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The influence of certain bacteria on the ripening of Cheddar cheese made from pasteurized milk

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THE INFLUENCE OF CERTAIN BACTERIA ON THE RIPENING OF CHEDDAR CHEESE MADE FROM PASTEURIZED MILK

By

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INTRODUCTION

Pasteurization of milk for Cheddar cheese making is probably the most important contribution to the cheese industry that has been made in recent years. From a public health standpoint the pasteurization of all milk for cheese making is of great significance since it minimizes the possibility of spreading infectious diseases through cheese. Pasteurization of milk for cheese making also aids in controlling undesirable fermentations due to organisms present in the raw milk and produces a more uniform product. From an economic standpoint, however, pasteurization adds to the cost of producing cheese: first, because of extra labor and equipment; and second, because it prolongs the ripening process. The extra cost involved in the manufacture of cheese from pasteurized milk is generally returned to the manufacturers in the form of more uniform quality, greater yield, and less loss in storage. In the pasteurization of milk several changes occur which not only prevent the typical cheese flavor from developing, but also materially increase the time necessary for proper ripening. Whether these changes are due to the destruction of the natural bacterial flora of the milk or to the partial destruction of enzymes, or perhaps both, is not known. If the increase in time required for the ripening of the cheese is due to the destruction of the natural bacterial flora of the milk by pasteurization, the addition of pure cultures of the essential bacteria should overcome this difficulty.

OBJECT

The primary object of the work carried out was to study the effect of certain bacteria on the nitrogenous decomposition and flavor development in Cheddar cheese made from pasteurized milk. The organisms investigated included proteolytic and lipolytic types.

Some attention was also given to the number of bacteria present in the cheese at various stages during ripening, the acid number of the fat in the cheese made with the various test organisms and the percentage of total flora made up of lactobacilli at various times during the making and ripening of the cheese.

GENERAL PROCEDURE

The effects of the cultures of bacteria were studied in six series of cheese made from pasteurized milk. Each series consisted of four lots of cheese, manufactured simultaneously from an original single lot of milk. One portion of the milk was used as a control while each of the three other portions was inoculated with a test organism or a mixture of test organisms in addition to the regular cultures. Each portion of milk was manufactured into two cheese, one ten pound longhorn for the chemical and bacteriological analyses, and one five pound young American for determining the keeping quality. Chemical and bacteriological analyses were made at the following intervals during the experimental periods: 3 days, 14 days, 28 days, 56 days, and 112 days. At the same intervals the cheese were scored for flavor, body and texture.

HISTORICAL

Part 1. The Influence of Pasteurization of Milk on the Ripening of Cheddar Cheese.

Klein and Kirsten (55), in 1898, added calcium chloride and a bacterial starter to pasteurized milk and were able to make fairly good Limburger and other soft cheeses. They used, in each 100 kilograms of skim milk, 100 to 125 ml. of a solution containing 40.0 per cent of calcium chloride.

Dean (21), as a result of experiments in the use of calcium chloride with pasteurized milk for Cheddar cheese making, stated that "the coagulum was of a soft weak nature and the cheese tended to be soft and porous." He also tried adding starter to the milk and ripening it for some time before setting and before adding calcium chloride, but no improvement was noticed over the use of calcium chloride alone. He summarized his work in the following statement: "On the whole, the results are not very satisfactory, and we shall require more light on the subject of making pasteurized milk cheese before we can recommend this method to Canadian cheesemakers."

Most of the defects observed in cheese factory milk, according to Sammis and Bruhn (78), are of bacterial origin. The desirability of pasteurization of milk for cheese making had often been stressed. They stated that the quality and behavior of pasteurized milk curd suggested that it lacked the acid which was normally produced in raw milk curd by the action of bacteria on milk sugar and also that heated milk coagulated poorly with rennet and did not expel moisture like raw milk curd. The difficulty of coagulation was overcome by the addition of calcium chloride solution to

the milk and both difficulties were overcome by the addition of an acid, preferably hydrochloric acid; 95 per cent of the hydrochloric acid passed out in the whey. Heating the milk to 160° to 165° F. flash was found sufficient to check effectively the bacterial action in milk for cheese making purposes. Bacterial counts showed that over 99 per cent of the total bacteria in the milk were destroyed at this temperature. The use of higher temperatures was shown to be objectionable on account of the effect upon the flavor, body and texture of the cheese.

Stevenson (83) concluded that a pasteurization temperature of 160° to 165° F. gave the best results. Below this temperature the bacterial efficiency decreased, and above this temperature the casein content of the milk was injured enough to cause a decrease in yield of cheese. He also found that good quality milk was just as essential in the manufacture of cheese from pasteurized milk as from raw milk, since only good milk will make the highest grade of cheese.

In studying the effect of pasteurization of milk for Cheddar cheese making, Murray (63) found that pasteurization caused milk to become more favorable to the attack of gas forming bacteria. To counteract this undesirable feature, precautions were taken to eliminate recontamination as far as possible.

Atkinson (3, 4), in 1924, confirmed the work of Murray. Marquardt and Hucker (59) found that cheese made from milk pasteurized at 142° to 145° F. for 30 minutes scored on an average 2.1 points above cheese made from similar raw milk. Pasteurization at this temperature did not affect the body and texture of the cheese or impart a pronounced cooked flavor if an active clean starter was used.

Price (68), in studies on the effect of various pasteurization temperatures on milk for Cheddar cheese, came to the conclusion that 145° F. for 30 minutes produced better quality cheese than did any other method of pasteurization.

Price and Prickett (69) found that milk pasteurized at 150° to 154° F., or at 160° to 165° F. flash, or at 145° F. for 30 minutes, produced higher quality cheese than identical milk not pasteurized. The flash methods of pasteurization were not as effective as the holding method.

Lane and Hammer (57) investigated the influence of pasteurization of milk on the nitrogenous decomposition in Cheddar cheese. They studied three series of cheese, each series consisting of three cheese, one made from pasteurized milk, one made from raw milk, and one made from 90 per cent pasteurized and 10 per cent raw milk. Changes in the nitrogen distribution in the cheese were determined by chemical analyses of cheese serum at intervals during ripening. They found that during the early stages of ripening there was very little variation in the amounts of the various fractions in the serums of the raw and pasteurized milk cheese. After longer periods of ripening the amounts of the various nitrogen fractions were definitely larger in the serum of the raw milk cheese than in the serum of the pasteurized milk cheese, which indicated that more rapid decomposition took place in the cheese made from raw milk. The cheese made from 90 per cent pasteurized and 10 per cent raw milk was usually intermediate between the cheese made from pasteurized and raw milk, as indicated by the various nitrogen fractions. They also found that cheese made from raw milk regularly scored higher on flavor after two months of ripening than did cheese made from pasteurized milk. The cheese made from pasteurized milk was gen-

erally characterized by a lack of flavor while the cheese made from the mixed milk developed a flavor quite similar to that of the raw milk cheese.

Part 2. The Influence of Bacteria on the Ripening of Cheddar Cheese.

The flavor development in Cheddar cheese has been the subject of much chemical and bacteriological research.

Russell (73), in 1896, made a study of a pure lactic culture of bacteria in cheese making. The culture he used was isolated from a ripening cheese, where it made up over 99 per cent of the bacteria in the cheese. He stated, "This organism is a bacillus of the lactic acid type that does not form gas or any objectionable by-products. No inherent virtue is claimed for this cheese germ as it is quite probable that other pure lactic ferments producing no undesirable by-products would be quite as efficient."

Russell (74) also studied the numbers of bacteria in Cheddar cheese at various stages during ripening. These periods included: "(a) a period of decrease in the number of bacteria in the green curd, which lasts for only a day or so; (b) a period of increase in numbers, in which the bacteria reach millions per gram; (c) a period of final decrease in bacteria, at first rapid but later more gradual until the germ content sinks to insignificant proportions when a point is reached where relatively few living bacteria remain." This investigator also stated, "The bacterial flora of cheese differ markedly from that of milk. In milk Streptococcus lactis predominates, but accompanying them are always liquefying and peptonizing organisms. In the ripening of cheese the peptonizing or casein digesting bacteria are quickly eliminated, while the gas producing bacteria disappear

more slowly. The generally accepted theory that the peptonizing or digesting bacteria are able to break down the casein in the cheese as they do in the milk is improbable because this type of bacteria fails to increase in the cheese and usually disappears before there is any evidence of physical change in the conditions of the casein. The same is true where cheese is made from pasteurized milk to which starters of the peptonizing organisms have been added."

Weigman (94) concluded from his work that lactic acid bacteria play an important part in cheese ripening, not in actually taking part in the ripening, but by directing the process in the right direction. This function consisted in eliminating certain forms of bacteria and fungi by means of the formation of lactic acid, and provided an acid medium upon which could thrive only such bacteria and fungi as could withstand the acid or consume it. The microorganisms which consumed the acid and prevented its accumulation in too strong a degree, took part in the peptonizing and flavor producing process that enabled other bacteria or fungi, whose activities were weakened by the acid, to continue their work.

The Bacterium casei group is active in breaking down the casein of milk to which calcium carbonate has been added, as was shown by Orla-Jensen (51). He came to the conclusion that the casein was not peptonized but was split directly into monoamino acids.

In studying the ripening of Cheddar cheese, Campbell (16) came to the following conclusions: (a) that the principle underlying the pure culture system was sound; (b) that though there were a number of different bacteria which produced lactic acid when grown in milk, one form was always found predominating in ripe milk, sour whey, and good cheese; (c) that for

the manufacture of Cheddar cheese this organism, and this one only, was required for the fermentation of both milk and curd, and also for the ripening process; (d) that the bacterium in the pure culture used in the investigation appeared to be identical with the bacterium that predominated in sour milk, sour whey, and good cheese, and all its characteristics agreed with the organism of Leichmann but it was not Bacillus acidi lactic.

Von Freudenrich (31) showed that practically only lactic acid producing organisms were found in ripening cheese, and that other bacteria occurred in numbers too small to mention. He stated it had been shown that these lactic acid bacteria were able to decompose and dissolve the casein, which proved that the lactic acid bacteria were the cause of the ripening of hard cheese.

Baier (7) concluded that certain relative proportions of the different kinds of bacteria were essential for the proper ripening of cheese and that the ripening process of a given type of cheese was not due to a single species of organism or to an accidental condition of affairs.

In comparing the bacterial content of different cheese, Harrison and Connell (44) found that there was a gradual decrease in numbers of bacteria after the cheese was 4 to 5 days old. They came to the conclusion that the high bacterial content was the chief factor in determining the flavor of cheese properly made from normal milk.

The function of the lactic acid bacteria in the manufacture and in the early stages of ripening of Cheddar cheese was studied by Harding (41). He stated "that when considering the flora of cheese, interest is commonly so centered upon the striking increase in the lactic acid types

that the presence of the other organisms is usually overlooked. While the number of liquefiers rarely amounts to more than one per cent of the total dur'ng the early history of Cheddar cheese, even under these circumstances their number is considerable. Furthermore, it is not unreasonable to suppose that an enzyme formed by this class of organisms will continue to act in the cheese even after the disappearance of the living cells."

The only group of bacteria constantly found in large numbers in Cheddar cheese, by Harding and Prucha (42), was the Bacterium lactis acidi group. They noted that the acid forming, liquefying organisms were present in cheese at all times but in numbers so small that it was suggested the group exerted little influence upon the ripening changes. The rate of the ripening process seemed to be independent of the number of germs present, except that in certain cheese a flora closely confined to acid producing forms was associated with a slower rate of ripening.

Hastings, Evans, and Hart (45), in their research on the ripening of Cheddar cheese, arrived at the following conclusions: (a) that if heavy inoculations of lactic bacilli were made into milk which contained a small number of Bacterium lactis acidi, the normal ecological balance would be destroyed and the result would be an abnormal cheese; (b) that if a culture of lactic bacilli was added to pasteurized milk instead of Bacterium lactis acidi, the ripening of the cheese would not be normal, and the result would be an increased rate of ripening with the production of an abnormal flavor in the cheese. They further stated that their work also indicates it is often useless to attempt to establish the role of any organism in cheese ripening by the addition of cultures to the milk to be used, since thereby the natural equilibrium is destroyed and the results

obtained indicate that the addition has injured the product; the conclusion is drawn that the organism added is not only unessential but is even harmful, although the organism may be an essential factor in the decomposition changes when developing in its natural sequence.

The influence of Bacterium casei in starters for pasteurized milk cheese was studied by Evans, Hastings, and Hart (26). In a series of cheese different varieties of Bacterium casei were added together with Bacterium lactis acidi. When variety "a" was added there was a tendency for the cheese to become "acid injured." The use of variety "b" was even more likely to bring about this condition. When variety "c" was used the cheese was almost certain to be ruined by the acid before it was a month old. They concluded that variety "a" and variety "b" were about equally distributed in normal Cheddar cheese where they usually occurred together and performed an active part in the ripening change. The introduction of this group as a starter, however, resulted in abnormally large numbers of Bacterium casei in the early ripening period, which were found to be detrimental to the cheese. The same investigators also isolated micrococci and liquefying bacteria from Cheddar cheese. When grown in milk the varieties of Bacterium casei, according to Hart, Hastings, Flint, and Evans (43), produced large quantities of the volatile acids, particularly acetic acid.

Evans (25) made a comparative study of the bacterial flora of raw milk cheese and pasteurized milk cheese to determine the origin of the characteristic Cheddar cheese flavor. The effective flora of raw milk cheese comprised the following four groups: (a) Bacterium lactis acidi; (b) Bacterium casei; (c) streptococci; and (d) micrococci. She came to the conclusion that good flavor does not depend upon the large predominance

of any one of these four groups, and that by preparing starters containing the four groups of bacteria the characteristic Cheddar flavor could be produced in pasteurized milk cheese. As commonly made (with a pure culture starter) from pasteurized milk, however, cheese does not contain the four groups named above and lacks the characteristic Cheddar flavor.

In discussing the relationship of bacteria to the quality of Cheddar cheese, Murray (63) stated that the bacterial flora of the raw milk was the most important factor in the production of good cheese, and that the characteristic aroma of choice Cheddar cheese was almost exclusively a feature resulting from interplay of bacterial activities.

Evans, Hastings, and Hart (26) were unable to obtain the characteristic Cheddar flavor in pasteurized milk cheese when starters composed of the Bacterium lactis acidi groups were used, but when cultures of certain streptococci isolated from raw milk cheese were added in addition to starters containing Bacterium lactis acidi the flavor was materially improved.

Evans (27) isolated two strains of streptococci from Cheddar cheese, Streptococcus X and Streptococcus kefir. The most pronounced biochemical characteristic which distinguished Streptococcus lactis from Streptococcus X and Streptococcus kefir was the small quantity of acetic acid which was produced in milk cultures. When the two latter organisms were inoculated into pasteurized milk to be made into cheese an improvement in flavor was noted and the protein breakdown was hastened.

In studying the types of bacteria present in commercial Cheddar cheese, Hucker (48) found that the better grades of cheese contained a distinctly different flora than the poorer grades. In the better types,

Streptococcus lactis and lactobacilli predominated, while in the poorer grades the spore forming and gram negative rods were present in the largest numbers. The presence of a large number of spore forming and gram negative rods in the poorer cheese indicated that these types were undesirable for the production of a higher grade of Cheddar cheese. The cocci and the streptococci other than Streptococcus lactis varied little in numbers in the different qualities of cheese.

In 1926, Hucker and Marquardt (49) studied the effect of several types of streptococci upon the flavor of the cheese when added to pasteurized milk, either in conjunction with a commercial starter or alone. They concluded that Streptococcus paracitrovorus improved the flavor of the cheese, while Streptococcus citrovorus had no effect upon the flavor. Streptococcus lactis was found to give as favorable results as commercial starter, whereas certain strains of proteolytic cocci, when used as a culture, produced a characteristic bitter flavor in the cheese.

Research by Hansen, Bendixen, and Theophilus (40) indicated that cheese made with Streptococcus citrovorus or Streptococcus paracitrovorus alone as starters produced a bitter flavor and had a weak body while cheese made with Streptococcus lactis as a starter did not develop a typical Cheddar flavor but had a good body and texture.

Whitehead (97) stated that representative strains of organisms of the colon group, if added to the milk immediately before the start of the process of cheese manufacture, had a deleterious influence on the flavor of Cheddar cheese, even when the inoculation was too small to produce gas holes in the cheese.

Haglund, Barthel, and Sandberg (36) found that the rapidity of the

ripening of hard cheese was directly dependent on the number of lactic acid bacteria in the cheese milk at the time of adding rennet. They claimed this fact supported the theory that the lactic acid streptococci exercised a direct, as well as an indirect, influence upon the ripening process. In a later report (37) they stated that the ripening was influenced by two factors, acidity and bacterial counts.

The influence of certain lactic acid streptococci on the chemical changes in Cheddar cheese during ripening was studied by Kelly (53). He found that the protein in the cheese made with strains of Streptococcus lactis and Streptococcus cremoris as cultures, underwent changes similar to those found in cheese made with commercial starters. He decided from this that acid production was the important function of a starter and that the starter had little direct action on the flavor and aroma. Kelly (54) later concluded that acid production was the chief function of a cheese starter and that satisfactory Cheddar cheese could be made with either Streptococcus lactis or Streptococcus cremoris.

