to produce the characteristic sweet flavor.

General effect of cultures on eye formation.

In the majority of cases, the cheese made in a series closely resembled each other in eye formation, whether or not propionic acid bacteria were added to the milk. With some series the eyes in the cheese made with added propionic acid organisms were the more desirable, while in others the eyes in the control cheese were the more desirable. In several instances no propionic acid bacteria were detected in 0.1 gram of the control cheese, yet the eye formation was equal to that obtained with propionic acid cultures.

Two lots of cheese to which propionic acid bacteria were added were nearly devoid of eyes, and only two or three eyes could be seen when the cheese were cut in half. The control cheese made from the same lot of milk, but without propionic acid bacteria added, contained many more eyes, although not as many as with some of the control cheese from other series. The cheese made with culture and which contained very few eyes developed the characteristic sweet flavor and the propionic acid organisms showed an appreciable increase in count during the ripening period.

Influence of Various Factors on Eye Formation.

Delayed salting.

Sodium chloride has an inhibitory effect on various microorganisms. In order to determine its influence on propionic acid bacteria, culture 43 was inoculated into a series of tubes containing tomato bouillon and various quantities of sodium chloride. As little as 0.5 per cent sodium chloride had an inhibitory effect and 10.0 per cent entirely checked the growth of the organisms. These results are in agreement with those obtained by Orla-Jensen (48).

Since the Swiss-type cheese investigated contained from 1.25 to 1.60 per cent sodium chloride, this concentration would be expected to check the action of the propionic acid organisms. However, the effect of sodium chloride on propionic acid bacteria in cheese may be different from its action on them in bouillon.

The usual salting procedure is to place the cheese in a 20 to 22 per cent sodium chloride solution the day following manufacture and hold for 48 hours. To study the influence of delayed salting on the propionic acid bacteria and eye formation, four series of cheese were made. Each series consisted of four cheese, two of which were made with propionic acid culture and two without. One of the cheese made with culture and one made without culture were salted soon after manufacture in the normal way. The other two cheese were held for 1 week at about 12.7°C. (55°F.) and then salted in the usual manner. The numbers of propionic acid bacteria in the cheese were determined at 30-day intervals, and the cheese were cut and examined at the end of 3 months.

With delayed salting, the cheese contained more eyes and the eyes extended nearer the surface than when the cheese were salted in the normal manner; also, they commonly had a slightly superior flavor. In general, the numbers of propionic acid bacteria increased in the cheese to a greater extent with the delayed salting. The objection to the 7-day delayed salting period was that the cheese had a slight tendency to flatten. Salting immediately after manufacture tended to produce a firmer surface and the cheese retained their original shapes better.

To overcome the disadvantage of the cheese flattening and yet obtain more satisfactory eye formation, a 2-day delayed salting period was investigated. This procedure slightly favored the production of more desirable eyes, since the eyes were better distributed, and the sweet flavor was somewhat more conspicuous. The numbers of propionic acid bacteria were slightly larger in the cheese made with delayed salting. The flattening of the cheese was not as noticeable as with the 7-day delayed salting period.

Temperature.

Most of the experimental cheese were ripened at about 10.0° to 12.7°C. (50° to 55°F.). A few of the cheese swelled more than was desirable during the ripening. In some instances the cheese which showed pronounced swelling contained eyes which were about 1 inch in diameter. Cheese having eyes of this size were objectionable from the standpoint of exterior and interior appearance of the cheese. In an attempt to control the overswelling, some of the cheese which were thought to contain eyes of sufficient size, as evidenced by swelling, were placed at 3.3° to 4.4°C. (38° to 40°F.). Holding the cheese at this temperature for the remainder of the curing period was effective in stopping further eye development.

In the manufacture of Swiss cheese, the cheese are usually held at 21.1°C. (70°F.) or higher for about the first 2 weeks of ripening to start development of eyes. In order to study the effect of a higher initial curing temperature on Swiss-type cheese, duplicate cheese were made with and without added propionic acid culture. One inoculated cheese and one control cheese were held at the regular curing temperature of about 10.0° to 12.7°C. (50° to 55°F.) and the other two cheese at about 21.1° to 22.2°C. (70° to 72°F.). Two series of cheese were used in this study. The first noticeable swelling occurred in 3 or 4 days when the cheese were held at 21.1° to 22.2°C. and in 3 or 4 weeks when held at 10.0° to 12.7°C. Some of the cheese held at 10.0° to 12.7°C. never showed evidence of swelling during the ripening period but eventually contained well formed eyes in about the desirable size and number. The cheese held at 21.1° to 22.2°C. for 1 week and then placed at 10.0 to 12.7°C. usually showed excessive swelling in 4 to 6 weeks and had to be placed at a lower temperature. The cheese cured at the higher temperature also tended to have eyes which were not quite as smooth as the eyes in the cheese held at 10.0° to 12.7°C. for the entire curing period of 3 months. The higher initial curing temperature might be advantageous in cases where it is difficult to obtain eye formation.

