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The effect of certain bacteria on the ripening of cheddar cheese made from pasteurized milk

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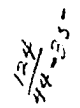
THE EFFECT OF CERTAIN BACTERIA ON THE RIPENING OF CHEDDAR CHEESE MADE FROM PASTEURIZED MILK

BY

C. BRONSON LANE



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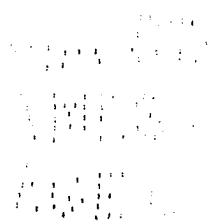

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INTRODUCTION

The chemical changes produced by bacteria during the normal ripening of cheddar cheese and the relation of these changes to the characteristic cheese flavor, have not been definitely established. The complexity of the compounds composing cheese, and the intricate physical properties of the cheese body, and the simultaneous progression of enzymatic and bacterial action in ripening cheese, all tend to make accurate studies of the cheese ripening process very difficult.

In many parts of the United States the milk delivered to cheese factories is frequently of poor quality. Such milk may contain various types of bacteria which not only cause undesirable flavors and textures in the cheese, but which are objectionable from the standpoint of public health. The education of producers in the proper handling of milk and the enforcement of certain quality standards now used in market milk sections, would insure milk of high quality for cheese making purposes. However, present economic conditions in the cheese industry compel the farmer to produce milk at a minimum cost and, as a result, the factors necessary in the production of high quality milk are likely to be neglected or ignored. Pasteurization of milk of poor quality is a logical

procedure to insure cheese of at least fair quality. Experience has shown, however, that cheddar cheese made from pasteurized milk rarely develops the full, characteristic flavor normally found in raw milk cheese of good quality. The pasteurized milk cheese, in addition to its usual lack of flavor, generally requires an extended ripening period to insure a normal degree of protein breakdown.

The destructive effect of pasteurization on certain inherent milk bacteria necessary for bringing about normal cheese ripening, probably accounts in part for the undesirable characteristics of pasteurized milk cheddar cheese. It is likely, therefore, that the addition of certain strains of bacteria to pasteurized milk used for cheese making, would produce desirable chemical changes in the cheese, with a corresponding improvement in flavor. The addition of special bacterial cultures to cheese in this manner also opens up many possibilities in connection with the development of new and desirable flavors in cheese of the cheddar type.

OBJECTS

The objects of the work dealing with the effect of certain bacteria on the ripening of cheddar cheese made from pasteurized milk, were as follows:

1. To develop a suitable method for obtaining juice or serum, to be used for analytical purposes, directly from cheddar cheese. The methods generally employed in determining the various soluble nitrogenous products in cheese, by analysis of water extracts of cheese, have not been entirely satisfactory.

2. To study the effect of adding 10 per cent raw milk to pasteurized milk used for making cheddar cheese on the nitrogen distribution in the cheese and on the flavor. It is probable that certain types of bacteria, important in cheese ripening, are destroyed by pasteurization, and that the addition of small amounts of raw milk to pasteurized milk used for making cheese may improve the bacterial flora from the standpoint of cheese ripening.

3. To study the effect of adding various bacteria to pasteurized milk used for making cheddar cheese on the nitrogen distribution in the cheese and on the flavor. The addition of milk cultures of various bacteria to pasteurized milk used

for making cheddar cheese opens up many possibilities from the standpoints of more rapid ripening and the development of desirable flavors of unusual type in cheese.

GENERAL PROCEDURE

The effect of certain bacteria on the ripening of cheddar cheese made from pasteurized milk was studied with 13 series of cheese. Each series contained three cheese, manufactured at the same time from equal portions of a single lot of milk. Usually one cheese was made from raw milk, one from pasteurized milk, and one from pasteurized milk plus a milk culture of a test organism, or 10 per cent raw milk. In some cases, however, all three of the cheese in a series were made from pasteurized milk, and each of two of the portions of milk was inoculated with a milk culture of a test organism. Changes in the nitrogen distribution in the cheese were determined by chemical analyses of cheese juice at various intervals during ripening. At the same periods, the cheese were examined organoleptically and scored for flavor.

HISTORICAL

PART 1. Methods of Securing Soluble Nitrogen Compounds From Cheese

The degree of ripeness in cheddar cheese is usually determined chemically by a study of its soluble nitrogen content. Up to the present time, the most widely used method of obtaining the soluble nitrogen from cheese has been that of water extraction. This method, which consists essentially of shaking cheese with warm water, filtering the mixture, and then analyzing the filtrate for products of protein hydrolysis, was first used with cheddar cheese in 1902 by Van Slyke and Hart (66). Since then, the water extraction method has been employed by practically all investigators who have worked with the soluble nitrogen constituents of cheddar cheese. Variations in the water extraction method were made by Whitehead (73) and later by Allen (2). Both of these investigators extracted the fat from cheese with ethyl ether, and then treated the residue with warm water.