Barthel and Sadler (10), working with commercial and other starters in chalked, sterilized milk over a period of two months, found that they did not form more soluble nitrogen than did pure cultures of strains of lactococci isolated from such starters. On the other hand, the former split off considerably larger amounts of amino acids than the latter. This circumstance must be regarded as still further emphasizing the importance of lactococci in the cheese ripening, as it was precisely in the form of starters that the lactic acid bacteria were added to the cheese milk.

Facetti (28), in a resumé of the results of experiments in the use of selected ferments in the cheese making industry, stated that the pure cul-

tures of lactic ferments (cocci bacilli, according to the type of cheese), when added to raw milk, have given results which have led to their adoption in practical cheese factories. He further stated that the problem confronting the bacteriologists is to determine which of the typical forms of lactic ferments at present known are to be used, and with what precautions. Such information is necessary in developing the process of manufacturing cheese from pasteurized milk, which is said to be still enveloped in uncertainty.

Barthel and Haglund (8), in testing the casein digesting powers of several strains of lactococci, one being a strain of Streptococcus cremoris and the other two being diplococcus strains, came to the conclusion that pure cultures of lactococci were always inferior to ordinary starters and that the possibility of shortening the time of cheese ripening by inoculation with strong casein digesting strains of lactococci had very small chance of success.

Lane (56) compared the effect of several organisms on the speed of ripening and the flavor of Cheddar cheese and found that certain strains of Lactobacillus casei, when added to pasteurized milk in addition to the regular starters, produced a mild buttery flavor and hastened the ripening. Aerobacter oxytocum and Streptococcus liquefaciens, when added to the milk for cheese making, produced a bitter flavor, but both organisms increased the rate of ripening. Streptococcus paracitrovorus produced a mild flavor during the early stage of ripening but had no effect on the hydrolysis of the proteins. An unidentified Micrococcus, when added to the milk for cheese making, slightly improved the flavor and somewhat hastened the ripening of the cheese.

Part 3. The Influence of Enzymes on the Ripening of Cheddar Cheese.

The importance of enzymes in the ripening of cheese was first investigated by Babcock, Russell, Vivian, and Hastings (5), who studied the effect of the enzyme galactase on the proteolytic changes in cheese. They stated that while galactase was of animal origin, it was specifically different from other animal ferments when the types of decomposition products formed were considered. Galactase showed a closer relationship to the bacterial enzymes produced by the digestive or liquefying organisms than to any other group. When these investigators compared the products formed in normally ripened cheese and in cheese where all factors other than the enzyme galactase were controlled, the decomposition products were very similar. They therefore concluded that galactase was the causal agent in the proteolytic changes in cheese ripening.

Babcock, Russell, and Vivian (6) stated that rennet exerted a digestive effect on the casein of cheese due to the presence of a peptic enzyme contained in the rennet extract, the action of which was intensified by the development of acid in the curd. The soluble nitrogenous products formed in Cheddar cheese by rennet were the albumoses and the higher peptones. They therefore concluded that increasing the amount of rennet extract used in cheese making did not increase the amount of soluble nitrogenous products by which was measured the progress of cheese ripening. Later Van Slyke and Hart (89) found that an increase in the amount of rennet used in cheese making increased the soluble nitrogenous compounds in the cheese. Barthel, Sandberg, and Haglund (12) were able to demonstrate that rennet was present in juice obtained from several varieties of well ripened cheese.

Bosworth (15) stated that the rennin action was probably a hydrolytic cleavage and may be considered the first step in the proteolysis of casein.

Van Slyke, Harding, and Hart (86) studied the effect of enzymes in cheese ripening and found that if cheese were made from milk to which chloroform had been added the cheese would not ripen normally. They noted that in such cheese the amounts of albumoses and peptones, when compared to amides, were relatively large, and concluded that both enzymes and bacteria were necessary in the ripening of cheese. Suzuki, Hastings, and Hart (82) stated that no enzyme capable of producing lactic acid or volatile acids could be isolated from cheese. They came to the conclusion that the acid normally found in cheese was formed by bacteria and not by enzymes.

In studies on a number of cheeses, Barthel and Sandberg (13) found catalase was present in all of them except one Cheddar cheese. Rogers (72) also noted the presence of enzymes in partially ripened cheese. Orla-Jensen (52) claimed that in the ripening of cheese the caseinous matter was partially peptonized and rendered soluble by means of the enzyme casease, which is very similar to trypsin, and that the microorganisms typical of cheese "fermentation" were more indirect than direct in their action.

Van Slyke and Hart (87) stated that the ripening process in normal Cheddar cheese, by which the insoluble nitrogen compounds change into soluble forms, did not begin with paracasein but with unsaturated paracasein lactate. They found that the water soluble nitrogen in cheese generally increased as the unsaturated paracasein decreased. Van Slyke and Hart (88), in studying the individual proteolytic compounds formed in cheese, obtained large amounts of paranuclein from young cheese, small amounts of histidine,

lysine, and putresine from middle-aged cheese, and putresine from old cheese. They concluded that these compounds were important in the formation of flavor in Cheddar cheese. Later Van Slyke and Hart (89) studied the factors affecting chemical changes in cheese ripening. They came to the conclusion that the accumulation of soluble nitrogen compounds in cheese appeared to diminish the action of the agents causing the changes so that cheese ripened less rapidly after the first period. They also found that the soluble nitrogen compounds in cheese increased quite closely in proportion to increase in temperature.

Virtanen (84) stated that the action of Bacterium casei was important in the mellowing of cheese, that the action took place in several phases, and that the proteolytic enzyme system of the bacterium consisted of three different proteolytic enzymes: (a) proteinase which decomposed the casein to a polypeptid stage; (b) polypeptidase which decomposed the polypeptids to single peptids, or, in special cases, to amino acids; (c) dipeptidase which was capable of decomposing dipeptids containing free amino or carboxyl groups. The decomposition of casein by the action of lactic acid bacteria thus took place by degrees and not without any transition directly to amino acids.

Price (68), working on the assumption that some inherent enzyme in milk was destroyed by pasteurization, added small amounts of well ripened cheese to pasteurized milk for cheese making. He found that the milk inoculated with cheese produced cheese having a very undesirable flavor.

Davis (19) studied the oxidation-reduction potentials of ripening Cheddar cheese. He came to the conclusion that cheese are not homogenous throughout "their mass," and that the zones of more highly oxidized condi-

tions exist near the surface. Davies, Davis, Dearden, and Mattick (20) concluded that variations in the amounts of pepsin and rennin had no effect on flavor, body, texture, or ripening of cheese, and that the addition of lactobacilli with the starter appeared to accelerate the protein degradation in the early stages of ripening.

Part 4. Methods of Obtaining the Soluble Nitrogen Compounds of Cheese.

The soluble nitrogen content of cheese has been used as a measure in determining the degree of ripeness. Van Slyke and Hart (88), in their early work, obtained the soluble nitrogen from cheese by the water extraction method. This method consisted of shaking a sample of cheese in warm water, filtering the mixture and then analyzing the filtrate for products of protein hydrolysis. Allen (1) claimed that results obtained by the water extraction method were unreliable as an index of the extent of protein decomposition. He stated that the percentage of nitrogen soluble in 80 per cent alcohol and the alcohol formol titration of a suspension of the fat free cheese, or of the aqueous or alcohol filtrate from such a suspension, may be taken as a more reliable index.

Sandberg, Haglund, and Barthel (75) stated that it is evident the extraction of a mass of cheese with water alters the physical-chemical equilibrium of the cheese. In several cheeses during ripening they observed protein substances having characteristics of globulins, which were in solution in the juice of cheese, but which were precipitated on extraction of the cheese with water. Van Dam (85) pointed out that since no standard method for determining chemical changes in cheese had been

adopted, there had been a great deal of variation in the procedures used by the various investigators. He showed that the temperature of the water employed, the amount of shaking, and the length of time allowed for the cheese to stand in contact with the water had considerable effect upon the amount of soluble nitrogen obtained in the extract.

Sörensen (81) proposed a method for the quantitative determination of the rate of proteolytic decomposition. It was based upon the assumption that proteolysis occurred as a hydrolytic cleavage with the formation of carboxyl and amino groups. The addition of formaldehyde, which with the amino group formed a methylene combination, allowed the determination of the carboxyl groups present at any stage in the proteolysis. The increase in the number of carboxyl groups could be determined by titration with an alkali from which, under the assumption that for each carboxyl a corresponding amino group is formed, could be calculated the nitrogen split off in the hydrolysis.

Gratz (33) came to the conclusion that the formol titration method was satisfactory for studying the proteolytic changes taking place in cheese. The results obtained by this procedure compared well with the figures obtained for the monoamino acids by precipitation with phosphotungstic acid.

Sandberg, Haglund, and Barthel (76) concluded that a determination of the degree of hydrolysis in cheese would be more exact if one worked directly with the juice extracted from the cheese rather than with a water extract of the cheese. They found that by submitting a mixture of ground cheese and fine sand to a relatively high pressure a cheese juice was obtained which, upon standing a time, separated into distinct layers of fat,

undissolved casein and cheese serum. This serum could then be analyzed directly for the various forms of soluble nitrogen.

Part 5. Precipitation of Protein and Protein Decomposition Products.

A large number of chemicals has been used by various investigators to separate the nitrogen containing compounds of different materials into various fractions. Such a separation has been used in studies on the decomposition products of milk, blood, water extract of cheese and juice pressed from cheese.

Ritthausen (71) used cupric hydroxide to precipitate the proteins from milk before determining the carbohydrate content. Sebelien (79), in a similar study, used tannic acid as a precipitating agent. Riddeal and Stewart (70) stated that the use of tannic acid for the precipitation of proteins gave unsatisfactory results and that they preferred the use of chlorine because it was simpler and gave results that were easy to duplicate. Allen and Searle (2) found bromine to be a more efficient precipitating agent than tannic acid. Simon (80) noted that a high concentration of trichloracetic acid is necessary for the complete precipitation of milk proteins.

Van Slyke and Hart (88) studied the water soluble nitrogen content of Cheddar cheese and used phosphotungstic acid and sulphuric acid, tannin plus sodium chloride, and bromine plus hydrochloric acid to separate the various proteolytic compounds found in cheese. In studying the group characteristics of the different amino acids, Van Slyke (91) found that phosphotungstic acid separated the amino acids into two fractions, the

bases (histidine, lysine, arginine, and cystine) being precipitated while the others remained in solution.

Welker and Marsh (95) used aluminum hydroxide as the precipitating agent for the determination of lactose in milk. Palmer and Scott (65) employed tannic acid in their studies on proteins in milk. Moir (60) came to the conclusion that trichloracetic acid removed the proteins from milk more completely than tannic acid or ethyl alcohol.

Dennis and Minot (23), in studying the non-protein nitrogen content of milk, used copper sulphate and copper acetate for removing all the proteins. Allen (1), working with water extract of cheese, employed ethyl alcohol to precipitate all the proteins except the amino acids. Sanders (77) employed trichloracetic acid as a protein precipitant in milk in the preparation of protein free filtrates for the determination of magnesium, calcium, and acid soluble phosphorus. Kelly (53) used the same reagent for precipitating proteins from water extract of cheese.

Eagles and Sadler (24), in studying the nitrogen distribution in Kingston cheese, used trichloracetic acid, phosphotungstic acid, and tannic acid to precipitate the protein and protein decomposition products in water extract of cheese. Lane and Hammer (57) employed trichloracetic acid, 95 per cent ethyl alcohol, phosphotungstic acid and tungstic acid for the precipitation of proteins and protein decomposition products from Cheddar cheese juice.

Many other investigators have used various precipitating agents to precipitate proteins in blood. Folin and Wu (30) used sodium tungstate. Folin (29) and also Greenwald (35) employed picric acid while Bock (14) used ethyl alcohol, trichloracetic acid, and colloidal iron. Van Slyke

and Meyer (93) used 95 per cent ethyl alcohol to precipitate proteins in blood so that the determination of the non-protein nitrogen would be facilitated. Domogalla, Juday, and Peterson (22), in studying the forms of nitrogen in certain lake waters, employed phosphotungstic acid, mercuric chloride, tannic acid, lead subacetate, sodium tungstate, potassium mercuric iodide, and trichloracetic acid.

Wasteneys and Borsook (96) successfully precipitated proteins and metaproteins with trichloracetic acid. They also used tannic acid to separate peptones from an enzymatic hydrolysate of proteins. Hiller and Van Slyke (46), using a number of precipitants, came to the conclusion that picric acid and tungstic acid were the most complete precipitants for protein intermediate products with the exception of amino acids. They found that ethyl alcohol precipitated the same substances as tungstic acid and picric acid, but the precipitation was not as complete. Wolf (98), using both ethyl alcohol and methyl alcohol for the precipitation of proteins in blood, found that ethyl alcohol precipitated slightly more protein material than did methyl alcohol.

METHOD

Manufacturing, Ripening, and Scoring of Cheese.

(a) Source and treatment of milk.

The milk used for making the experimental cheese came from the Iowa State College herd. It contained about 3.0 per cent fat and about 8.6 per cent solids-not-fat. The bacterial content of the milk varied somewhat but was never less than 20,000 bacteria per ml. and exceeded 100,000 per ml. only once. When the methylene blue reduction test was used, reduction required from 7 to 9 hours and, if the samples were held at 37° C. (99° F.) for 24 hours, a considerable portion of the milk solids was proteolyzed.

(b) Manufacture and ripening of cheese.

Approximately 600 pounds of milk were used for each series of cheese. The milk was heated in a horizontal coil pasteurizer to 62.8° C. (145° F.) and held for 30 minutes, cooled to 4.4° C. (40° F.), put into 10 gallon milk cans (each can containing 75 pounds of milk), and placed in cold storage at 1° C. (34° F.) until the following morning. The milk was then divided equally between four small cheese vats so that each contained 150 pounds of milk. The milk in each vat was warmed to 22.2° C. (72° F.) and 2.0 per cent of a butter culture (122) and 0.5 per cent of a pure culture of *Lactobacillus casei* (L4) in milk were added. The butter culture used was the type that produced considerable flavor and aroma and usually contained

from 0.8 to 0.85 per cent acid, calculated as lactic acid. In addition to the butter culture and the pure culture of Lactobacillus casei each of three of the vats was inoculated with a milk culture of a test organism. After the addition of the cultures, the temperature of the milk was raised to 30.0° C. (86° F.) and, when the desired acidity had developed (usually about 0.19 to 0.21 per cent), commercial vegetable cheese color was added at the rate of 20 ml. per 1000 pounds of milk and commercial rennet at the rate of 100 ml. per 1000 pounds of milk. The same lots of color and rennet were used in all the trials. Coagulation of the milk usually began 8 to 10 minutes after the addition of the rennet and was complete in 30 minutes.

The curd was cut with three-sixteenth inch curd knives and allowed to stand 8 to 10 minutes, after which it was slowly heated to 40.0° C. (104° F.) and held at that temperature until the curd was firm and the acidity of the whey had reached about 0.16 to 0.18 per cent. The heating of the curd from 30.0° C. (86° F.) to 40.0° C. (104° F.) was completed in 60 to 90 minutes, depending on the acidity of the whey after cutting the curd. When the curd reached the proper degree of firmness, the whey was drained and the curd allowed to mat. The curd was turned frequently during the matting process, and when the acidity of the whey draining from the curd reached 0.45 to 0.6 per cent the curd was milled. The curd was forked frequently during the 30 minute interval between milling and salting. Salt was added at the rate of 2.5 pounds per 100 pounds of curd. When the salt was completely dissolved, which usually required from 30 to 40 minutes, the curd from each of the four vats was made into two cheese, one longhorn and one young American. The cheese were placed in the cheese press for one hour after which they were removed, dressed, and then replaced in the press

for 16 hours. After removal from the press the cheese were marked with serial numbers and the date of manufacture and placed at 13.5° C. (57° F.) for 48 hours, after which they were paraffined and placed in the curing room at 13.5° C. (56° F.) for ripening.

(c) Preparation of cultures.

The butter culture was prepared in pasteurized milk using an incubation of about 16 hours at 21.1° C. (70° F.).

Cultures of the test organisms and of Lactobacillus casei were prepared in flasks of sterile milk by inoculating the milk with pure cultures of the respective organisms. The following incubation conditions were used in preparing the cultures:

Lactobacillus casei (L4)	37° C.	(98.6° F.)	3 days
Micrococcus (unidentified)	21° C.	(70° F.)	7 days
Streptococcus liquefaciens	21° C.	(70° F.)	4 days
Alcaligenes viscosus (non-ropy strain)	21° C.	(70° F.)	4 days
Achromobacter lipolyticum	21° C.	(70° F.)	4 days
Pseudomonas fluorescens	21° C.	(70° F.)	4 days
Pseudomonas fragi	21° C.	(70° F.)	4 days
Lipolytic acid forming organism (No.12)	21° C.	(70° F.)	4 days
Lipolytic inert organism (No.18)	21° C.	(70° F.)	4 days

The test cultures were examined microscopically, both before their inoculation into the sterile milk and before being added to the milk in the cheese vats, in order to be certain that there was an abundance of organisms present. All of the organisms used were isolated from dairy products at the Dairy Industry Department of Iowa State College.

(d) Examination and scoring of cheese.

The cheese were scored at intervals of 3, 14, 28, 56, and 112 days during the ripening period by Professor E. F. Goss of the Dairy Industry Department of Iowa State College. The standard cheese score card, allowing 45 points for flavor, was used. Chemical and bacteriological analyses were also made on the cheese at the same intervals. Since most of the cheese made in the United States is consumed before it reaches an age of 112 days, no attempt was made to continue the analyses beyond this period.