Effect of Addition of Propionic Acid Bacteria on Volatile Acidity of Cheese and Acid Number of Cheese-fat.

The most important character of the propionic acid bacteria is their ability to ferment lactic acid or lactates with the production of propionic and acetic acids and carbon dioxide. Propionic and acetic acids are volatile so it seemed possible that a determination of the volatile acidity of Swiss-type cheese would give some indication of the activity of these organisms in the cheese. Propionic and acetic acids are also soluble in fat, and if present in appreciable amounts would tend to increase the titration value of the fat.

Volatile acidities and acid numbers were determined on 45 samples of cheese which were 3 months old. Four samples of domestic Swiss cheese and one of imported Swiss cheese were also analyzed for volatile acidities and acid numbers in order to make comparisons between the various cheeses; these cheese were about 1 year old.

Table 6 presents the results of the comparisons. With the experimental cheese large variations occurred in volatile acidities of the cheese and in acid numbers of the cheese-fat. The cheese made with the addition of propionic acid culture was always higher in volatile acidity and the cheese-fat had a higher acid number than the cheese made from the same lot of milk to which no culture was added. Moreover, in most instances it contained more volatile acids and more acid in the fat than the domestic or imported Swiss cheese. Good quality domestic Swiss cheese had high volatile acidities and the cheese-fat had high acid numbers compared to domestic Swiss cheese of poorer quality. The one imported Swiss cheese which was of good quality, had a slightly lower volatile acidity than the good quality domestic Swiss cheese and a lower acid number on the fat.

Table 7 gives a detailed comparison of the flavors of three series of experimental Swiss-type cheese with the volatile acidities of the cheese and the acid numbers of the cheese-fat.

Table 6. Comparison of Volatile Acidity of Cheese with Acid Number of Fat.

Experimental Swiss-type and domestic and imported Swiss cheese.

Type of cheese	Ml. N/20 volatile acids per 400 grams cheese			Acid number of fat
	1st 500 ml. distillate	2nd 500 ml. distillate	Sum	
Experimental; no culture added	19.40	11.00	30.40	1.35
Experimental; culture added	255.40	125.80	381.20	7.82
Experimental; no culture added	42.20	26.20	68.40	1.51
Experimental; culture added	196.40	118.00	314.40	10.22
Experimental; no culture added	29.00	13.50	42.50	2.73
Experimental; culture added	128.00	73.75	201.75	9.55
Experimental; no culture added	33.75	14.25	48.00	2.41
Experimental; culture added	277.50	153.75	431.25	13.15
Experimental; no culture added	26.50	13.25	39.75	3.35
Experimental; culture added	241.50	124.25	365.75	13.40
Domestic Swiss; good quality	138.40	98.80	237.20	10.37
Domestic Swiss; good quality	118.50	102.50	221.00	9.47
Domestic Swiss; fair quality	37.00	35.50	72.50	7.00
Domestic Swiss; poor quality	37.50	27.50	65.00	7.59
Imported Swiss; good quality	105.00	70.00	175.00	7.58