Methods for the Study of the Nitrogen Distribution in Cheese by Chemical Analyses of Cheese Serum.

(a) Methods of obtaining the cheese serum.

The method used in obtaining the cheese serum was that developed by Barthel, Sandberg, and Haglund (11). In brief, the method consisted of submitting finely shredded cheese mixed with fine sea sand to a relatively high pressure in a hydraulic laboratory press. The press used for obtaining the serum was manufactured by Fred L. Carver, mechanical engineer, 347 Hudson Street, New York City. The press was adjusted so that any pressure could be obtained up to 25,000 pounds per square inch.

Lane and Hammer (57) found that when the cheese was prepared by shredding with a fine vegetable grater it was much easier to get a satisfactory mixture of the cheese and sand than when the cheese was ground because the ground cheese had a tendency to stick together, making it very difficult to get a uniform mixture of the cheese and sand. These investigators also found that the type of cloth used to line the metal cylinder was very im-

portant. They used muslin, canvas, and linen. The muslin allowed some of the cheese solids and sand to escape with the serum and the canvas absorbed too much of the serum. A strong, closely woven linen proved to be the most successful of the materials tried for lining the cylinder. A felt pad was placed in the bottom of the cylinder and another on top of the cheese and sand mixture before it was placed in the press for removal of the serum.

Lane (56), in an attempt to improve the procedure used in securing the cheese serum, found that if the cheese samples were left at room temperature several hours before shredding, the cheese serum was more easily obtained. If the cheese was too cold there was a tendency for a part of it to pass through the linen cloth when the pressure was applied.

To obtain sufficient cheese serum from a sample of cheese for all the analyses, 400 grams of finely shredded cheese were mixed with 800 grams of fine, clean, sea sand and put into the cylinder ready for removal of the cheese serum. The pressure was then applied slowly by the aid of the pump handle. As the pressure was gradually increased, the cheese serum was forced out of the cylinder through small holes located in grooves on the outside of it and allowed to run down to the base plate from which it drained into a beaker. The pressure necessary to obtain the serum varied somewhat according to the age of the cheese. With fresh cheese and with old well ripened cheese, less pressure was necessary than with partially ripened cheese. The serum started flowing when about 3000 pounds per square inch were applied. The pressure was then gradually increased until the desired amount of serum had been obtained. Rarely was it necessary to apply more than 12,000 to 15,000 pounds per square inch, although

when the cheese was from 4 to 8 weeks of age as high as 20,000 pounds per square inch were used occasionally. It usually required about one hour to obtain 20 ml. of cheese serum, the amount necessary for the chemical analyses. The fat came first when the pressure was applied, followed by a mixture of fat, cheese serum and some cheese solids. The mixture of fat, serum and cheese solids was placed in a 200 ml. separatory funnel and held in an incubator at 37° C. (99° F.) for about one hour to facilitate the separation of the various fractions. The fat formed a layer at the top, the serum formed the bottom layer, and the cheese solids formed a layer between the two. The serum was then drawn off, filtered through a paper, which was placed in a long stemmed, glass funnel, and collected in a large test tube. The test tube was corked tightly, but the cork contained a hole large enough to permit the funnel stem to pass through. This precaution was taken to prevent evaporation of the serum during filtering. The test tube containing the cheese serum was held at about 1.7° C. (35° F.) until the analyses of the serum could be started. The fat forming the top layer in the separatory funnel was removed, filtered through paper at 37° F. (99° F.) and used for determining the acid value of the fat. The heavy precipitate, consisting of protein material, was discarded after the filtration of the serum and fat. The cheese serum, in its final form as used for the chemical analyses, was an amber colored, transparent fluid, very salty to the taste.

(b) Chemical analyses of cheese serum.

Quantitative determinations were made of the total nitrogen, the amino nitrogen, and the various fractions of protein and protein decom-

position products which were soluble or insoluble in trichloracetic acid, ethyl alcohol, or phosphotungstic acid. The procedures used were as follows:

Total nitrogen. One ml. of serum was analyzed for total nitrogen by the Kjeldahl method.

Amino nitrogen. One ml. of serum was analyzed by the Van Slyke gasometric method (91).

Trichloracetic acid soluble and insoluble nitrogen fractions. One ml. of serum was treated with 44 ml. of water and 5 ml. of 20 per cent aqueous trichloracetic acid. After standing 8 to 10 hours, or over night, at 21° C. (70° F.), the mixture was filtered through filter paper and the precipitate washed with a trichloracetic acid solution containing 45 ml. of water and 5 ml. of 20 per cent aqueous trichloracetic acid. The solution used for washing the precipitate contained the same concentration of reagent as that used in the precipitation. The filtrate and precipitate were then transferred to Kjeldahl flasks and analyzed separately for nitrogen.

Ethyl alcohol soluble and insoluble nitrogen fractions. One ml. of serum was treated with 9 ml. of water and 85 ml. of 95 per cent ethyl alcohol. After standing 8 to 10 hours, or over night, at 21° C. (70° F.), the mixture was filtered through filter paper and the precipitate washed with an ethyl alcohol solution containing 10 ml. of water and 85 ml. of 95 per cent ethyl alcohol. The filtrate and precipitate were then transferred to Kjeldahl flasks and analyzed separately for nitrogen.

Phosphotungstic acid soluble and insoluble nitrogen fractions. One ml. of serum was treated with 49 ml. of water, 15 ml. of 25 per cent (by volume) aqueous sulphuric acid, and 10 ml. of 10 per cent aqueous phosphotungstic

acid. After standing 8 to 10 hours, or over night, at 21° C. (70° F.), the mixture was filtered through filter paper and the precipitate washed with a phosphotungstic acid solution containing 50 ml. of water, 15 ml. of 25 per cent aqueous sulphuric acid, and 10 ml. of 10 per cent aqueous phosphotungstic acid. The filtrate and precipitate were then transferred to Kjeldahl flasks and analyzed separately for nitrogen.

Acid Value of Fat.

The acid value of the fat obtained from the cheese was determined as follows: 20 grams of fat were weighed into a 250 ml. flask, 50 ml. of neutral 95 per cent ethyl alcohol added, the mixture brought up to a boil and then titrated while hot with N/10 sodium hydroxide, using phenolphthalein as the indicator.

Bacteriological Analyses of the Cheese.

Bacterial counts were made on all the experimental cheese at 3, 14, 28, 56, and 112 days of age in order to study the total numbers of bacteria, as well as the numbers of proteolytic and lipolytic organisms present at the various stages of ripening.

(a) Total bacteria.

The method used in determining the total bacteria was as follows: A small portion of cheese was taken from several sections of the cheese with a sterile knife and 1 gram weighed out on a piece of sterile parchment paper. This was put into a sterile mortar with 5 to 6 ml. of a sodium citrate solu-

tion and thoroughly mixed with a pestle until all the cheese was dissolved or in fine suspension. The mixture was then poured into a sterile test tube and the mortar rinsed with 3 to 4 ml. of sodium citrate solution. The sodium citrate solution was made up as follows: 2 grams of sodium citrate were added to 90 ml. of distilled water; after dissolving the sodium citrate, the solution was measured into test tubes (each tube containing 9 ml.), and the tubes were stoppered with cotton, sterilized, cooled, and put into a refrigerator until used.

The sample of cheese that had been treated with sodium citrate solution was diluted in sterile water blanks and plated in dilutions of 1 - 1,000,000, 1 - 10,000,000, 1 - 50,000,000, and 1 - 100,000,000, using both beef infusion agar and tomato juice agar. The plates were incubated for 4 to 5 days at 21° C. (70° F.) for total bacterial counts.

(b) Proteolytic bacteria.

The sample of cheese was also plated on a special medium in order to determine the number of proteolytic bacteria present; dilutions of 1 - 100, 1 - 1000, and 1 - 10,000 were used. To each petri dish was added 1 ml. of sterile milk and 1 ml. of cheese dilution; these were mixed and 10 ml. of beef infusion agar added. The plates were allowed to cool, and then incubated at 37° C. (99° F.) for 12 to 14 hours after which the proteolytic organisms were counted. If a longer incubation was used, there was a tendency for the proteolytic areas to enlarge and cover other bacterial colonies close by, making it impossible to determine accurately the number of proteolytic bacteria present. The addition of milk to the agar gave it a milky appearance and the proteolytic bacteria were easily counted as they formed

a small clear area around each colony.

(c) Lipolytic bacteria.

For the lipolytic bacteria, dilutions of 1 - 100 and 1 - 1000 were used. To each petri dish was added 1 ml. of cheese dilution, 0.5 ml. of fat emulsion, 0.5 ml. of nile blue sulphate solution, and 10 ml. of beef infusion agar. After all the materials had been added to the petri dish they were thoroughly mixed. The plates were then incubated at 21° C. (70° F.) for 4 to 5 days before being counted. With the medium employed the fat globules near lipolytic colonies become blue in color and can be easily distinguished from the unhydrolyzed globules which are pink. The fat emulsion was made by dissolving 0.5 gram of agar in 100 ml. with distilled water, adding 2 or 3 grams of Wesson oil and sterilizing. After removal from the sterilizer, the mixture was shaken frequently while cooling in order to emulsify the Wesson oil uniformly throughout the agar. The nile blue sulphate was made up as a 0.1 per cent solution in distilled water and sterilized. The final concentration of nile blue sulphate in the agar was approximately 1 - 20,000.

Microscopic Study of the Bacterial Flora in Cheese Serum.

Approximately 0.01 ml. of cheese serum was spread uniformly over an area of one square centimeter and allowed to dry. The film of cheese serum was then stained with methylene blue and examined. A count was made of all the bacteria present and also of the organisms having a morphology suggesting lactobacilli; the percentage of the total flora made up of the latter organisms was then calculated.

EXPERIMENTAL

Effect of Adding Various Organisms Alone or in Combinations to Pasteurized Milk Used for Making Cheddar Cheese on the Nitrogenous Decomposition and Flavor Development in the Cheese.

The effect of adding various organisms to pasteurized milk used for making Cheddar cheese on the nitrogenous decomposition in the cheese and the flavor development was studied with six series of cheese, each containing four lots. Two per cent of a butter culture (122) and 0.5 per cent of a milk culture of Lactobacillus casei (L4) were used in all lots of milk because, in the work of Lane and Hammer (58), this combination of organisms produced a satisfactory cheese when used with pasteurized milk. These cultures alone were used in making the control cheese, while with the other three lots of cheese in each series one or more test organisms were also employed. The test organisms used were as follows: Streptococcus liquefaciens, an unidentified Micrococcus, an inert lipolytic organism, Pseudomonas fragi, an acid forming lipolytic organism, Pseudomonas fluorescens, Alcaligenes viscosus, and Achromobacter lipolyticum. The various organisms were added to the pasteurized milk in the form of milk cultures, using the following percentages: 0.05 per cent with S. liquefaciens, the inert lipolytic organism, Ps. fragi, the acid forming lipolytic organism, Ps. fluorescens, A. viscosus or A. lipolyticum and 0.5 per cent with the unidentified Micrococcus.

Effect of S. liquefaciens, an Unidentified Micrococcus or Both.

Table 1 presents the data on the nitrogenous decomposition and flavor development in two series of cheese in which S. liquefaciens, an unidentified Micrococcus or both were used in addition to the regular cultures.

Series 1. When the cheese in series 1 were only 3 days old there was very little variation in the total nitrogen in the serums of the four cheese. The total variation at this period was from 5.0 to 5.2 ml., reported as milliliters of N/10 acid equivalent to the nitrogen in 1 ml. of cheese serum. As the ripening progressed there was an increase in the total nitrogen in the serums of all the cheese and the differences between the four cheese in the series became slightly greater. After 112 days of ripening the total nitrogen varied from 19.6 to 20.6 ml. of N/10 acid. The most rapid increase in total nitrogen in the serums occurred during the early stages of the ripening period. The increase during the period from the 3rd to the 14th day was over 100 per cent. After the 14th day the rate of increase in the total nitrogen was materially reduced. The serums of the control cheese and the cheese made with S. liquefaciens did not differ materially in total nitrogen at any stage during the ripening. After 112 days of ripening the serum of the cheese made with the unidentified Micrococcus or with a mixture of S. liquefaciens and the Micrococcus was only very slightly higher in total nitrogen than was the serum of the control cheese.

A steady increase in the various nitrogen fractions occurred in the serums of all the cheese as the ripening progressed. In the young cheese the amounts of nitrogen in the fractions soluble in trichloracetic acid,

ethyl alcohol, or phosphotungstic acid showed little variation with the serum of the various cheese. As the ripening progressed the differences in the amounts of the various fractions in the serum were greater but even after 112 days of ripening the variations were still small.

The amounts of nitrogen in the fractions insoluble in the various reagents generally showed a steady increase in the serums throughout the ripening period. The only exception was when trichloracetic acid was used as the precipitating agent; then the insoluble fraction increased during the first 28 days and after that gradually decreased so that at 112 days it was only slightly greater than at 3 days. In the 3 day old cheese there was no significant variation in these insoluble fractions. Although the variations found in the insoluble fractions were small they indicate that the control cheese contained a slightly smaller insoluble fraction in the serum when ethyl alcohol or phosphotungstic acid were used as precipitating agents, than the cheese made with the test organisms. The cheese made from pasteurized milk inoculated with both S. liquefaciens and the unidentified Micrococcus was highest in the insoluble fractions of nitrogen.

Increases in the amounts of amino nitrogen in the serums were shown by all of the cheese as the ripening progressed. During the early stages of ripening the variations in the amounts of amino nitrogen in the four cheese were relatively small; after longer periods of ripening larger differences existed. In all cases the three cheese containing the test organisms showed more amino nitrogen in the serum after 28 days than the control cheese.

The moisture contents of the four cheese in the series varied but

little at any stage during the ripening.

The data on the effect of the various organisms on the flavor development in the cheese show that the cheese made with the test organisms scored higher in flavor than the control cheese. There was no apparent difference in the flavor scores of the cheese made with S. liquefaciens, the unidentified Micrococcus or both of these organisms. After a period of ripening a sour flavor developed in all the cheese and was most pronounced in the control.

Table 1

Effect of *S. liquefaciens*, an unidentified Micrococcus or both on the nitrogenous decomposition and flavor d
Series 1

Serial number of cheese	Age of cheese days	Test organisms used	Moisture per cent	ml. of N/10 acid equiv. to nitrogen in 1 ml. of cheese serum							
				Total nitrogen in ml.N/10 acid	Nitrogen fractionated into soluble and insoluble fractions with						Amino nitrogen Mgs.
					Trichloracetic acid		Ethyl alcohol		Phosphotungstic acid		
					Sol.	Insol.	Sol.	Insol.	Sol.	Insol.	
1-1	3	None.	38.9	5.1	3.1	2.0	1.2	3.9	1.0	4.0	.68
1-2	3	*S. liquefaciens*	38.6	5.0	3.0	1.9	1.1	3.8	1.1	3.9	.64
1-3	3	Micrococcus	38.7	5.0	3.1	1.9	1.1	3.9	1.1	3.8	.72
1-4	3	*S. liquefaciens* and Micrococcus	38.9	5.2	3.4	1.7	1.2	4.0	1.2	4.0	.68
1-1	14	None.	37.8	10.9	7.1	3.8	3.8	7.1	2.4	8.4	.90
1-2	14	*S. liquefaciens*	37.2	10.7	7.2	3.6	3.2	7.5	2.3	8.5	.86
1-3	14	Micrococcus	37.4	10.8	7.1	3.6	3.1	7.8	1.9	9.0	.94
1-4	14	*S. lique.* and Micro.	37.9	11.0	7.4	3.6	3.1	7.9	2.0	9.0	.93
1-1	28	None.	37.2	14.0	10.6	3.3	4.6	9.5	3.4	10.6	1.73
1-2	28	*S. liquefaciens*	37.0	14.1	10.7	3.5	4.1	10.1	2.4	11.9	1.73
1-3	28	Micrococcus	37.3	12.9	10.0	3.7	3.5	9.5	2.4	10.5	1.71
1-4	28	*S. lique.* and Micro.	37.5	14.0	10.4	3.6	4.2	9.7	3.6	10.4	2.07
1-1	56	None.	37.0	16.9	14.6	2.4	5.8	11.1	4.0	13.0	3.9
1-2	56	*S. liquefaciens*	37.1	16.4	13.5	3.0	5.9	10.5	4.6	12.0	4.13
1-3	56	Micrococcus	37.2	16.6	13.7	2.9	5.8	10.8	3.0	13.6	4.24
1-4	56	*S. lique.* and Micro.	37.2	17.8	14.7	3.1	6.2	11.7	3.7	14.0	4.13
1-1	112	None.	36.4	19.6	17.2	2.5	9.6	9.9	6.8	12.7	5.12
1-2	112	*S. liquefaciens.*	36.5	19.6	17.0	2.6	7.7	11.0	6.0	13.7	6.53
1-3	112	Micrococcus	36.2	20.6	18.0	2.7	8.4	12.1	6.7	13.6	6.21
1-4	112	*S. lique.* and Micro.	36.6	20.6	17.9	2.6	8.2	12.5	6.5	14.0	6.26

Table 1

nidentified Micrococcus or both on the nitrogenous decomposition and flavor development in the cheese.
Series 1

	Moisture per cent	Total nitrogen in ml.N/10 acid	ml. of N/10 acid equiv. to nitrogen in 1 ml. of cheese serum: Nitrogen fractionated into soluble and insoluble fractions with Trichloracetic acid Sol.	Trichloracetic acid Insol.	Ethyl alcohol Sol.	Ethyl alcohol Insol.	Phosphotungstic acid Sol.	Phosphotungstic acid Insol.	Amino nitrogen Mgs.	Flavor score of cheese	Remarks on cheese flavor
	38.9	5.1	3.1	2.0	1.2	3.9	1.0	4.0	.68		
	38.6	5.0	3.0	1.9	1.1	3.8	1.1	3.9	.64		
	38.7	5.0	3.1	1.9	1.1	3.9	1.1	3.8	.72		
d	38.9	5.2	3.4	1.7	1.2	4.0	1.2	4.0	.68		
	37.8	10.9	7.1	3.8	3.8	7.1	2.4	8.4	.90	37.0	Sour.
	37.2	10.7	7.2	3.6	3.2	7.5	2.3	8.5	.86	38.5	Sl. fermented.
	37.4	10.8	7.1	3.6	3.1	7.8	1.9	9.0	.94	39.5	Lacks flavor.
ɔ.	37.9	11.0	7.4	3.6	3.1	7.9	2.0	9.0	.93	38.5	Sl. fermented.
	37.2	14.0	10.6	3.3	4.6	9.5	3.4	10.6	1.73	38.0	Sl. sour.
	37.0	14.1	10.7	3.5	4.1	10.1	2.4	11.9	1.73	39.5	Sl. sour.
	37.3	12.9	10.0	3.7	3.5	9.5	2.4	10.5	1.71	39.0	" "
ɔ.	37.5	14.0	10.4	3.6	4.2	9.7	3.6	10.4	2.07	39.0	" "
	37.0	16.9	14.6	2.4	5.8	11.1	4.0	13.0	3.9	36.5	Sour.
	37.1	16.4	13.5	3.0	5.9	10.5	4.6	12.0	4.13	37.5	Sl. sour.
	37.2	16.6	13.7	2.9	5.8	10.8	3.0	13.6	4.24	38.0	" "
ɔ.	37.2	17.8	14.7	3.1	6.2	11.7	3.7	14.0	4.13	37.0	" "
	36.4	19.6	17.2	2.5	9.6	9.9	6.8	12.7	5.12	36.5	Sour.
	36.5	19.6	17.0	2.6	7.7	11.0	6.0	13.7	6.53	38.5	Sl. sour.
	36.2	20.6	18.0	2.7	8.4	12.1	6.7	13.6	6.21	37.5	" "
ɔ.	36.6	20.6	17.9	2.6	8.2	12.5	6.5	14.0	6.26	37.5	" "

Series 2. There was a slight variation in the total nitrogen in the serums of the four cheese in series 2 when the cheese were 3 days old. At this period the total variation in the four cheese was from 4.8 to 5.5 ml. reported as milliliters of N/10 acid equivalent to the nitrogen in 1 ml. of cheese serum. As the ripening progressed, there was a gradual increase in the total nitrogen in the serums of all four of the cheese and the differences between the cheese became greater. After 112 days of ripening the total nitrogen varied from 17.0 to 21.2 ml. of N/10 acid. The greatest increase in the total nitrogen occurred during the first 14 days of the ripening period. During the period from the 3rd to the 14th day the increase was about 100 per cent. After this period the rate of increase in the total nitrogen was materially reduced. The total nitrogen in the serums of the four cheese differed very little during the early stage of ripening, but the cheese made with the test cultures contained more nitrogen in the serums than the control cheese after 112 days of ripening.

As the ripening progressed there was a steady increase in the various nitrogen fractions in the serums of all the cheese. In the young cheese the amount of nitrogen in the fractions soluble in trichloracetic acid, ethyl alcohol or phosphotungstic acid showed little variation in the four cheese in the series. As the ripening period progressed the differences in the amount of these fractions in the serums were greater. In general, there was a gradual increase in the soluble fractions of nitrogen throughout the 112 days of ripening. Late in the ripening period the serum from the control cheese was relatively low in the soluble fractions as well as in total nitrogen; this may have been due to the fact that the cheese was excessively high in moisture.

The amounts of nitrogen in the fractions insoluble in the various reagents showed no significant variation with the 3 day old cheese. Generally, there was an increase in the insoluble fractions as the ripening progressed. However, when trichloracetic acid was used as the precipitating agent the insoluble fractions increased during the first 28 days and then gradually decreased so that at 112 days the fraction was only slightly greater than at 3 days. The variations found in the serums from the four cheese do not indicate that the test cultures exerted any significant influence on the insoluble nitrogen fractions.

All of the cheese showed increases in the amounts of amino nitrogen in the serums as the ripening progressed. There was considerable variation in the amounts of amino nitrogen among the four cheese throughout the ripening period. The serums from the three cheese containing the test cultures were definitely higher in amino nitrogen than the control cheese after 112 days of ripening.

The moisture content of the three cheese inoculated with the test cultures varied little, but the control cheese was excessively high in moisture throughout the ripening period.

The flavor scores of the four cheese in series 2 varied considerably and the three cheese containing the test organisms scored higher on flavor than did the control cheese. There was no apparent difference in the flavor scores of the three cheese containing the test cultures. All the cheese scored highest during the early period of the ripening and as the ripening progressed there was a tendency for a bitter flavor to develop and cause a decrease in flavor score.

Since series 2 was a duplicate of series 1, the variations in the

results obtained in the two series should be small. The data on the cheese in series 1 and 2 show that in general the addition of the test organisms, S. liquefaciens, an unidentified Micrococcus or both, increased the total nitrogen and the amino nitrogen in the serum of the cheese and also improved the flavor.

Table 1 (Cont.)

Series 2

Serial number of cheese	Age of cheese days	Test organisms used	Moisture per cent	ml. of N/10 acid equiv. to nitrogen in 1 ml. of cheese serum: Total nitrogen in ml.N/10 acid	Nitrogen fractionated into soluble and insoluble fractions with: Trichloracetic acid Sol.	Trichloracetic acid Insol.	Ethyl alcohol Sol.	Ethyl alcohol Insol.	Phosphotungstic acid Sol.	Phosphotungstic acid Insol.	Amino nitrogen Mgs.
2-1	3	None.	42.0	4.8	2.9	1.8	0.9	4.0	1.3	1.3	.66
2-2	3	S. liquefaciens	39.6	5.5	3.4	2.1	1.1	4.4	1.1	4.4	.77
2-3	3	Micrococcus	40.0	5.4	3.2	2.2	1.1	4.3	1.2	4.2	.99
2-4	3	S. lique. and Micro.	40.0	5.4	3.3	2.1	1.2	4.3	1.1	4.3	.83
2-1	14	None.	41.2	10.8	7.8	3.0	2.7	8.2	2.4	8.4	.91
2-2	14	S. liquefaciens	39.2	11.0	8.0	3.2	2.8	8.2	2.1	9.2	.95
2-3	14	Micrococcus	39.2	11.1	7.2	3.8	3.4	7.7	2.2	9.1	1.20
2-4	14	S. lique. and Micro.	38.3	10.9	7.0	3.8	3.0	7.9	2.0	8.8	1.01
2-1	28	None.	40.9	13.7	9.9	3.7	3.4	10.3	2.6	11.1	1.54
2-2	28	S. liquefaciens	38.7	14.6	11.3	3.4	4.5	10.0	2.3	12.4	1.98
2-3	28	Micrococcus	38.7	15.1	12.0	3.2	4.5	10.8	3.0	12.1	1.92
2-4	28	S. lique. and Micro.	38.3	13.1	9.8	3.2	4.3	8.9	2.1	11.0	1.87
2-1	56	None.	40.0	15.0	11.6	3.3	3.8	11.3	2.6	12.4	2.31
2-2	56	S. liquefaciens	37.3	16.1	13.6	2.5	5.9	10.2	3.3	12.8	4.41
2-3	56	Micrococcus	37.3	17.5	14.9	2.6	4.2	13.2	3.0	14.5	4.36
2-4	56	S. lique. and Micro.	37.0	16.4	13.9	2.6	5.4	11.1	3.1	13.3	4.74
2-1	112	None.	40.0	17.0	14.0	3.1	5.3	11.8	3.0	14.0	4.28
2-2	112	S. liquefaciens	37.0	20.9	18.8	2.0	8.8	11.9	6.5	14.3	6.69
2-3	112	Micrococcus	37.1	20.1	17.4	2.8	8.3	11.7	5.8	14.2	5.99
2-4	112	S. lique. and Micro.	37.1	21.2	19.0	2.3	8.4	12.6	5.6	14.6	6.85

Table 1 (Cont.)

Series 2

ed	Moisture per cent	Total nitrogen in ml.N/10 acid	ml. of N/10 acid equiv. to nitrogen in 1 ml. of cheese serum: Nitrogen fractionated into soluble and insoluble fractions with Trichloracetic acid Sol.	Trichloracetic acid Insol.	Ethyl alcohol Sol.	Ethyl alcohol Insol.	Phosphotungstic acid Sol.	Phosphotungstic acid Insol.	Amino nitrogen Mgs.	Flavor score of cheese	Remarks on cheese flavor
	42.0	4.8	2.9	1.8	0.9	4.0	1.3	1.3	.66		
	39.6	5.5	3.4	2.1	1.1	4.4	1.1	4.4	.77		
	40.0	5.4	3.2	2.2	1.1	4.3	1.2	4.2	.99		
ro.	40.0	5.4	3.3	2.1	1.2	4.3	1.1	4.3	.83		
	41.2	10.8	7.8	3.0	2.7	8.2	2.4	8.4	.91	35.0	Sour.
	39.2	11.0	8.0	3.2	2.8	8.2	2.1	9.2	.95	38.0	Sl. sour.
	39.2	11.1	7.2	3.8	3.4	7.7	2.2	9.1	1.20	38.0	" "
ro.	38.3	10.9	7.0	3.8	3.0	7.9	2.0	8.8	1.01	38.5	" "
	40.9	13.7	9.9	3.7	3.4	10.3	2.6	11.1	1.54	35.0	" "
	38.7	14.6	11.3	3.4	4.5	10.0	2.3	12.4	1.98	39.0	Good.
	38.7	15.1	12.0	3.2	4.5	10.8	3.0	12.1	1.92	38.5	Sl. sour.
ro.	38.3	13.1	9.8	3.2	4.3	8.9	2.1	11.0	1.87	39.0	" "
	40.0	15.0	11.6	3.3	3.8	11.3	2.6	12.4	2.31	35.0	Sour.
	37.3	16.1	13.6	2.5	5.9	10.2	3.3	12.8	4.41	36.0	Bitter.
	37.3	17.5	14.9	2.6	4.2	13.2	3.0	14.5	4.36	36.0	"
ro.	37.0	16.4	13.9	2.6	5.4	11.1	3.1	13.3	4.74	36.0	"
	40.0	17.0	14.0	3.1	5.3	11.8	3.0	14.0	4.28	36.0	"
	37.0	20.9	18.8	2.0	8.8	11.9	6.5	14.3	6.69	36.5	
	37.1	20.1	17.4	2.8	8.3	11.7	5.8	14.2	5.99	36.5	Bitter.
ro.	37.1	21.2	19.0	2.3	8.4	12.6	5.6	14.6	6.85	36.5	"

Effect of an Inert Lipolytic Organism, Ps. fragi or an Acid Forming Lipolytic Organism.

The data on the nitrogenous decomposition and flavor development in two series of cheese in which an inert lipolytic organism, Ps. fragi or an acid forming lipolytic organism was used in addition to the regular cultures are given in Table 2.

Series 3. There was considerable variation in the total nitrogen in the serums of the four cheese in series 3 when the cheese was 3 days old. The total variation at this period was from 4.5 to 6.6 ml., reported as milliliters of N/10 acid equivalent to the nitrogen in 1 ml. of cheese serum. As the ripening progressed there was an increase in the total nitrogen in the serums of all the cheese. After 112 days of ripening the total nitrogen varied from 20.1 to 22.8 ml. of N/10 acid. The most rapid increase in the total nitrogen in the serums occurred in the period between the 3rd and 14th day of ripening. The increase during this period was about 100 per cent in the control cheese and in the cheese made with the inert lipolytic organism and with Ps. fragi, while the cheese made with the acid forming lipolytic organism only increased about 80 per cent. After the 14th day the rate of increase in the total nitrogen in the serums was substantially reduced. The serums of the control cheese and the cheese made with Ps. fragi did not differ materially in total nitrogen during the first 28 days of ripening. After this period the cheese made with Ps. fragi increased so that it contained the greatest amount of nitrogen in the serum of any cheese in the series. The cheese made with the inert lipolytic organism contained more nitrogen in the serum

throughout most of the ripening period than did the control cheese. Cheese made with the acid forming lipolytic organism was much higher in nitrogen than the control cheese, but after 112 days the difference between the two cheeses was somewhat less.

In general there was a steady increase in the various nitrogen fractions in the serums of all the cheese as the ripening progressed. Among the four cheese in the series there was considerable variation, during the first 14 days of ripening, in the amounts of nitrogen in the fractions soluble in trichloracetic acid, but not in the fractions soluble in ethyl alcohol or phosphotungstic acid. There was a consistent increase in the soluble fractions of nitrogen throughout the ripening period. After 112 days of ripening the soluble fraction of nitrogen in the serum from the control cheese was slightly lower, when trichloracetic acid was used as the precipitating agent, than in the serums from the cheese made with the test organisms. There was very little difference in the soluble nitrogen fraction in the serums from the four cheese when ethyl alcohol or phosphotungstic acid was used as the precipitating agent.

The amount of nitrogen in the fractions insoluble in the various reagents usually showed a steady increase in the serums throughout the ripening period. In the young cheese there was no significant variation in the insoluble fractions of nitrogen with any of the precipitating agents. When trichloracetic acid was used as the precipitating agent the insoluble fraction did not increase after the 28th day of ripening. With ethyl alcohol or phosphotungstic acid used as the precipitating agent there was a consistent increase in the nitrogen in the insoluble fraction throughout the ripening period. After 112 days the differences

in the nitrogen content of the insoluble fraction of the serums from the four cheese in the series were too small to be of any significance.

Increases in the amounts of amino nitrogen in the serums as the ripening progressed were shown by all the cheese. There was considerable variation in the amounts of amino nitrogen in the four cheese, during the early period of ripening as well as in the latter period. The control cheese and the cheese made with the inert lipolytic organism were considerably lower in amino nitrogen in the serum at 3 days of age than the other two cheese in the series, while the control cheese and the cheese made with the acid forming lipolytic organism were low after 112 days of ripening. At this time the cheese made with Ps. fragi was considerably higher in amino nitrogen in the serum than any of the other three cheese in the series.

There was very little variation in the moisture content of the four cheese at any period during ripening.

The data on the effect of the various organisms on the flavor development in the cheese show that in general the control cheese scored higher in flavor than the cheese made with the test organisms. During the early part of the ripening period the control cheese and the cheese made with the inert lipolytic organism received about the same flavor scores. The cheese made with Ps. fragi and the acid forming lipolytic organism received the lowest flavor score throughout the 112 day ripening period. The control cheese was not criticized at any period during ripening, while the cheese made with Ps. fragi and the cheese made with the acid forming lipolytic organism were criticized for being sour and bitter, respectively.