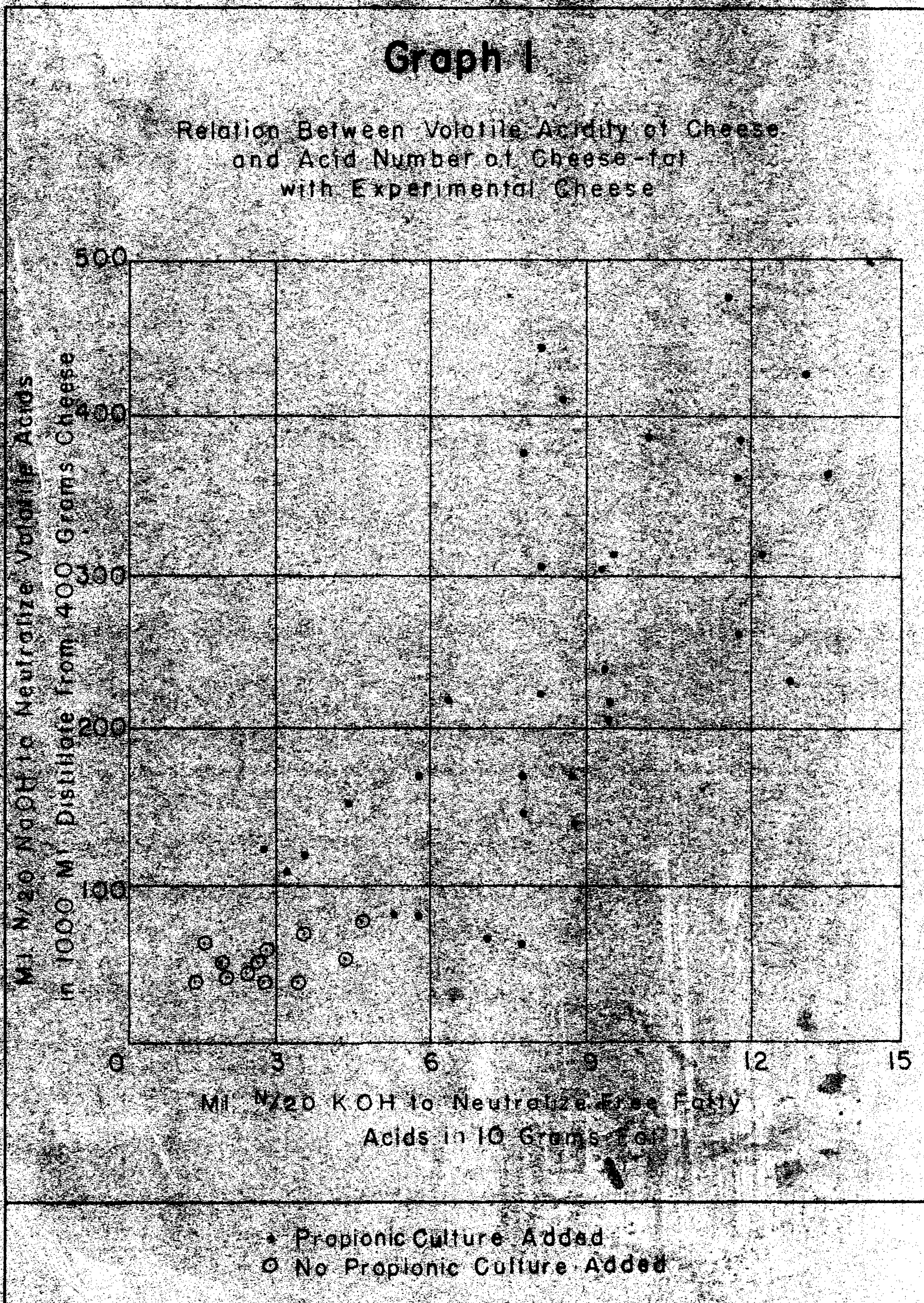
Table 7. Comparison of Flavor of Swiss-type Cheese with Volatile Acidity of Cheese and Acid Number of Fat.

Cheese no.	Flavor	Ml. N/20 volatile acids per 400 grams cheese: 1st 500 ml. distillate	Ml. N/20 volatile acids per 400 grams cheese: 2nd 500 ml. distillate	Ml. N/20 volatile acids per 400 grams cheese: Sum	Acid number of fat
25-1	Sweet	128.00	73.75	201.75	9.55
25-2	Fair	56.50	31.25	87.75	5.95
25-3	Fair	52.50	32.50	85.00	5.22
25-4	Poor	29.00	13.50	42.50	2.73
26-1	Very sweet	277.50	153.70	431.20	13.15
26-2	Sweet	179.00	108.00	287.00	11.00
26-3	Fair	77.00	48.00	125.00	2.45
26-4	Poor	31.00	13.75	44.75	2.23
27-1	Very sweet	241.50	123.25	364.75	13.40
27-2	Sweet	145.00	88.25	233.25	12.88
27-3	Good	161.25	99.25	260.50	11.66
27-4	Poor	26.50	13.25	39.75	3.35

Cheese having a very sweet flavor always contained a large quantity of volatile acids and the cheese-fat always had a high acid number. With a decrease in the sweet flavor of the cheese, as evidenced by the cheese having a fair or poor flavor, the volatile acidities of the cheese and acid numbers of the cheese-fat decreased. In most cases, the cheese having the highest volatile acidity in the series was considered to have the most desirable flavor.

Graph 1 shows the relation between the volatile acidity of cheese and the acid number of cheese-fat. It is based on the results given in Tables 5 and 6, together with additional data. The experimental cheese made without added propionic acid culture fall in a rather small area at the lower left-hand portion of the graph, indicating a low volatile acidity and a low acid number. The experimental cheese made with propionic acid culture had a general tendency to have a high volatile acidity and a high acid number. In some cases cheese having nearly the same volatile acidity showed considerable variation in acid numbers. For example, two cheese having volatile acidities of about 225, had acid numbers of 6.5 and 12.5.

In several instances the distillates obtained from steam distilling cheese were analyzed for types of volatile acids. The distillates did not contain all of the volatile acids present in the cheese because only 1 liter of distillate was collected. Propionic and acetic acids were found to make up


Graph I
Relation Between Volatile Acidity of Cheese and Acid Number of Cheese-fat with Experimental Cheese
Ml N/20 NaOH to Neutralize Volatile Acids in 1000 Ml Distillate from 400 Grams Cheese
500
400
300
200
100
0
3
6
9
12
15
Ml. N/20 KOH to Neutralize Free Fatty Acids in 10 Grams Fat
• Propionic Culture Added
⊙ No Propionic Culture Added

about 94 per cent of the volatile acids. The other 6 per cent was probably contributed by higher molecular weight acids. Propionic and acetic acid were present in the ratio of approximately four to one.

pH of Swiss-type Cheese.

Measurement of the pH of cheese is of comparatively recent origin when contrasted with other methods of measuring acidity. The pH has been used as a basis for predicting the quality of Swiss cheese that would result after aging, and a pH value of 5.2 when the cheese is 24 hours old appears to be the most desirable (17, 22). It also has been shown that various defects, such as checking of the rind, are due to large differences in pH between the interior of the cheese and the portion just beneath the rind (7).

At various stages in the manufacture of Swiss-type cheese from pasteurized milk with the addition of propionic acid bacteria, pH determinations were made on several series of cheese.

Table 8 gives the pH values on four lots of cheese and the corresponding titratable acidities. In two of the trials, the fresh milk had a pH of 6.58 and the corresponding titratable acidities were 0.14 and 0.16. The addition of 1 per cent regular cheese culture to the milk decreased the pH and increased the titratable acidity but not by constant values. Allowing the milk to ripen for about 30 minutes before adding

Table 8. Relation Between pH and Titratable Acidity During the Manufacture of Swiss-type Cheese.

Stage of manufacture	Trial 1		Trial 2		Trial 3		Trial 4	
	pH	% acid	pH	% acid	pH	% acid	pH	% acid
Fresh milk	6.72	0.12	6.69	0.14	6.58	0.16	6.58	0.14
Milk after adding starter	6.65	0.16	6.52	0.17	6.55	0.17	6.55	0.16
Milk after ripening	6.55	0.17	6.48	0.18	6.52	0.18	6.51	0.17
Whey after cutting	6.50	0.12	6.47	0.11	6.51	0.12	6.49	0.11
Whey at draining time	6.45	0.12	6.46	0.12	6.47	0.10	6.44	0.12
Fresh curd	6.52		6.49		6.51		6.48	

the rennet further decreased the pH and increased the titratable acidity. At the end of the ripening period there was very little difference in pH or titratable acidity between the various batches; the difference in pH being 0.07 and in titratable acidity 0.01.

After cutting the curd, the whey showed a slight decrease in pH and a large decrease in titratable acidity as compared with the milk after ripening. The whey decreased in pH slightly from the time of cutting the curd until draining, while the titratable acidity remained about the same. Between the time of cutting the curd and draining the whey, about one-third of the whey was drawn off and replaced with hot water. The pH of the fresh curd was very constant and ranged from 6.48 to 6.52.

The relationship between the pH and the quality of the cheese was studied with eight series of cheese. Each series consisted of four cheese made from the same lot of milk. Propionic acid culture was used in making three of the cheese, the fourth being the control. The series were selected because they showed distinct differences in quality; the cheese falling into four definite groups classed as excellent, good, fair and poor.

Table 9 gives the data obtained. The variation in pH between all the cheese examined was very small, the maximum pH being 6.20 and the minimum 5.53 (difference 0.67). There was much overlapping of the pH values of the excellent and those of the poor cheese. Only three of the eight cheese classed as poor had pH values that did not fall within the pH

Table 9. Comparison of pH of Cheese with Quality of Swiss-type Cheese.

Series no.	Quality of cheese			
	Excellent	Good	Fair	Poor
	pH	pH	pH	pH
10	6.01	6.01	5.84	5.84
11	6.07	5.93	5.96	6.03
12	5.91	6.01	5.53	5.61
13	5.72	6.06	6.06	5.89
16	5.88	5.70	5.53	5.54
20	6.11	5.93	5.79	5.71
25	5.76	5.78	5.79	5.94
26	5.82	5.78	6.20	5.97
pH range	5.72-6.11	5.70-6.06	5.53-6.20	5.54-6.03

range of the excellent cheese. Determinations were also made on other cheese not represented in the table, and the pH values do not vary from the ranges given.

There was no apparent relation between the pH and the quality of the cheese.

It should be noted that the Swiss-type cheese upon which these determinations were made is kept very low in acid throughout the manufacturing process. Draining a portion of the whey and replacing it with water makes the cheese low in lactose so that the subsequent lactic acid production is rather limited. In the manufacture of regular Swiss cheese in the kettle, considerably more acid is developed in the manufacturing process.

Defects Encountered in Swiss-type Cheese.

In the manufacture of Swiss-type cheese from pasteurized milk with the addition of propionic acid bacteria, various defects were encountered. A lack of flavor was sometimes noted in cheese to which propionic acid culture had been added. When this was the case, however, the propionic acid bacteria failed to grow extensively in the cheese and were present in rather small numbers. Cheese having too many and too large eyes were most commonly encountered during the summer months. This was probably due to a lack of adequate temperature control in the curing room since sufficient refrigeration could not be obtained. During these months, both the control cheese and the inoculated cheese often had excessive eye formation.