Table 2

Effect of an inert lipolytic (No. 18) Ps. fragi or a lipolytic acid former (No. 12) or both on the nitrogenous development in the cheese.
Series 3

Serial number of cheese	Age of cheese	Test organisms used	Moisture per cent	ml. of N/10 acid equiv. to nitrogen in 1 ml. of cheese corrected: Total nitrogen in ml.N/10 acid	Nitrogen fractionated into soluble and insoluble fractions with: Trichloracetic acid Sol.	Trichloracetic acid Insol.	Ethyl alcohol Sol.	Ethyl alcohol Insol.	Phosphotungstic acid Sol.	Phosphotungstic acid Insol.	Amino nitrogen Mgs.
	days										
3-1	3	None.	39.8	4.5	2.8	1.7	1.50	3.1	1.0	3.8	.88
3-2	3	Inert lipolytic (No. 18)	39.8	5.1	3.3	1.8	1.95	3.2	1.0	4.1	.89
3-3	3	Ps. fragi	39.5	4.8	3.3	1.7	1.60	3.4	0.9	3.4	1.26
3-4	3	Lipolytic acid former (No. 12)	39.3	6.8	4.8	2.0	1.65	3.9	1.1	5.5	1.21
3-1	14	None.	37.4	9.3	6.0	3.2	3.50	5.7	2.0	7.2	1.00
3-2	14	Inert lipolytic, No.18	38.3	10.0	7.6	2.5	3.00	6.0	1.9	8.0	0.54
3-3	14	Ps. fragi	38.2	9.9	7.3	2.5	3.60	6.2	1.5	8.3	1.00
3-4	14	Lipo.acid former,No.12	38.3	11.9	9.2	2.7	4.10	7.8	1.5	10.4	2.60
3-1	28	None.	37.3	13.1	10.3	2.8	3.70	9.4	2.0	11.1	2.21
3-2	28	Inert lipolytic, No.18	38.0	13.6	9.8	3.8	3.80	9.8	2.2	11.4	2.35
3-3	28	Ps. fragi	38.3	13.7	10.1	3.7	4.30	9.4	1.6	13.0	2.40
3-4	28	Lipo.acid former,No.12	38.3	13.6	10.8	3.0	3.50	10.1	1.9	11.8	2.25
3-1	56	None.	37.3	16.0	13.5	2.5	4.30	11.7	2.5	13.5	3.79
3-2	56	Inert lipolytic, No.18	37.6	16.0	13.0	3.0	4.50	11.5	2.3	13.7	3.83
3-3	56	Ps. fragi	37.7	16.7	13.9	2.8	4.80	11.9	2.0	14.7	3.57
3-4	56	Lipo.acid former,No.12	37.9	16.5	14.2	2.3	4.20	12.3	2.3	14.2	3.84
3-1	112	None.	37.2	20.1	17.0	3.0	6.0	12.0	7.1	13.0	5.30
3-2	112	Inert lipolytic, No.18	37.7	22.3	19.0	3.2	9.8	12.5	6.2	14.0	7.01
3-3	112	Ps. fragi	37.7	22.8	19.7	2.9	9.7	12.8	6.2	14.5	8.85
3-4	112	Lipo.acid former,No.12	37.6	21.7	19.5	2.1	8.4	13.2	8.0	13.6	6.97

Table 2

...c (No. 18) Bo. fungi or a lipolytic acid former (No. 12) or both on the nitrogenous decomposition and flavor development in the cheese.
Series 3

...s used	Moisture per cent	Total nitrogen in ml.N/10 acid	ml. of N/10 acid equiv. to nitrogen in 1 ml. of cheese serum: Nitrogen fractionated into soluble and insoluble fractions with Trichloracetic acid Sol.	Insol.	Ethyl alcohol Sol.	Insol.	Phosphotungstic acid Sol.	Insol.	Amino nitrogen Mgs.	Flavor score of cheese	Remarks on cheese flavor
	39.8	4.5	2.8	1.7	1.50	3.1	1.0	3.8	.82		
...ic	39.2	5.1	3.3	1.8	1.95	3.2	1.0	4.1	.89		
	39.0	4.8	3.3	1.7	1.80	3.4	0.9	3.4	1.26		
...d former	39.3	6.6	4.6	2.0	1.85	3.9	1.1	5.5	1.21		
	32.4	9.3	6.0	3.2	3.50	5.7	2.0	7.3	1.00	39.0	
...ic, No.18	38.3	10.0	7.6	2.5	3.00	6.0	1.9	8.0	2.34	39.5	
	38.2	9.9	7.3	2.5	3.60	6.2	1.5	8.3	1.90	37.0	Sour.
...mer,No.12	38.0	11.9	9.2	2.7	4.10	7.8	1.5	10.4	2.60	36.0	Bitter.
	38.3	13.1	10.3	2.8	3.70	9.4	2.0	11.1	3.21	38.5	
...ic, No.18	38.0	13.6	9.8	3.8	3.80	9.8	2.2	11.4	3.88	38.0	
	38.2	13.7	10.1	3.7	4.30	9.4	1.6	13.0	2.40	37.5	Sl. sour.
...mer,No.12	38.2	13.6	10.8	3.0	3.50	10.1	1.9	11.8	2.25	38.5	Sl. bitter.
	37.8	16.0	13.5	2.5	4.30	11.7	2.5	13.5	3.79	38.0	
...ic, No.18	37.8	16.0	13.0	3.0	4.90	11.5	2.3	13.7	3.88	38.0	
	37.7	16.7	13.9	2.8	4.60	11.9	2.0	14.7	3.57	37.0	Sl. sour.
...mer,No.12	37.9	16.5	14.2	2.3	4.80	12.3	2.3	14.2	3.94	37.0	Bitter.
	38.2	20.1	17.0	3.0	6.0	12.0	7.1	13.0	6.38	38.0	
...ic, No.18	38.7	22.3	19.0	3.2	8.8	12.5	6.2	14.0	7.01	37.0	
	38.7	22.8	19.7	2.9	9.7	12.8	6.2	14.5	6.80	36.0	
...mer,No.12	38.6	21.7	19.5	2.1	8.6	13.2	8.0	13.6	6.97	36.5	

Series 4. There was some variation in the total nitrogen in the serums of the four cheese in series 4 when the cheese were 3 days old. The total variation at this time was from 4.0 to 4.9 ml. on the basis of the milliliters of N/10 acid equivalent to the nitrogen in 1 ml. of cheese serum. As the ripening progressed there was a steady increase in the total nitrogen in the serums of all four of the cheese and the differences between the cheese became somewhat greater. The variation in the total nitrogen after 112 days was from 19.5 to 22.3 ml. of N/10 acid. The greatest increase in the total nitrogen took place during the first 14 days of the ripening period; from the 3rd to the 14th day the increase was about 100 per cent. After this period the rate of increase in the total nitrogen was materially reduced. There was no significant variation in the total nitrogen in the serums from the four cheese at any stage during ripening, the percentage difference being about the same at 112 days as after 3 days of ripening.

There was a steady increase in the various nitrogen fractions in the serums of all the cheese as the ripening progressed. At 3 days the amount of nitrogen in the fractions soluble in trichloracetic acid, ethyl alcohol or phosphotungstic acid showed small variations in the four cheese in the series. As the ripening progressed the differences in the amount of these fractions in the serums were greater and there was a gradual increase in the soluble fractions of nitrogen throughout the 112 day period of ripening. During the latter part of the ripening period the serums from the cheese made with Ps. fragi and the acid forming lipolytic organism were lower in soluble nitrogen than the serums from the control cheese and from the cheese made with the inert

lipolytic organism, when trichloracetic acid was used as the precipitating agent. Cheese made with the inert lipolytic organism gave the highest soluble fraction of nitrogen in the serum, with all the precipitating agents after 112 days of ripening.

The amount of nitrogen in the fractions insoluble in the various precipitating agents showed a steady increase throughout the ripening period. The greatest increases in the insoluble fractions of nitrogen in the serums occurred during the first 14 days of ripening. There were no significant variations in the insoluble fractions in the young cheese regardless of precipitating agent used, and in general there were no differences in the insoluble fractions of nitrogen in the serums from the four cheese in the series that could be attributed to the test organisms used.

A gradual increase in the amounts of amino nitrogen in the serums as the ripening progressed was shown by all of the cheese. There was very little difference in the amino nitrogen content of the serums from the four cheese during the early stages of ripening, but as the ripening continued the variations became greater. After 112 days of ripening there was not a great deal of difference in the amino nitrogen content of the serums from the control cheese and the cheese made with the acid forming lipolytic organism but the values were considerably lower than those of the serums from the cheese made with the inert lipolytic organism or Ps. fragi.

The moisture content varied very little in the four cheese in the series at any period during the ripening.

The data on the effect of the various organisms on the flavor development show that the control cheese and the cheese made with the inert

lipolytic organism scored about the same throughout the ripening period. These cheese scored considerably higher, especially during the first half of the ripening period, than the cheese made with Ps. fragi or the acid forming lipolytic organism. After 112 days of ripening there was but little difference in the flavor score of all four cheese in the series. Throughout most of the ripening period the cheese made with the acid forming lipolytic organism was criticized for being bitter and the other three cheese for being sour.

Since series 4 was a duplicate of series 3, the results obtained in the two series should not vary greatly. A comparison of the cheese in the two series shows that the cheese made with Ps. fragi and the acid forming lipolytic organism scored lower in flavor than the control cheese or the cheese made with the inert lipolytic organism.

Table 2 (Cont.)

Series 4

Serial number of cheese	Age of cheese days	Test organisms used	Moisture per cent	ml. of N/10 acid equiv. to nitrogen in 1 ml. of cheese serum: Total nitrogen in ml.N/10 acid	Nitrogen fractionated into soluble and insoluble fractions with: Trichloracetic acid Sol.	Trichloracetic acid Insol.	Ethyl alcohol Sol.	Ethyl alcohol Insol.	Phosphotungstic acid Sol.	Phosphotungstic acid Insol.	Amino nitrogen Mgs.
4-1	3	None.	38.4	4.80	3.2	1.6	1.7	3.1	1.0	3.8	.98
4-2	3	Inert lipolytic,No.18	37.6	4.90	3.4	1.6	1.7	3.3	1.0	3.9	1.08
4-3	3	Ps. fragi	37.4	4.00	2.5	1.5	1.3	2.8	.9	3.2	.92
4-4	3	Lipo.acid former,No.12	37.6	4.1	2.7	1.3	1.3	2.8	1.0	3.1	.98
4-1	14	None.	37.5	9.3	6.9	2.4	3.2	6.0	1.7	7.5	2.02
4-2	14	Inert lipolytic, No.18	37.3	10.0	7.3	2.7	3.5	6.4	1.9	8.0	2.14
4-3	14	Ps. fragi	37.0	7.9	5.7	2.2	3.1	4.8	2.5	5.3	1.59
4-4	14	Lipo.acid former,No.12	37.4	8.4	6.0	2.3	3.2	5.1	1.8	6.5	2.08
4-1	28	None.	37.0	12.6	9.7	2.9	4.7	7.8	2.8	9.7	2.62
4-2	28	Inert lipolytic, No.18	37.1	14.0	10.8	3.3	4.5	9.3	2.6	11.5	2.88
4-3	28	Ps. fragi	36.9	12.2	9.4	2.7	4.7	7.4	3.1	9.0	2.77
4-4	28	Lipo.acid former,No.12	37.2	12.3	9.5	2.8	4.7	7.5	3.0	9.3	2.98
4-1	56	None.	36.2	15.2	12.3	3.0	5.6	9.7	3.2	12.1	3.29
4-2	56	Inert lipolytic, No.18	36.1	17.1	14.3	2.9	5.5	11.7	3.1	14.1	3.81
4-3	56	Ps. fragi	36.5	14.1	11.3	2.9	4.4	9.7	3.3	10.7	3.33
4-4	56	Lipo.acid former,No.12	36.7	13.9	10.7	3.0	5.2	8.7	3.2	10.9	3.40
4-1	112	None.	35.3	20.6	16.4	4.3	7.2	13.3	6.0	14.8	6.9
4-2	112	Inert lipolytic, No.18	35.4	22.3	18.0	4.5	8.3	13.9	7.5	14.7	7.35
4-3	112	Ps. fragi	35.4	20.2	15.7	4.4	7.5	12.3	6.3	13.5	7.97
4-4	112	Lipo.acid former,No.12	35.5	19.5	15.0	4.4	7.2	12.1	6.1	13.2	5.88

Table 2 (Cont.)

Series 4

used	Moisture per cent	ml. of N/10 acid equiv. to nitrogen in 1 ml. of cheese serum: Total nitrogen in ml.N/10 acid	Nitrogen fractionated into soluble and insoluble fractions with: Trichloracetic acid Sol.	Trichloracetic acid Insol.	Ethyl alcohol Sol.	Ethyl alcohol Insol.	Phosphotungstic acid Sol.	Phosphotungstic acid Insol.	Amino nitrogen Mgs.	Flavor score of cheese	Remarks on cheese flavor
	38.4	4.80	3.2	1.6	1.7	3.1	1.0	3.8	.98		
,No.18	37.6	4.90	3.4	1.6	1.7	3.3	1.0	3.9	1.08		
	37.4	4.00	2.5	1.5	1.3	2.8	.9	3.2	.92		
r,No.12	37.6	4.1	2.7	1.3	1.3	2.8	1.0	3.1	.98		
	37.5	9.3	6.9	2.4	3.2	6.0	1.7	7.5	2.02	39.0	
, No.18	37.3	10.0	7.3	2.7	3.5	6.4	1.9	8.0	2.14	39.0	
	37.0	7.9	5.7	2.2	3.1	4.8	2.5	5.3	1.59	37.5	Sl. sour.
r,No.12	37.4	8.4	6.0	2.3	3.2	5.1	1.8	6.5	2.08	37.0	Sl. bitter.
	37.0	12.6	9.7	2.9	4.7	7.8	2.8	9.7	2.62	38.0	Sl. sour.
, No.18	37.1	14.0	10.8	3.3	4.5	9.3	2.6	11.5	2.88	38.5	" "
	36.9	12.2	9.4	2.7	4.7	7.4	3.1	9.0	2.77	37.0	Sour.
r,No.12	37.2	12.3	9.5	2.8	4.7	7.5	3.0	9.3	2.98	37.0	Sl. bitter.
	36.2	15.2	12.3	3.0	5.6	9.7	3.2	12.1	3.29	38.0	Sl. sour.
, No.18	36.1	17.1	14.3	2.9	5.5	11.7	3.1	14.1	3.81	38.0	" "
	36.5	14.1	11.3	2.9	4.4	9.7	3.3	10.7	3.33	36.5	Sour.
r,No.12	36.7	13.9	10.7	3.0	5.2	8.7	3.2	10.9	3.40	37.0	Sl. bitter.
	35.3	20.6	16.4	4.3	7.2	13.3	6.0	14.8	6.9	37.5	Sl. sour.
, No.18	35.4	22.3	18.0	4.5	8.3	13.9	7.5	14.7	7.35	38.0	" "
	35.4	20.2	15.7	4.4	7.5	12.3	6.3	13.5	7.97	36.5	Sour.
r,No.12	35.5	19.5	15.0	4.4	7.2	12.1	6.1	13.2	5.88	37.0	Sl. bitter.

Effect of Ps. fluorescens, A. viscosus or A. lipolyticum.

Table 3 presents the data on the nitrogenous decomposition and flavor development in two series of cheese in which Ps. fluorescens, A. viscosus or A. lipolyticum was used in addition to the regular cultures.

Series 5. When the cheese in series 5 were 3 days old there was a slight variation in the total nitrogen in the serums from the four cheese. The total difference at this period was from 5.2 to 6.4 ml. on the bases of the milliliters of N/10 acid equivalent to the nitrogen in 1 ml. of cheese serum. As the ripening progressed there was an increase in the total nitrogen in the serums of all the cheese and the differences between the four cheese became slightly greater. After 112 days of ripening the total nitrogen varied from 22.1 to 24.4 ml. of N/10 acid. The greatest increase in the total nitrogen in the serums from the four cheese in the series occurred during the period from the 3rd to the 14th day of ripening. The increase during this period was about 60 per cent for the control cheese and about 80 or more per cent for the cheese made with the test organisms. After 112 days of ripening the serum from the control cheese contained the greatest amount of nitrogen per milliliter of any of the cheese in the series.

A steady increase occurred in the various nitrogen fractions in the serums of all the cheese as the ripening progressed. In the 3 day old cheese the amounts of nitrogen in the fractions soluble in trichloracetic acid, ethyl alcohol or phosphotungstic acid showed little variations in the serums of the various cheese. As the ripening of the cheese advanced the differences in the amounts of the fractions in the serums increased,

but the variations were small even after 112 days of ripening.

The amounts of nitrogen in the fractions insoluble in the various reagents generally showed a steady increase in the serums throughout the ripening period. The only exception was when trichloracetic acid was used as the precipitating agent, then the insoluble fraction increased up to the 56th day and then decreased so that after 112 days of ripening it was about the same as at 14 days. In the young cheese there were no significant variations in the insoluble fractions. The variations found in the insoluble fractions in the serums from the four cheese in the series were small and probably not significant.

Increases in the amounts of amino nitrogen in the serums as the ripening progressed were shown by all of the cheese. The small variations found in the amino nitrogen content of the serums from the four cheese during the early stages of ripening did not change materially as the ripening progressed. There were no differences in the four cheese as far as amino nitrogen in the serum was concerned, that could be attributed to the test organisms used.

The moisture content of the four cheese varied very little at any period during the ripening.

The data on the effect of the various organisms on the flavor development in the cheese show that in general the cheese with the test cultures scored slightly higher than the control cheese. During the early part of the ripening period the cheese made with the test culture Ps. fluorescens scored highest. As the ripening progressed the variations became less and the differences after 112 days of ripening were probably not significant.