An objectionable defect, encountered several times, was the presence of small irregular holes near the rind on one side of a cheese. Figures 3 and 4 illustrate this defect. It is thought to be due to cold curd which did not mat properly. Since the defect was only present on one side of the cheese, it may be that this conforms to the top of the original curd mass. The top surface would cool rather quickly when only a small amount of curd is present in the vat, which was the case when making experimental cheese.

Defects due to objectionable organisms were also encountered. These involved bacteria which should have been killed by pasteurization so they presumably gained entrance to the milk by recontamination, and also organisms which are not destroyed by pasteurization so that they may have survived the heat treatment.

Two of the series of cheese contained *Escherichia-Aerobacter* organisms in large numbers. These cheese had developed many small eyes when 2 days old. One cheese from each series was examined, and there were approximately 60,000,000 *Escherichia-Aerobacter* organisms per gram in each of the samples that was plated on violet red bile agar. During the curing process, pronounced swelling occurred. The cheese were cut when 3 months old and contained numerous small ragged eyes. Figures 5 and 6 illustrate the exterior and interior of the cheese. The flavor of these cheese was some-

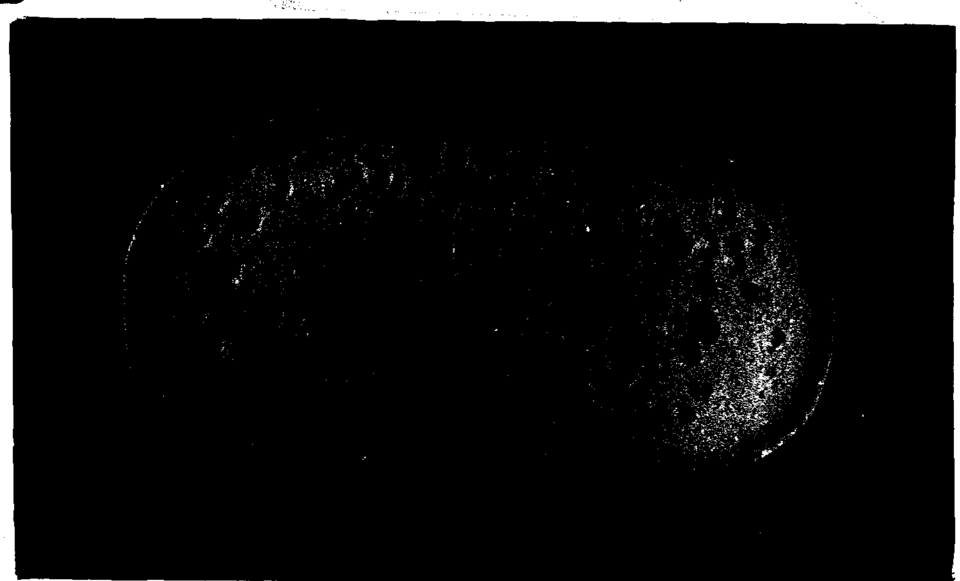


Figure 3.
Swiss-type cheese showing small irregular holes near the rind; probably due to cold curd.

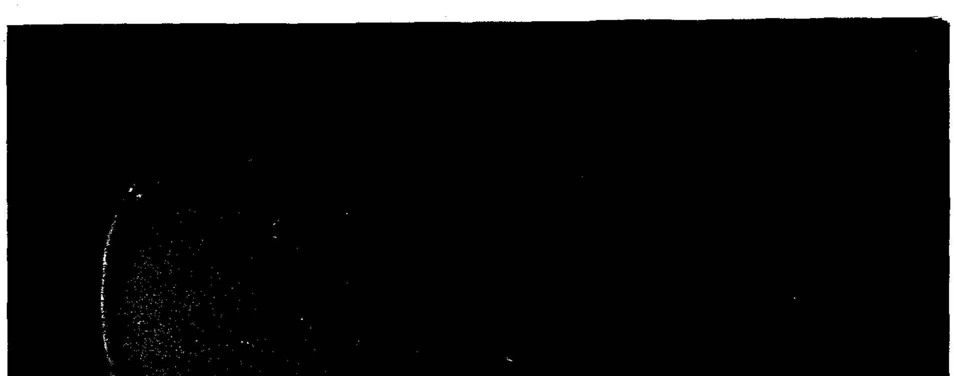


Figure 4.
Swiss-type cheese showing small irregular holes near the rind; probably due to cold curd.

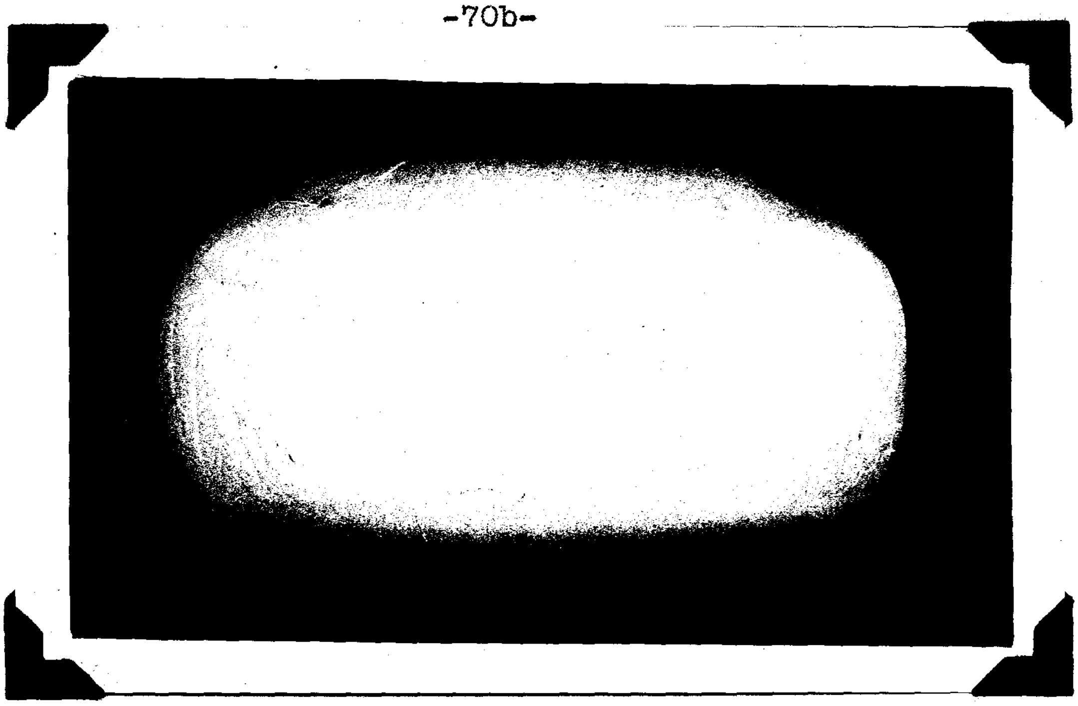


Figure 5.
Exterior of a Swiss-type cheese showing excessive swelling due to <u>Escherichia</u>-<u>Aerobacter</u> organisms.

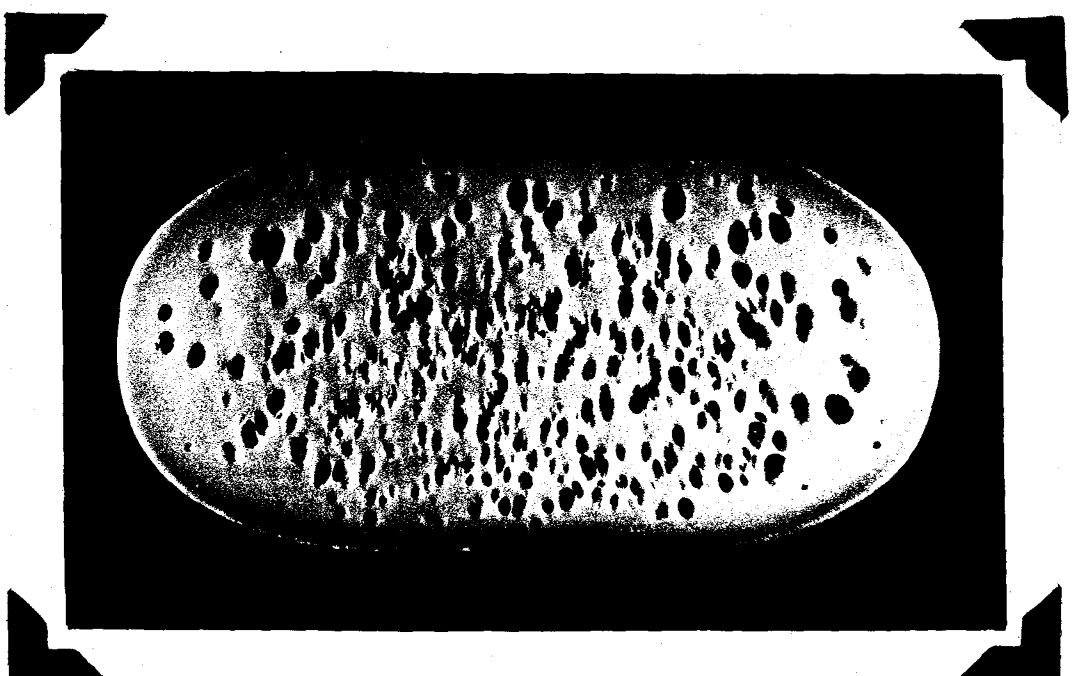


Figure 6.
Interior of a Swiss-type cheese showing numerous, small, ragged eyes due to <u>Escherichia</u>-<u>Aerobacter</u> organisms.