Table 3

Effect of Ps. fluorescens, A. viscosus or A. lipolyticum on the nitrogenous decomposition and flavor de
Series 5

Serial number of cheese	Age of cheese days	Test organisms used	Moisture per cent	ml. of N/10 acid equiv. to nitrogen in 1 ml. of cheese serum: Total nitrogen in ml.N/10 acid	Nitrogen fractionated into soluble and insoluble fractions with: Trichloracetic acid Sol.	Trichloracetic acid Insol.	Ethyl alcohol Sol.	Ethyl alcohol Insol.	Phosphotungstic acid Sol.	Phosphotungstic acid Insol.	Amino nitrogen Mgs.
5-1	3	None.	38.6	6.4	4.6	1.8	2.0	4.3	1.5	4.8	1.02
5-2	3	Ps. fluorescens	39.0	5.5	3.7	1.8	1.9	3.5	1.0	4.6	.97
5-3	3	A. viscosus	38.4	5.7	3.4	2.2	2.0	3.7	1.0	4.8	1.07
5-4	3	A. lipolyticum	37.8	5.2	3.6	1.6	1.9	3.2	1.0	4.2	1.07
5-1	14	None.	37.8	10.2	7.8	2.4	2.0	8.2	1.9	8.3	2.18
5-2	14	Ps. fluorescens	38.0	10.0	7.0	3.0	2.2	7.8	1.7	8.3	1.85
5-3	14	A. viscosus	37.5	10.3	7.6	2.7	2.0	8.3	1.7	8.6	1.74
5-4	14	A. lipolyticum	37.2	9.7	7.0	2.7	2.0	7.7	1.8	7.9	2.28
5-1	28	None.	36.8	15.2	11.5	3.8	5.5	9.6	3.4	12.0	2.63
5-2	28	Ps. fluorescens	37.2	16.4	12.5	3.9	5.8	10.6	3.5	13.0	3.18
5-3	28	A. viscosus	36.8	16.1	12.9	3.2	5.6	10.6	3.2	13.0	2.74
5-4	28	A. lipolyticum	36.7	16.5	13.9	2.6	4.2	12.4	3.7	12.8	2.78
5-1	56	None.	36.4	19.6	16.1	3.5	6.5	13.0	4.2	15.3	3.00
5-2	56	Ps. fluorescens	36.2	18.0	14.4	3.6	6.0	12.0	3.9	14.0	3.42
5-3	56	A. viscosus	36.4	18.0	14.5	3.5	6.6	11.4	3.5	14.5	3.10
5-4	56	A. lipolyticum	36.0	18.9	15.6	3.4	6.5	12.5	3.8	15.2	3.14
5-1	112	None.	35.3	24.4	21.8	2.6	11.9	12.4	7.6	16.9	6.01
5-2	112	Ps. fluorescens	35.7	22.1	19.5	2.8	8.9	13.5	6.6	15.4	5.28
5-3	112	A. viscosus	35.5	23.1	21.2	1.9	10.7	12.6	7.2	16.2	6.37
5-4	112	A. lipolyticum	35.4	23.5	20.6	3.1	9.5	14.0	7.1	16.5	6.32

Table 3

viscosus or A. lipolyticum on the nitrogenous decomposition and flavor development in cheddar cheese.
Series 5

ml. of N/10 acid equiv. to nitrogen in 1 ml. of cheese serum										
		Nitrogen fractionated into soluble and insoluble fractions with								
		Trichloracetic acid		Ethyl alcohol		Phosphotungstic acid				
ed	Moisture per cent	Total nitrogen in ml.N/10 acid	Sol.	Insol.	Sol.	Insol.	Sol.	Insol.	Amino nitrogen Mgs.	Flavor score of cheese	Remarks on cheese flavor
	38.6	6.4	4.6	1.8	2.0	4.3	1.5	4.8	1.02		
	39.0	5.5	3.7	1.8	1.9	3.5	1.0	4.6	.97		
	38.4	5.7	3.4	2.2	2.0	3.7	1.0	4.8	1.07		
	37.8	5.2	3.6	1.6	1.9	3.2	1.0	4.2	1.07		
	37.8	10.2	7.8	2.4	2.0	8.2	1.9	8.3	2.18	35.5	Bitter and sour.
	38.0	10.0	7.0	3.0	2.2	7.8	1.7	8.3	1.85	38.0	Lacks flavor.
	37.5	10.3	7.6	2.7	2.0	8.3	1.7	8.6	1.74	36.0	Sour.
	37.2	9.7	7.0	2.7	2.0	7.7	1.8	7.9	2.28	37.0	Sour.
	36.8	15.2	11.5	3.8	5.5	9.6	3.4	12.0	2.63	35.5	Bitter.
	37.2	16.4	12.5	3.9	5.8	10.6	3.5	13.0	3.18	37.5	Lacks flavor.
	36.8	16.1	12.9	3.2	5.6	10.6	3.2	13.0	2.74	36.5	Sour.
	36.7	16.5	13.9	2.6	4.2	12.4	3.7	12.8	2.78	37.0	Sl. sour.
	36.4	19.6	16.1	3.5	6.5	13.0	4.2	15.3	3.00	36.0	Bitter.
	36.2	18.0	14.4	3.6	6.0	12.0	3.9	14.0	3.42	37.5	Lacks flavor.
	36.4	18.0	14.5	3.5	6.6	11.4	3.5	14.5	3.10	37.5	Sl. sour.
	36.0	18.9	15.6	3.4	6.5	12.5	3.8	15.2	3.14	37.0	Sour.
	35.3	24.4	21.8	2.6	11.9	12.4	7.6	16.9	6.01	36.5	Sl. bitter.
	35.7	22.1	19.5	2.8	8.9	13.5	6.6	15.4	5.28	37.0	Lacks flavor.
	35.5	23.1	21.2	1.9	10.7	12.6	7.2	16.2	6.37	37.0	" "
	35.4	23.5	20.6	3.1	9.5	14.0	7.1	16.5	6.32	36.0	Sour.

Series 6. There was very little difference in the total nitrogen of the serums of the four cheese in series 6 when the cheese were 3 days old. The variations in the total nitrogen at this time were from 4.7 to 5.6 ml., reported as milliliters of N/10 acid equivalent to the nitrogen in 1 ml. of cheese serum. As the cheese increased in age there was an increase in the total nitrogen in the serums of all the cheese. The total nitrogen varied from 18.4 to 18.6 ml. of N/10 acid after 112 days of ripening. The greatest increase in the total nitrogen occurred during the first 14 days of ripening, the increase from the 3rd to the 14th day being from about 60 to about 80 per cent; the smallest increases in the nitrogen content were in the control cheese and in the cheese made with Ps. fluorescens while the larger increases were in the cheese made with A. viscosus or A. lipolyticum. There was also a considerable increase in total nitrogen during the period from the 14th to the 28th day of ripening, but the per cent increase was less than during the earlier period. The variations in the total nitrogen in the serums from the four cheese were small throughout the ripening period.

There was a steady increase in the various fractions of nitrogen in the serums of all the cheese as the ripening progressed. There was practically no variation in the amount of nitrogen in the fractions soluble in trichloracetic acid, ethyl alcohol or phosphotungstic acid with the four cheese in the series at any period during ripening. The greatest increase in the soluble fractions of nitrogen in the serums usually came during the first 28 days of ripening. There were no variations in the amounts of nitrogen in the soluble fraction in the serum of the four cheese in the series that could be attributed to the test organisms used.

The amount of nitrogen in the fractions insoluble in the various reagents usually showed a steady increase in the serums throughout the ripening period. There were no significant variations in the insoluble fractions of nitrogen in the serums when the cheese were 3 days old. When trichloracetic acid was used as the precipitating agent there was a small but gradual increase in the insoluble fractions of nitrogen in the serum throughout the ripening period, with ethyl alcohol or phosphotungstic acid used as the precipitating agent there was an increase in the insoluble fractions of nitrogen in the serums throughout the 112 day ripening period, but the greatest increase occurred during the first 28 days of ripening. The variations found in the insoluble fractions of nitrogen in the serums from the four cheese show that the differences between the control cheese and that made with the test cultures are too small to be of any importance.

There was a steady increase in the amount of amino nitrogen in the serums from the four cheese as the ripening progressed. The small variations found in the amino nitrogen content of the serums from the four cheese in the series at all stages of ripening were too small to be of any importance.

The moisture content of the four cheese in the series showed very little variation at any stage during ripening.

The results on the effect of the various organisms on the flavor development in the cheese show that during the early stages of ripening, the cheese made with the test organisms scored higher than the control cheese. As the ripening progressed the flavor in the control cheese improved and after 28 days of ripening the variation in the flavor scored from the four

cheese in the series was not significant.

There was considerable difference in the nitrogen content of the cheese in series 5 and 6, although they were made under identical conditions. There was no material difference in the total nitrogen content of the serums from the two series of cheese at 3 days, but as the ripening progressed the variations became greater. The amino nitrogen content of the serums from the two series of cheese showed small variations during the early period of ripening, but as the ripening progressed the differences increased. The flavor scores in the two series are in very close agreement at all periods during ripening; while there are some small differences in the flavor scores in the cheese within the series, they are too small tc be of any importance.

Table 3 (Cont.)

Series 6

Serial number of cheese	Age of cheese days	Test organisms used	Moisture per cent	ml. of N/10 acid equiv. to nitrogen in 1 ml. of cheese serum: Total nitrogen in ml.N/10 acid	Nitrogen fractionated into soluble and insoluble fractions with: Trichloracetic acid Sol.	Trichloracetic acid Insol.	Ethyl alcohol Sol.	Ethyl alcohol Insol.	Phosphotungstic acid Sol.	Phosphotungstic acid Insol.	Amino nitrogen Mgs.	Flav scor of chee
6-1	3	None.	39.3	5.1	3.3	1.8	1.7	3.4	.9	4.2	.87	
6-2	3	Ps. fluorescens	39.5	5.6	3.4	2.1	2.0	3.5	1.0	4.5	.92	
6-3	3	A. viscosus	39.1	5.0	3.5	1.6	1.7	3.4	.9	4.2	.87	
6-4	3	A. lipolyticum	38.8	4.7	3.2	1.6	1.7	3.0	.7	3.9	.92	
6-1	14	None.	38.5	8.7	6.3	2.3	3.3	5.3	1.4	7.3	1.85	35.
6-2	14	Ps. fluorescens	38.6	8.8	6.5	2.3	3.4	5.6	1.5	7.4	1.68	37.
6-3	14	A. viscosus	38.2	9.0	6.8	2.3	3.5	5.4	1.5	7.4	1.63	36.
6-4	14	A. lipolyticum	38.1	8.5	6.5	2.1	3.3	5.3	1.3	7.3	1.80	37.
6-1	28	None.	37.3	13.1	11.0	2.0	4.0	9.0	3.0	10.2	2.72	36.
6-2	28	Ps. fluorescens	37.5	13.4	10.6	2.8	4.1	9.3	2.7	10.6	2.28	36.
6-3	28	A. viscosus	37.5	14.2	12.2	2.0	4.0	10.1	2.7	11.4	2.39	36.
6-4	28	A. lipolyticum	36.9	13.0	10.0	3.1	4.5	8.8	2.7	10.3	2.33	37.
6-1	56	None.	36.8	16.0	12.9	3.1	5.1	11.0	2.9	13.0	3.33	36.
6-2	56	Ps. fluorescens	36.8	16.1	12.9	3.2	5.0	11.1	2.9	13.2	2.83	37.
6-3	56	A. viscosus	37.2	15.8	12.7	3.1	5.0	10.7	2.5	12.4	2.78	37.
6-4	56	A. lipolyticum	36.8	16.0	12.7	3.2	5.1	10.8	2.4	12.5	3.16	37.
6-1	112	None.	36.1	18.6	14.5	3.9	8.2	11.2	4.4	14.2	5.00	36.
6-2	112	Ps. fluorescens	36.3	18.4	15.0	3.3	7.0	11.6	4.0	14.4	5.97	37.
6-3	112	A. viscosus	36.7	18.5	15.2	3.5	7.1	11.2	4.5	14.1	5.55	37.
6-4	112	A. lipolyticum	36.2	18.4	14.4	4.0	7.0	11.5	4.5	14.0	5.39	36.

Table 3 (Cont.)

Series 6

ed	Moisture per cent	Total nitrogen in ml.N/10 acid	ml. of N/10 acid equiv. to nitrogen in 1 ml. of cheese serum: Nitrogen fractionated into soluble and insoluble fractions with Trichloracetic acid Sol.	Trichloracetic acid Insol.	Ethyl alcohol Sol.	Ethyl alcohol Insol.	Phosphotungstic acid Sol.	Phosphotungstic acid Insol.	Amino nitrogen Mgs.	Flavor score of cheese	Remarks on cheese flavor
	39.3	5.1	3.3	1.8	1.7	3.4	.9	4.2	.87		
	39.5	5.6	3.4	2.1	2.0	3.5	1.0	4.5	.92		
	39.1	5.0	3.5	1.6	1.7	3.4	.9	4.2	.87		
	38.8	4.7	3.2	1.6	1.7	3.0	.7	3.9	.92		
	38.5	8.7	6.3	2.3	3.3	5.3	1.4	7.3	1.85	35.0	Sour.
	38.6	8.8	6.5	2.3	3.4	5.6	1.5	7.4	1.68	37.0	Sour.
	38.2	9.0	6.8	2.3	3.5	5.4	1.5	7.4	1.63	36.0	Fermented.
	38.1	8.5	6.5	2.1	3.3	5.3	1.3	7.3	1.80	37.0	Sour.
	37.3	13.1	11.0	2.0	4.0	9.0	3.0	10.2	2.72	36.0	Sour.
	37.5	13.4	10.6	2.8	4.1	9.3	2.7	10.6	2.28	36.5	Sour.
	37.5	14.2	12.2	2.0	4.0	10.1	2.7	11.4	2.39	36.5	Sour.
	36.9	13.0	10.0	3.1	4.5	8.8	2.7	10.3	2.33	37.5	Sl. sour.
	36.8	16.0	12.9	3.1	5.1	11.0	2.9	13.0	3.33	36.5	" "
	36.8	16.1	12.9	3.2	5.0	11.1	2.9	13.2	2.83	37.0	Fermented.
	37.2	15.8	12.7	3.1	5.0	10.7	2.5	12.4	2.78	37.0	Fermented.
	36.8	16.0	12.7	3.2	5.1	10.8	2.4	12.5	3.16	37.0	Sl. sour.
	36.1	18.6	14.5	3.9	8.2	11.2	4.4	14.2	5.00	36.5	Sl. sour.
	36.3	18.4	15.0	3.3	7.0	11.6	4.0	14.4	5.97	37.0	Sl. fermented.
	36.7	18.5	15.2	3.5	7.1	11.2	4.5	14.1	5.55	37.0	" "
	36.2	18.4	14.4	4.0	7.0	11.5	4.5	14.0	5.39	36.5	Sl. sour.

Effect of Adding Various Organisms Alone or in Combinations to Pasteurized Milk for Making Cheddar Cheese on the Total Number, Proteolytic and Lipolytic Bacteria in the Cheese.

The total counts on beef infusion agar and on tomato juice agar agreed very closely throughout the ripening period. The most significant differences occurred late in the ripening period and then, with a number of the cheese, the counts on beef infusion agar were higher than the counts on tomato juice agar. Because of the close agreement of the counts, the following discussion deals only with the data obtained on beef infusion agar.

The Numbers of Bacteria per Gram of Cheese Made From Pasteurized Milk Inoculated with S. liquefaciens, an Unidenti- Micrococcus or Both.

Table 4 presents the data from series 1 and 2 on the numbers of total and proteolytic bacteria per gram of cheese at various stages during ripening.

With series 1 the total count on the control cheese at 3 days of age was 1,200,000,000 per gram. This number increased so that at 14 days and at 28 days there were 1,800,000,000 bacteria per gram, after which there was a decrease to 780,000,000 at 112 days.

The cheese made from the milk inoculated with S. liquefaciens gave somewhat higher counts than the control cheese during the early part of the ripening period, but the count was not significantly different after the 112 days. This cheese contained 1,800,000,000 bacteria per gram at 3 days, 4,700,000,000 at 14 days and 720,000,000 after 112 days of ripening.

When the unidentified Micrococcus was used as the test culture the numbers of total bacteria differed only slightly from the control cheese. The counts were 1,850,000,000 per gram at 3 days, 2,100,000,000 at 28 days and 840,000,000 after 112 days.

The cheese made from the milk inoculated with both S. liquefaciens and the unidentified Micrococcus contained more bacteria per gram than the control cheese throughout the experimental period. The numbers were 1,650,000,000 per gram at 3 days, 3,500,000,000 at 14 days, and 1,020,000,000 after 112 days. This cheese did not get as high in total counts as the cheese made with S. liquefaciens alone during the early part of ripening but, after 112 days of ripening, contained a greater number of bacteria than any other cheese in the series.

The control cheese in series 1 contained very few proteolytic bacteria. The number was less than 100 per gram up to the 14th day; after this there was an increase so that there were 2,800, 4,000 and 2,500 per gram, respectively, at 28, 56 and 112 days. The cheese made with S. liquefaciens as the test culture contained large numbers of proteolytic bacteria at all stages during ripening. There were 35,500,000 bacteria per gram at 3 days, the count increased to 80,000,000 at 28 days, and then decreased to 11,000,000 after 112 days of ripening. The numbers of proteolytic bacteria per gram of cheese when the unidentified Micrococcus was used as the test culture were much lower than with the cheese containing S. liquefaciens, but higher than with the control. There were 1,000 per gram up to the 14th day, 141,000 at 28 days and 62,000 after 112 days of ripening. When both S. liquefaciens and the unidentified Micrococcus were used the numbers of proteolytic bacteria were 20,000,000

per gram of cheese at 3 days, 45,000,000 at 28 days and 12,000,000 after 112 days. These counts were in general agreement with those obtained when S. liquefaciens alone was added to the milk.

As shown by the data in Table 4, the total bacterial counts on the cheese in series 2 did not vary materially from those on the cheese in series 1, although there was less of a tendency toward unusually high counts in the cheese containing S. liquefaciens. The control cheese contained 1,000,000,000 bacteria per gram at 3 days, 2,120,000,000 at 14 days and 410,000,000 after 112 days of ripening.

The cheese made from milk inoculated with S. liquefaciens gave a bacterial count of 1,500,000,000 bacteria per gram at 3 days, 2,250,000,000 at 14 days and 550,000,000 after 112 days; this cheese contained a slightly higher number of bacteria per gram of cheese throughout the experimental period than did the control cheese.

When the unidentified Micrococcus was used as the test culture, the total bacterial counts were 1,600,000,000 per gram at 3 days, 1,850,000,000 at 14 days, and 850,000,000 after 112 days of ripening.