what unclean but was only slightly objectionable; the control cheese had a more pronounced unclean flavor then the cheese made with propionic acid bacteria, the latter developed the characteristic sweet flavor typical of Swiss-type cheese and perhaps this helped to mask the unclean flavor. The defect was undoubtedly due to contamination from cans which were used to transport the milk from the pasteurizing room to the cheese-making room. The two lots of cheese were made on successive days, and on these days there was an irregularity in the can washing. Moreover, on subsequent days when the cans were properly steamed just prior to use, the defect was eliminated.

One series of six cheese developed a flavor and odor resembling butyric acid during the ripening. The flavor and odor were not so objectionable early in the ripening process but became more intense later. This defect was present in the control cheese as well as in the cheese made with propionic acid bacteria. These cheese contained many anaerobic spore-forming rods (genus Clostridium). A pure culture isolated from cheese fermented lactic acid under anaerobic conditions with the production of butyric acid; the culture was grown in a broth containing 0.5 per cent yeast extract and 1 per cent sodium lactate and the butyric acid was identified by the partition method of Osburn, Wood and Werkman (49).

Members of the genus Clostridium in the spore stage are not killed by pasteurization and if present in the milk in large numbers might cause a defect which is beyond the control of the cheesemaker. The defect due to the *Clostridium* organisms was much more objectionable than that due to the *Escherichia-Aerobacter* organisms because the intense flavor and odor of butyric acid increased with age.

DISCUSSION

The presence of relatively large numbers of propionic acid bacteria in aged Iowa Swiss-type cheese of satisfactory quality, as well as in domestic Swiss, suggests that there is a correlation between the numbers of propionic acid bacteria and the characteristic sweet flavor. It also suggests the possibility of adding cultures of the organisms to milk used in the manufacture of Swiss-type cheese. Although a few of the Cheddar cheese examined contained rather large numbers of propionic acid bacteria, the lack of correlation between the numbers of these organisms and the cheese quality shows that the fermentation in this type of cheese is quite different than in Swiss-type.

The desirability of adding certain strains of propionic acid bacteria (genus Propionibacterium) to pasteurized milk used for the manufacture of Swiss-type cheese is indicated by the production of the characteristic sweet flavor with this procedure. The importance of the action of these organisms in the cheese is emphasized by the fact that the cultures which were most satisfactory from the standpoint of flavor development were those which grew best in the cheese and also by the fact that when the control cheese had a sweet flavor, relatively large numbers of the organisms were present in it.

Cheese having considerable of the characteristic sweet flavor regularly had a high volatile acidity which evidently was due to the production of propionic and acetic acids from lactic acid or lactates by the propionic acid bacteria. This relationship is in agreement with the conclusions of Babel and Hammer (3) who associated the sweet flavor of Swiss-type cheese with propionates, calcium propionate presumably being the most important because of the relative abundance of calcium with which the propionic acid arising from the fermentation of lactic acid can combine. The fact that increased amounts of a satisfactory propionic acid culture added to the milk used in the manufacture of Swiss-type cheese resulted in an increase in the degree of sweet flavor further indicates that certain products formed by these organisms are important flavor contributors.

The cheese made in a series (from milk that had been pasteurized in one lot and then divided into four portions) and cured under the same conditions generally had similar eye formation, although one of the cheese was a control while the other three were made with added propionic acid culture. Propionic acid organisms undoubtedly affect eye formation to some extent since cheese made with large inoculations of propionic acid organisms frequently developed too large eyes while smaller inoculations resulted in satisfactory eye formation. However, various other organisms can undoubtedly produce eyes in Swiss-type cheese because in several instances cheese had about the desired number of eyes and no propionic acid bacteria could be detected in 0.1 gram portions.

Although delaying the salting for 7 days following manufacture was desirable from the standpoint of flavor and eye formation, the tendency of the cheese to flatten makes the procedure impractical. With a 2-day delayed salting period the flattening was less. Since the cheese used in these experiments were about 15 pounds in weight there probably was less flattening than if a heavier cheese had been made.

The data obtained from pH determinations on aged Iowa Swiss-type cheese did not indicate any correlation between pH and cheese quality. Although this is not in agreement with the results of other investigators, it should be noted that only slight variations in pH existed among all the samples of Swiss-type cheese examined and also that only a small amount of acid is developed in the cheese due to the low concentration of lactose.

The use of pasteurized milk in the manufacture of Swiss-type cheese should be beneficial from the standpoint of making cheese of more uniform quality because pasteurization tends to destroy certain organisms capable of bringing about undesirable fermentations. The presence of large numbers of Escherichia-Aerobacter organisms are particularly troublesome in the manufacture of Swiss-type cheese.