With both S. liquefaciens and the unidentified Micrococcus used in the cheese, the total bacterial counts were 1,700,000,000 per gram at 3 days, 2,300,000,000 at 14 days and 290,000,000 after 112 days.

The numbers of proteolytic bacteria in the cheese in series 2 were considerably lower than with the cheese in series 1. In the control cheese the number was less than 100 per gram of cheese up to 56 days and was 1,000 per gram at 112 days. The cheese made with S. liquefaciens contained 23,000,000 proteolytic bacteria per gram at 3 days, 46,000,000 at 14 days and 2,480,000 at 112 days. The cheese containing the unidentified

Micrococcus gave a proteolytic bacteria count of 1,000 per gram at 3 days, 18,000 at 28 days and 10,000 at 112 days. When both S. liquefaciens and the unidentified Micrococcus were used in the cheese, the numbers of proteolytic bacteria were 30,000,000 at 3 days, 32,000,000 at 14 days and 2,500,000 at 112 days.

If a comparison is made of the results obtained in series 1 and 2 it is evident that, while there were some variations in the general changes in the numbers of total bacteria, nevertheless the trends in the two series were very definitely the same. There was a rapid increase in the number of bacteria per gram of cheese in the early periods of ripening, followed by a rapid decrease so that at 28 days the bacterial counts were about the same as at 3 days and, finally, there was a slower decrease until the end of the 112 days of ripening. In each series, the numbers of proteolytic bacteria in the cheese made with the test organisms were strikingly higher than the numbers in the control cheese.

Table 4

Numbers of bacteria at various stages of ripening in cheese made with different test org

Series 1

Age of cheese in days	Test organisms added: None — Bacteria per gram			S. liquefaciens — Bacteria per gram			Micrococcus — Bacteria per gram			S.
	Total on			Total on			Total on			
	Beef infusion agar*	Tomato juice agar*	Proteolytics	Beef infusion agar*	Tomato juice agar*	Proteolytics*	Beef infusion agar*	Tomato juice agar*	Proteolytics	Be fu a
3	1200000	1100000	100	1800000	1750000	35500	1850000	2000000	1000	16
14	1800000	2200000	100	4700000	6500000	56000	1800000	2200000	1000	35
28	1800000	2100000	2800	2200000	1900000	80000	2100000	2550000	141000	22
56	850000	900000	4000	1200000	1150000	7770	1000000	1200000	50000	12
112	780000	920000	2500	720000	700000	11000	840000	780000	62000	10

Series 2

3	1000000	1200000	100	1500000	1700000	23000	1600000	1500000	1000	17
14	2120000	2140000	100	2250000	2330000	46000	1850000	2000000	2100	23
28	1370000	1280000	100	2050000	1150000	41000	1000000	1020000	18000	12
56	1000000	1000000	100	1200000	850000	5000	950000	1000000	7000	9
112	410000	140000	1000	550000	350000	2480	850000	630000	10000	2

* 000 omitted.

Table 4

cteria at various stages of ripening in cheese made with different test organisms.

Series 1

Test organisms added								
S. liquefaciens Bacteria per gram			Micrococcus Bacteria per gram			*S. liquefaciens* and Micrococcus Bacteria per gram		
Total on			Total on			Total on		
Beef infusion agar*	Tomato juice agar*	Proteolytics*	Beef infusion agar*	Tomato juice agar*	Proteolytics	Beef infusion agar*	Tomato juice agar*	Proteolytics*
1800000	1750000	35500	1850000	2000000	1000	1650000	2000000	20000
4700000	6500000	56000	1800000	2200000	1000	3500000	3800000	43000
2200000	1900000	80000	2100000	2550000	141000	2200000	1950000	45000
1200000	1150000	7770	1000000	1200000	50000	1200000	1600000	5500
720000	700000	11000	840000	780000	62000	1020000	520000	12000

Series 2

1500000	1700000	23000	1600000	1500000	1000	1700000	1800000	30000
2250000	2330000	46000	1850000	2000000	2100	2300000	2380000	32000
2050000	1150000	41000	1000000	1020000	18000	1280000	1280000	31000
1200000	850000	5000	950000	1000000	7000	900000	850000	9000
550000	350000	2480	850000	630000	10000	290000	350000	2500

The Numbers of Bacteria per Gram of Cheese Made From Pasteurized Milk Inoculated with an Inert Lipolytic Organism, Ps. fragi or a Lipolytic Acid Forming Organism.

The data from series 3 and 4 on the numbers of total and lipolytic bacteria per gram of cheese at various periods during ripening are given in Table 5.

The total count on the control cheese in series 3 at 3 days of age was 2,500,000,000 bacteria per gram, after which there was a decrease to 800,000,000 at 112 days.

In the cheese made from the milk inoculated with the inert lipolytic organism the numbers of total bacteria differed only slightly from the control cheese. The count was 2,800,000,000 per gram at 3 days; this was followed by a reduction in numbers to 620,000,000 at 112 days.

When Ps. fragi was used as the test organism the numbers of total bacteria were slightly higher than in the control cheese early in the ripening period and lower late in the ripening period. The highest count of 3,000,000,000 bacteria per gram was obtained when the cheese was 3 days of age; this was followed by a steady decrease so that there were only 600,000,000 per gram at 112 days.

The cheese made from the milk inoculated with the lipolytic acid forming organism was lower in bacterial counts than the control cheese. The counts were 1,000,000,000 per gram at 3 days, 1,500,000,000 at 14 days and 330,000,000 at 112 days.

The control cheese in series 3 contained less than 100 lipolytic bacteria per gram at all stages throughout the ripening period. The cheese made with the inert lipolytic organism also contained less than

100 lipolytic bacteria per gram of cheese during the first 28 days of ripening; the count then increased to 1,000 per gram at 56 days and decreased to less than 100 per gram at 112 days. When Ps. fragi was used as the test culture the number of lipolytic bacteria was 4,000 per gram at 3 days. There was then an increase to 23,000 per gram at 56 days and a decrease to 5,000 lipolytic bacteria per gram at 112 days. When the lipolytic acid forming organism was used as the test culture, the number of lipolytic bacteria per gram was much greater than in any of the other cheese in series 3. At 3 days the count was 22,000 per gram; this was followed by an increase to 100,000 per gram at 56 days and then a decrease to 1,000 per gram after 112 days of ripening.

The first three cheese in series 3, that is, the control cheese and the cheese made with the test culture, inert lipolytic organism and Ps. fragi, started out with high total counts, followed by a steady decrease until the end of the ripening period, while the cheese made with the lipolytic acid forming organism increased in total count up to the 14th day and then decreased in total count until the end of the ripening period.

The bacterial counts in the cheese at 3 days were much lower in series 4 than in series 3, except when the lipolytic acid former was used as the test culture. In general, all the cheese in series 4 showed a large increase in bacterial counts during the first 14 days, followed by a steady decrease in bacterial counts during the balance of the ripening period. At 3 days the control cheese contained 1,300,000,000 bacteria per gram; this number increased to 2,300,000,000 at 14 days and then decreased to 600,000,000 at 112 days.

The cheese made from the milk inoculated with the inert lipolytic organism gave bacterial counts of 1,200,000,000 per gram at 3 days, 1,350,000,000 at 14 days and 780,000,000 after 112 days of ripening.

When Ps. fragi was used as the test culture, the total bacterial counts were 1,000,000,000 per gram at 3 days, 2,750,000,000 at 14 days and 820,000,000 after ripening 112 days.

With the lipolytic acid forming organism used in the cheese the total bacterial counts were 1,000,000,000 per gram at 3 days, 2,000,000,000 at 14 days and 580,000 at 112 days.

The control cheese in series 4 contained less than 100 lipolytic bacteria per gram at 3 days. At 14 days the count was 1,000 per gram, after which it decreased to less than 100 per gram at 28 days and remained less than 100 lipolytic bacteria per gram during the remainder of the ripening period. When the inert lipolytic organism was used, the numbers of lipolytic organisms per gram of cheese were 4,000 at 3 days, 51,000 at 14 days and less than 100 after 112 days of ripening. The cheese made from milk inoculated with Ps. fragi contained 16,000 lipolytic bacteria per gram at 3 days, 73,000 at 14 days, and less than 100 per gram after 112 days of ripening. When the lipolytic acid forming organism was used as the test culture the numbers of lipolytic bacteria per gram were 16,000 at 3 days, 73,000 at 14 days and less than 100 per gram at 112 days.

A comparison of the results obtained on series 3 and 4 shows that there were considerable variations in the total bacterial counts on the cheese from the two series during the early stages of ripening. After 112 days of ripening, the variations in bacterial counts on the cheese from

the two series did not show any significant variations. In general, the cheese made with the lipolytic acid forming organism gave a slightly lower total bacterial count after 112 days than did the other cheese in the two series, while there were no material variations in the total counts on the other three cheese in each series. Usually there was a rapid increase in the total counts during the early period of ripening followed by a rapid decrease for a short period of time, the decrease then became more gradual. When the lipolytic organisms were used as the test cultures, the lipolytic counts were usually high during the early stages, but nearly always less than 100 per gram after 112 days of ripening.

Table 5

Numbers of bacteria at various stages of ripening in cheese made with different test o

Series 3

Age of cheese in days	Test organisms added: None — Bacteria per gram			Inert lipolytic (No. 18) — Bacteria per gram			Ps. fragi — Bacteria per gram			L
	Total on			Total on			Total on			
	Beef infusion agar*	Tomato juice agar*	Lipolytics	Beef infusion agar*	Tomato juice agar*	Lipolytics	Beef infusion agar*	Tomato juice agar*	Lipolytics	B f
3	2500000	2850000	100	2800000	2350000	100	3000000	3750000	4000	1
14	2250000	1500000	100	1350000	1750000	100	2900000	3100000	12000	1
28	1750000	1900000	100	1500000	1250000	100	1300000	1000000	21000	1
56	1600000	1500000	100	1300000	1200000	10000	1000000	900000	23000	
112	800000	420000	0	620000	440000	100	600000	360000	5000	

Series 4

3	1300000	900000	100	1200000	900000	4000	1000000	850000	16000	1
14	2300000	1250000	1000	1350000	1000000	51000	2750000	2800000	73000	2
28	1900000	1300000	100	1000000	900000	50000	2500000	1300000	67000	1
56	600000	640000	100	440000	880000	2000	800000	840000	9000	
112	600000	420000	100	780000	570000	100	820000	350000	100	

* 000 omitted.

Table 5

of bacteria at various stages of ripening in cheese made with different test organisms.

Series 3

Test organisms added									
	Inert lipolytic (No. 18) Bacteria per gram			Ps. fragi Bacteria per gram			Lipolytic acid former (No. 12) Bacteria per gram		
	Total on			Total on			Total on		
lytics	Beef infusion agar*	Tomato juice agar*	Lipolytics	Beef infusion agar*	Tomato juice agar*	Lipolytics	Beef infusion agar*	Tomato juice agar*	Lipolytics
100	2800000	2350000	100	3000000	3750000	4000	1000000	1750000	22000
100	1350000	1750000	100	2900000	3100000	12000	1500000	1350000	47000
100	1500000	1250000	100	1300000	1000000	21000	1300000	1300000	60000
100	1300000	1200000	10000	1000000	900000	23000	900000	750000	100000
0	620000	440000	100	600000	360000	5000	330000	480000	1000

Series 4

100	1200000	900000	4000	1000000	850000	16000	1000000	800000	16000
.000	1350000	1000000	51000	2750000	2800000	73000	2000000	1500000	73000
100	1000000	900000	50000	2500000	1300000	67000	1800000	1200000	67000
100	440000	880000	2000	800000	840000	9000	990000	720000	6000
100	780000	570000	100	820000	350000	100	580000	200000	100

The Numbers of Bacteria per Gram of Cheese Made from Pasteurized Milk Inoculated with Ps. fluorescens, A. viscosus or A. lipolyticum.

Table 6 presents the results from series 5 and 6 on the numbers of total and lipolytic bacteria per gram of cheese at various stages in the ripening.

The total counts on the control cheese in series 5 were 1,000,000,000 bacteria per gram at 3 days of age, 2,000,000,000 at 28 days and then there was a decrease to 1,100,000,000 per gram after 112 days of ripening.

The cheese made from the milk inoculated with Ps. fluorescens as the test culture contained 700,000,000 bacteria per gram at 3 days, 3,500,000,000 at 14 days, and then decreased to 600,000,000 per gram after 112 days of ripening. At 14 or 28 days of age the control cheese was much lower in total count than the cheese made with the test organism, while at 3 days or 112 days it was higher in total counts than the cheese containing the test organism.

When A. viscosus was used as the test culture the total numbers of bacteria were 2,500,000,000 at 3 days, 3,400,000,000 at 14 days and 580,000,000 at 112 days. The cheese made with this organism was considerably higher in total numbers of bacteria per gram than the control cheese during the first part of ripening but was lower after 112 days.

The cheese made from milk inoculated with A. lipolyticum was higher in total bacterial counts during the first 14 days, and lower after 112 days, than the control cheese. The bacterial counts of the cheese made with A. lipolyticum were 2,000,000,000 per gram at 3 days of age, this

number decreased to 200,000,000 after 112 days.

The control cheese from series 5 contained less than 100 lipolytic bacteria per gram at all stages during ripening. The cheese made with Ps. fluorescens gave a lipolytic count of less than 100 per gram at 3 days; the count then increased to 60,000 per gram at 56 days and finally decreased to less than 100 per gram at 112 days. When A. viscosus was used as the test organism there were 65,000 lipolytic bacteria per gram of cheese at 3 days, 110,000 per gram at 14 days and 1,000 per gram at 112 days. The cheese made with A. lipolyticum gave a lipolytic count of less than 100 per gram during the first 14 days, 100 per gram up to 56 days and 2,000 per gram after 112 days of ripening.

The total bacterial counts in series 6 varied considerably from the counts in series 5. There was a tendency for the total counts to be lower in series 6 than in series 5, especially during the latter part of the ripening period. At 3 days of age the control cheese contained 1,000,000,000 bacteria per gram; this number increased to 2,300,000,000 at 14 days and then decreased to 190,000,000 after 112 days.

The cheese made with Ps. fluorescens as the test organism contained 1,200,000,000 bacteria per gram at 3 days of age, 4,000,000,000 per gram at 14 days and 430,000,000 at 112 days.

When A. viscosus was used as the test culture the total bacterial counts were 950,000,000 per gram at 3 days, 1,500,000,000 at 14 days and 270,000,000 at 112 days.

The cheese made from the milk inoculated with A. lipolyticum gave bacterial counts of 1,300,000,000 per gram at 3 days, 2,100,000,000 at 14 days and 110,000,000 after 112 days of ripening.

The number of lipolytic bacteria in the control cheese was less than 100 per gram at 3 days of age; there was an increase to 1,000 per gram at 14 days and then a decrease to less than 100 per gram at 56 days. The cheese made with Ps. fluorescens as the test organism gave lipolytic counts of 7,000 per gram at 3 days, 17,000 at 14 days and then decreased to less than 100 per gram at 112 days. When A. viscosus was used the numbers of lipolytic bacteria were 8,500 per gram at 3 days, 31,000 at 28 days and less than 100 per gram at 112 days. With A. lipolyticum as the test organism the numbers of lipolytic bacteria were 10,000 per gram at 3 days, 21,000 at 28 days and less than 100 per gram after 112 days of ripening.

A comparison of the results obtained in series 5 and 6 shows that there were considerable differences in the total bacterial counts in the two series during the early stages of ripening, especially in the cheese made with the test cultures. There was also considerable variation during the latter part of ripening, the total bacterial counts being lower on the cheese in series 6 than on the cheese in series 5. The highest total counts obtained in the two series were on cheese made with Ps. fluorescens.

In general, the number of lipolytic bacteria in the cheese inoculated with the test cultures gave relatively high lipolytic counts during the first half of the ripening period, but usually contained less than 100 lipolytic bacteria per gram of cheese after 112 days of ripening.

Table 6

Numbers of bacteria at various stages of ripening in cheese made with different test organisms

Series 5

Age of cheese in days	Test organisms added: None — Bacteria per gram — Total on Beef infusion agar*	Tomato juice agar*	Lipolytics	Ps. fluorescens — Bacteria per gram — Total on Beef infusion agar*	Tomato juice agar*	Lipolytics	A. viscosus — Bacteria per gram — Total on Beef infusion agar*	Tomato juice agar*	Lipolytics	Beef fusi aga
3	1000000	950000	0	700000	750000	0	2500000	2000000	65000	2000
14	1000000	1000000	0	3500000	2900000	16000	3400000	4000000	110000	1600
28	2000000	1500000	0	3200000	2700000	20000	2800000	3000000	80000	1900
56	1580000	1310000	0	1650000	1140000	60000	1300000	840000	14000	1260
112	1100000	940000	0	600000	600000	100	580000	450000	1000	200

Series 6

3	1000000	850000	100	1200000	1000000	7000	950000	750000	8500	1300
14	2300000	1400000	1000	4000000	2000000	17000	1500000	1500000	20000	2100
28	1520000	990000	1000	1000000	1100000	16000	790000	550000	31000	720
56	400000	300000	100	560000	840000	15000.	780000	750000	26000	800
112	190000	160000	100	430000	470000	100	270000	240000	100	110

* 000 omitted.

Table 6

' bacteria at various stages of ripening in cheese made with different test organisms.