Pasteurization would limit the numbers of these organisms and thereby prevent them from causing defective cheese unless they gained entrance to the milk subsequent to the heating. Organisms belonging to the genus Clostridium also cause defective Swiss-type cheese and if present in the spore stage would not likely be destroyed by pasteurization. Therefore, pasteurization would not be a means of entirely controlling the initial bacterial flora of the milk for cheese manufacture but would aid materially in controlling defects due to certain species.

SUMMARY AND CONCLUSIONS

Propionic acid bacteria were found in various cheeses, including Iowa Swiss-type, domestic Swiss, Cheddar and canned Cheddar. Swiss-type cheese having a characteristic sweet flavor generally contained relatively large numbers of propionic acid bacteria and cheese with a poor flavor generally contained few or no propionic acid bacteria in 0.1 gram. All the samples of domestic Swiss cheese contained rather large numbers of propionic acid bacteria. About 85 per cent of the samples of Cheddar cheese (of both good and poor quality) contained propionic acid bacteria; there was no correlation between the numbers of these organisms and the quality of the cheese. One sample of canned Cheddar cheese which had eyes similar to those in Swiss cheese contained a rather large number of propionic acid bacteria.

Eighteen strains of propionic acid organisms were used in the manufacture of Swiss-type cheese from pasteurized milk. Several of the cultures were rather consistent in the type of flavor produced while others were variable. Results of comparisons indicated that certain cultures rather regularly produced cheese having either an excellent or good flavor. None of the cultures were consistent in producing good eye formation.

In most instances, cultures which increased appreciably in number during the ripening period produced cheese having considerable sweet flavor.

Additional trials made with cultures which gave satisfactory results in the original comparisons indicated that certain cultures were very consistent in producing an excellent or good flavor. The culture selected as best produced cheese having an excellent or good flavor in 16 of 17 trials.

A comparison of various amounts of propionic acid culture showed that by increasing the number of propionic acid bacteria added to the milk, a corresponding increase was obtained in the degree of sweet flavor produced. Cheese receiving an inoculation of 100 ml. of culture per 100 pounds of milk were occasionally criticized as being too sweet. With an inoculation of 25 ml. of culture per 100 pounds of milk, the cheese were considered to have about the desired sweet flavor. The cheese which received an inoculation of 100 ml. of culture per 100 pounds of milk had a tendency to develop eyes which were a little larger than was desirable, but this was not true in all cases. Cheese receiving an inoculation of 5 ml. of culture per 100 pounds of milk gave the best eyes in the majority of instances.

In several series the control cheese, to which no propionic acid culture was added, developed as good a flavor as the cheese made with propionic acid culture.

When this was the case, propionic acid bacteria were found in the cheese in large numbers.

Frequently, the four cheese in a series closely resembled each other in eye formation although one cheese was a control while the others were made with propionic acid bacteria added to the milk. Cheese in which no propionic acid bacteria could be detected in 0.1 gram sometimes developed eyes equal to those in cheese made with added propionic acid culture.

When Swiss-type cheese were not salted until 7 days after manufacture, the cheese contained more eyes and the eyes extended nearer the surface than when the cheese were salted in the normal way (the day following manufacture). Delayed salting also tended to produce cheese having more sweet flavor. However, the delayed salting tended to cause the cheese to flatten, which was not the case when the cheese were salted in the normal way. A 2-day delayed salting period slightly favored the production of more desirable eyes and the sweet flavor was somewhat more conspicuous; the cheese also retained their original shapes better.

Excessive swelling, due to the production of many large eyes, could be stopped by placing the cheese in a cold room at about 3.3° to 4.4°C. (38° to 40°F.). Swiss-type cheese held at about 21.1° to 22.2°C. (70° to 72°F.) showed first signs of swelling in 3 or 4 days while with holding at about 10.0° to 12.7°C. (50° to 55°F.) the first signs of swelling were evident in 3 or 4 weeks. Cheese held for a time at the higher temperature tended to have eyes which were not quite as smooth as the eyes in the cheese held at the lower curing temperature.

The addition of propionic acid bacteria to pasteurized milk made into cheese gave cheese with a higher volatile acidity and the cheese-fat contained more free acids than cheese made from milk to which no propionic acid bacteria were added.

The pH determinations on Swiss-type cheese, aged 3 months, indicated there was no definite correlation between pH and quality. There was very little difference between the pH values of all cheese examined.

The defect encountered most frequently in Swiss-type cheese was poor eye formation. Two series of cheese contained large numbers of <u>Escherichia</u>-<u>Aerobacter</u> organisms; they were more objectionable from the standpoint of eye formation than flavor since the eyes were small and numerous. One series of cheese contained large numbers of anaerobic spore-forming rods; they were more objectionable from the standpoint of flavor than eye formation.

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