Series 5

Test organisms added									
	Ps. fluorescens Bacteria per gram			A. viscosus Bacteria per gram			A. lipolyticum Bacteria per gram		
	Total on			Total on			Total on		
›lytics	Beef infusion agar*	Tomato juice agar*	Lipolytics	Beef infusion agar*	Tomato juice agar*	Lipolytics	Beef infusion agar*	Tomato juice agar*	Lipolytics
0	700000	750000	0	2500000	2000000	65000	2000000	2100000	0
0	3500000	2900000	16000	3400000	4000000	110000	1600000	1500000	0
0	3200000	2700000	20000	2800000	3000000	80000	1900000	1300000	0
0	1650000	1140000	60000	1300000	840000	14000	1260000	880000	0
0	600000	600000	100	580000	450000	1000	200000	220000	2000

Series 6

100	1200000	1000000	7000	950000	750000	8500	1300000	950000	10000
.000	4000000	2000000	17000	1500000	1500000	20000	2100000	1500000	13000
.000	1000000	1100000	16000	790000	550000	31000	720000	600000	21000
100	560000	840000	15000	780000	750000	26000	800000	320000	21000
100	430000	470000	100	270000	240000	100	110000	120000	100

Acid Value of Fat of Cheese Made With Different Cultures.

The acid values of the fat of the experimental cheese were determined at various stages of ripening in an attempt to obtain information on the factors influencing the acidity of the fat in Cheddar cheese. The values were expressed as milliliters of N/10 sodium hydroxide required to neutralize 20 grams of the fat. The data are presented in Table 7.

With series 1 and 2 the acid values were determined only after 56 and 112 days. There was an increase in the acid values from one examination to the next with each of the cheese in these two series. In general, the variations in the acid values for the different cheese in each series were small and the variations between the acid value of the control cheese and those of the cheese containing the test organisms were too small to be of significance. The cheese in series 1 had slightly higher acid values than the cheese in series 2, both with the control cheese and with the cheese containing test organisms. In series 2 the variations in the acid values were greater than in the other series. With series 2 the highest acid value of 5.0 ml. was obtained on the cheese containing S. liquefaciens, the lowest acid value of 4.0 ml. was obtained on the cheese made with S. liquefaciens and the Micrococcus, while values of 4.4 ml. and 4.0 ml., respectively, were obtained on the control cheese, and the cheese made with the unidentified Micrococcus.

With series 3 to 6, inclusive, the acid values were determined at each examination of the cheese. In general, there was an increase in acid value during the ripening for each of the cheese studied.

There was some variation in the acid values of the fat from the

cheese in series 3. The control cheese and the cheese made with the inert lipolytic organism and Ps. fragi as test cultures did not show much variation; with each there was a small and rather regular increase in the acid values from the 3rd to the 112th day of ripening. The cheese made with the lipolytic acid forming organism showed the greatest increase in the acid value of any of the cheese in series 3. With this series the highest acid value of 5.7 ml. was obtained on the cheese made with the lipolytic acid forming organism, the lowest value of 4.2 ml. was obtained on the control cheese, while the cheese made with the inert lipolytic organism and Ps. fragi gave acid values of 4.7 ml. and 4.9 ml., respectively.

The acid values in series 4 showed some variation from those in series 3, both with the control cheese and with the cheese containing the test organisms. In general, the cheese in series 4 were lower in acid values than the cheese in series 3. With the former series the variations in the acid values on the control cheese and the cheese made with the inert lipolytic organism and Ps. fragi were too small to be of any significance even after 112 days of ripening. The cheese made with the lipolytic acid forming organism was slightly higher in acid value than the control cheese, but the variation is probably of little importance.

The greatest variations in the acid values of the cheese fat occurred in series 5 and 6. In series 5 the variations were small during the first 56 days of ripening. After this there were considerable increases in the acid values with the cheese containing the test organisms while there was no increase with the control cheese. After 112 days of ripening the highest acid value of 6.7 ml. was obtained on the cheese containing Ps. fluorescens and also on the cheese containing A. viscosus,

while acid values of 5.4 ml. and 4.3 ml., respectively, were obtained on the cheese made with A. lipolyticum and on the control cheese.

The greatest differences in the acid values of the fat from the cheese occurred in series 6. While the variations were very small during the first half of the ripening period, they were very noticeable after 112 days of ripening. The control cheese was the lowest in the series, with an acid value of 4.0 ml., the cheese made with Ps. fluorescens was high with a value of 7.8 ml., while the cheese made with A. viscosus and A. lipolyticum as the test cultures had acid values of 5.5 and 5.8 ml., respectively.

From the data presented it is evident that the acid values of the fat in the cheese were not materially influenced by S. liquefaciens, the Micrococcus, the inert lipolytic organism, or Ps. fragi, while the lipolytic acid forming organism, Ps. fluorescens, A. viscosus and A. lipolyticum tended to increase the acid value of the fat in the cheese.

Table 7

Acid values on fat of cheese made with different cultures.

Series and number of cheese	Test culture used*	(ml. of N/10 NaOH required to neutralize 20 grams of fat after) 3 days	14 days	28 days	56 days	112 days
1-1	Control cheese				4.1	5.2
1-2	S. liquefaciens				4.2	5.3
1-3	Micrococcus				4.0	4.9
1-4	S. liquefaciens, Micrococcus				4.2	5.3
2-1	Control cheese				3.7	4.4
2-2	S. liquefaciens				4.0	5.0
2-3	Micrococcus				3.9	4.8
2-4	S. liquefaciens, Micrococcus				3.5	4.0
3-1	Control cheese	2.9	3.3	3.5	4.0	4.2
3-2	Inert lipolytic (No. 18)	3.0	3.5	4.1	4.5	4.7
3-3	Ps. fragi	3.0	3.8	4.8	4.9	4.9
3-4	Lipolytic acid former (No. 12)	2.8	3.4	4.6	4.5	5.7
4-1	Control cheese	3.0	3.0	3.2	3.4	3.8
4-2	Inert lipolytic (No. 18)	2.7	3.1	3.9	4.1	4.1
4-3	Ps. fragi	2.8	3.4	4.0	4.0	4.3
4-4	Lipolytic acid former (No. 12)	3.3	3.9	4.8	5.0	4.9
5-1	Control cheese	2.8	3.4	4.3	4.4	4.3
5-2	Ps. fluorescens	2.8	3.3	4.5	5.2	6.7
5-3	A. viscosus	2.8	3.3	4.1	4.4	6.7
5-4	A. lipolyticum	2.9	3.5	4.1	4.3	5.4
6-1	Control cheese	2.8	3.3	3.5	4.0	4.0
6-2	Ps. fluorescens	2.7	3.4	4.0	4.3	7.8
6-3	A. viscosus	2.9	3.5	4.2	4.4	5.5
6-4	A. lipolyticum	3.0	3.6	4.1	5.6	5.8

*Butter culture (122) and L. casei (L4) were used in all cheese.

Percentage of the Total Flora Made Up of Lactobacilli at Various Times During the Making and Ripening of the Cheese.

The work of various investigators has shown the importance of Lactobacilli in the flora of Cheddar cheese. An attempt was made to get a general idea of the prominence of these organisms in the experimental cheese made from pasteurized milk since a culture of L. casei was regularly added to the milk. The general procedure used was to make microscopic examinations at various times during the making and ripening process. Slides were prepared of the original pasteurized milk, of the milk after the cultures had been added and of the ripened milk. These slides were made by spreading a drop of milk over a portion of a clean glass slide and allowing it to dry. After cutting the curd and until the curd was too tough to permit it, the slides were prepared by placing a small piece of curd between two clean glass slides, squeezing it into a thin layer and drying. After the curd had become too tough to use in the preparation of a slide, a small amount of serum was squeezed out of the curd, spread on a slide, and dried. Cheese serum was used for the preparation of the slides after the cheese had been pressed; the slides were prepared by placing a drop of cheese serum on a clean slide, spreading, and drying. The prepared slides were stained with methylene blue and examined under the microscope. The Lactobacilli were distinguished on the basis of general morphology only; while this procedure is not entirely satisfactory, the appearance of Lactobacilli in milk and Cheddar cheese is sufficiently characteristic so that useful information can be obtained on the basis of morphology alone. The Lactobacilli and total bacteria in a number of fields were counted and the percentage of the total flora made up of Lactobacilli was calculated.

The data obtained are presented in Table 8.

In no case did the microscopic examination reveal the presence of Lactobacilli in the pasteurized milk. This does not mean there were no Lactobacilli present in the milk, but rather that the number was so small the finding of them was practically impossible.

In general, there was a small increase in the percentage of Lactobacillus present from the time the culture was added to the pasteurized milk until the cheese was pressed. This was followed by an increase during the ripening of the cheese so that after 28 days approximately 50 per cent of the bacterial flora of the cheese serum was made up of Lactobacilli.

With series 1 and 2 the conspicuous increase in the Lactobacilli occurred between the 14th and 28th day. With series 3 and 4 the striking increase was between the 3rd and the 14th day, while with series 5 it was during the first 3 days, and with series 6 between the 14th and 28th day.

The Lactobacilli present in the cheese after 112 days of ripening usually made up about 90 per cent of the total number of bacteria present.

Table 8

Percentage of the total flora made up of lactobacilli at various stages during the making and ripening of the cheese.

Series and number of cheese	Per cent of the total organisms represented by lactobacilli in the											
	Milk			Curd				Cheese				
	Original	After adding culture	After ripening the milk	At cutting	At dipping	At milling	At pressing	At 3 Days	At 14 Days	At 28 Days	At 56 Days	At 112 Days
1-1	0	2	1.5	2	2	4	6	8	11	50	73	93
1-2	0	1	1	1	2.5	3	5	6	9	52	77	90
1-3	0	1	1	1	3	2	3	6	11	50	71	89
1-4	0	1	1	2	2	3	2.5	5	10	47.5	72	88
2-1	0	1	1	1	5	5	6	7	10	47	74	91
2-2	0	1	1	1.5	1	3	3	6	7	53	77	92
2-3	0	.5	1	3	3	5	4	7	9	57	68	93
2-4	0	.5	2	2	2	3	4	6	8	52	62	89
3-1	0	.5	1	1	1.5	1	3	6	37	51	69	91
3-2	0	1	1	1	2	2	3	7	28	50	77	90
3-3	0	1	1	1	3	2	4	8	40	52	69	93
3-4	0	1	1	2	2	2	2.5	7	29	53	73	92
4-1	0	0	1	2	2	4	5	5	30	50	65	91
4-2	0	0	1	1	3	3	4	6	27	40	60	89
4-3	0	0	1	1	2	3	5	6	30	52	62	90
4-4	0	0	2	1	2	3	5	7	32	45	58	92
5-1	0	0	2	2	4	6	5	20	29	45	68	91
5-2	0	1	1	2	2	5	10	14	25	50	69	93.5
5-3	0	2	1	2	3	6	12	21	27	52	58	93
5-4	0	1	2	3	5	5	10	23	30	50	64	89
6-1	0	1	2	2	2	6	5	9	12	49	75	91
6-2	0	0	1	1	3	4	5	10	13	53	69	88.5
6-3	0	1	1	3	2	5	6	9	11	51	67	89.5
6-4	0	0	2	2	1	6	6	8	17	48	67	91

DISCUSSION OF RESULTS

The results obtained on the control cheese agreed in general with those reported by Lane and Hammer (58); the flavor scores were slightly lower, the total nitrogen about the same and the amino nitrogen slightly higher. There was a tendency for a sour flavor to develop in the cheese inoculated with L. casei (L4) regardless of the moisture content or method used in the manufacture of the cheese. This result agrees with the findings of Evans, Hastings and Hart (26).

The addition of S. liquefaciens, the unidentified Micrococcus or both of these test cultures did not materially increase the protein decomposition in the cheese. While both cultures are proteolytic and were present in the cheese throughout the experimental period in rather large numbers, the results do not indicate that either of these organisms was of great importance as far as the protein decomposition was concerned. This result is similar to that obtained by Russell (74). The addition of these organisms definitely increased the flavor score of the resulting cheese, which agrees with the results obtained by Lane (56).

The test organisms used in series 3, 4, 5 and 6 were all lipolytic organisms. The inert lipolytic organisms when used did not influence the flavor score of the resulting cheese but did increase the protein decomposition of the cheese as measured by the nitrogen content of the cheese serum. Ps. fragi and the lipolytic acid forming organism both decreased the flavor scores of the resulting cheese but had no material influence on the protein decomposition in the cheese. Ps. fluorescens, A. viscosus and

A. lipolyticum when used as the test organisms had no influence on the flavor development or on the protein decomposition in the resulting cheese. Collins and Hammer (18) found that some of these organisms, Ps. fragi, Ps. fluorescens and A. lipolyticum when introduced into butter produced a rancid flavor in a short time. There was no evidence of a rancid flavor, however, in any of the cheese made with any one of the lipolytic test organisms.

The proteolytic bacteria, that is, S. liquefaciens and the Micrococcus, were present in fairly large numbers in the cheese after 112 days of ripening. This does not agree with the findings of Russell (73) who stated that in the ripening of cheese the peptonizing or casein digesting bacteria are quickly eliminated; nor with Hastings, Evans and Hart (45) who reported that while liquefying and inert bacteria were always present in milk in small numbers, there was no evidence that growth of these organisms ever occurred during the ripening process of the cheese.

The lipolytic cultures used in these experiments all increased greatly in numbers in the cheese during the early stages of ripening, and were always present, although in small numbers, after 112 days. These results are similar to those obtained by Collins and Hammer (18), who worked with some of the same lipolytic organisms in butter. The rancid flavor developed by these organisms in butter did not develop in the cheese.

The acid value of the fat from the experimental cheese was materially affected by the test organisms used. When the test cultures of Ps. fluorescens, A. viscosus, A. lipolyticum or the lipolytic acid forming organism were used there was a material increase in the acid values of the fat in the cheese. The increase in the acid values, however, did not seem to produce any off flavor or influence the flavor score to any appreciable extent

in the cheese.

The microscopic examination showed that as the cheese ripened there was a gradual change in the bacterial flora. In the early stages the Lactobacillus made up a very small percentage of the total number of organisms present. This condition gradually changed so that after the cheese had ripened 112 days the percentage of Lactobacillus was usually around 90 per cent of the total flora in the cheese. These results agree with those reported by Evans, Hastings and Hart (26), who reported that there was a rapid increase in Lactobacilli during ripening followed by a rapid decrease in the numbers of S. lactis.

SUMMARY AND CONCLUSIONS

The work reported involved a study of the effect of certain bacteria on the ripening of Cheddar cheese made from pasteurized milk. All the experimental cheese were compared with control cheese made by adding a pure culture of L. casei (L4) and a butter culture (122) to the pasteurized milk, because, according to the work of Lane and Hammer (58), the addition of certain strains of L. casei to pasteurized milk used for making Cheddar cheese appeared to have a desirable effect on the nitrogenous decomposition, the flavor development and the uniformity of the resulting cheese. These cultures were also used in the milk inoculated with the various test organisms.

1. Within the limits of the study, as imposed by the numbers of cheese made and the scope of the chemical analysis, the inoculation of small amounts of milk cultures of the test organisms into the pasteurized milk appeared to have the following effects on the resulting cheese:

a. S. liquefaciens improved the flavor of the resulting cheese but did not materially influence the nitrogen in the cheese.

b. An unidentified Micrococcus improved the flavor of the resulting cheese but did not materially influence the nitrogen decomposition in the cheese.

c. When both S. liquefaciens and the unidentified Micrococcus were added to the milk the flavor was improved and there was a small increase in the total nitrogen in the cheese serum.

d. An inert lipolytic organism (No. 18) did not influence the flavor development but increased the total nitrogen in the cheese serum.

e. Ps. fragi decreased the flavor score of the resulting cheese, but did not materially influence the nitrogenous decomposition in the cheese.

f. The lipolytic acid forming organism (No. 12) decreased the flavor score of the resulting cheese, but did not influence the nitrogenous decomposition in the cheese.

g. Ps. fluorescens did not materially affect the flavor score or the nitrogenous decomposition in the resulting cheese.

h. A. viscosus did not affect the flavor score or the nitrogenous decomposition of the resulting cheese.

i. A. lipolyticum did not affect the flavor score or the nitrogenous decomposition of the resulting cheese.

2. The total bacterial counts on the cheese, as determined with beef infusion agar, agreed closely throughout the entire ripening period with the counts as determined on tomato juice agar, regardless of the cultures used in making the cheese.

3. In general, the number of bacteria per gram of cheese was highest at 14 days; the maximum count was followed by a rapid decrease to the 28th day and then by a slower but steady decrease to the 112th day of ripening.

4. The cheese made from milk inoculated with the proteolytic test organisms were strikingly higher in numbers of proteolytic bacteria than the control cheese.

5. The cheese made from milk inoculated with lipolytic test organisms

were nearly always high in numbers of lipolytic organisms during the first half of the ripening period, after which the numbers decreased so that at the end of 112 days there were only a few present.

6. The acid values of the fat of the cheese were materially increased when the following test organisms were used: The lipolytic acid forming organism (No. 12), Ps. fluorescens, A. viscosus and A. lipolyticum. The following test organisms did not materially affect the acid values: S. liquefaciens, the unidentified Micrococcus and the inert lipolytic organism (No. 18).

7. Lactobacilli made up only a small percentage of the flora of the very young cheese but as the ripening progressed the flora changed so that, after 112 days, about 90 per cent of the bacteria in the cheese were Lactobacilli.

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