Barthel, Sandberg, and Haglund (6) explained and demonstrated how inaccurate results may be obtained when the water extraction method is used. They stated that the

addition of large quantities of water upsets, to some extent, the physical-chemical equilibrium existing in the cheese. A decrease of the salt concentration takes place which enables certain casein decomposition products to become more soluble, while other substances, having the character of globulins, are precipitated. This change in the solubility equilibrium of the nitrogenous compounds, according to the data reported by Sandberg, Haglund, and Barthel (51) accounts for the unreliable results obtained by chemical analysis of a water extract of cheese. These investigators further explained that the nitrogenous materials to be extracted per unit weight of cheese may vary due to the lack of uniformity in the percentages of fat existing in different lots of cheese.

Since no standardized water extraction method has been adopted, there have been variations in the procedures used by different investigators. van Dam (58) pointed out that variations in the temperature of the water used, and differences in the methods of shaking the cheese and water mixtures, have considerable effect upon the amounts of soluble nitrogen found in the water extracts.

With these sources of error in view, Barthel, Sandberg, and Haglund (5) developed a method for obtaining the soluble nitrogen materials from cheese. They found that by submitting a mixture of finely divided cheese and sand to relatively high pressures, a cheese juice or serum was obtained which could be

analyzed directly for various forms of soluble nitrogen. Sandberg, Haglund, and Barthel (51), after a comparative study of the water extraction and direct methods of securing the soluble nitrogen from cheese, concluded that more accurate results are obtained by analysis of cheese juice than by analysis of a water extract of cheese.

PART 2. Precipitation of Proteins and Protein Decomposition Products

Numerous chemicals have been used to precipitate proteins and individual protein decomposition products from blood, milk, water extracts of cheese, and other materials.

As early as 1877, Ritthausen (46) precipitated milk proteins with cupric hydroxide in the quantitative determination of carbohydrates in milk. Tannic acid, as a milk protein precipitant, was used by Sibilien (54) and later by Palmer and Scott (43) in the chemical analysis of milk. Wasteneys and Borsook (70) used tannic acid to separate peptones from an enzymatic hydrolysate of proteins. Proteins and meta-proteins were successfully precipitated by them with trichloracetic acid. In studying the nitrogen distribution in Kingston cheese by chemical analyses of water extracts of cheese, Eagles and Sadler (10) also used tannic and trichloracetic acids as well as phosphotungstic acid to

precipitate proteins and individual protein hydrolysates.

In 1897, Rideal and Stewart (45) used chlorine water to separate proteins from water extracts of meat, while Allen and Searle (1) used bromine water for the same purpose. Van Slyke and Hart (65) precipitated peptones from water extracts of cheddar cheese with bromine water. Other chemicals used by him to precipitate peptones were tannin with sodium chloride, and phosphotungstic acid.

In 1901, Simon (56) used trichloracetic acid to separate milk proteins. He pointed out that a high concentration of this acid in the milk-acid mixture is necessary to obtain complete precipitation. Bock (7), in 1916, compared the merits of trichloracetic acid, ethyl alcohol, and collodial iron for precipitating blood proteins. He concluded that, of the three reagents, trichloracetic acid gives the most complete precipitation. Later, in 1919, Greenwald (19) determined that trichloracetic acid is more desirable for precipitating blood proteins than methyl alcohol, because of the higher concentration of intermediate products in the trichloracetic acid filtrates.

Trichloracetic acid, tannic acid, and ethyl alcohol were compared by Moir (40) as precipitants for milk proteins. He concluded that the proteins are more completely removed with trichloracetic acid. Hiller and Van Slyke (30) studied the efficiencies of a number of precipitants, including

trichloracetic acid, using Witte peptone as a representative mixture of protein and protein decomposition products. They found that, of the reagents used, tungstic acid and picric acid are the most complete precipitants for protein intermediate products with the exception of amino-acids, while trichloracetic acid precipitates proteins only. They further discovered that ethyl alcohol precipitates the same materials as tungstic and picric acids, but is not so complete in its action. Trichloracetic acid was also used by Roe and Kahn (47) in the preparation of protein-free blood serum in the colorimetric determination of blood calcium; by Sanders (52) for preparing protein-free filtrates in the determination of calcium, magnesium, and acid-soluble phosphorus in milk; by Kelly (34) for precipitating proteins from water extracts of cheddar cheese; and by Sanders (53) as a precipitant for milk proteins.

The mechanism of milk protein precipitation by trichloracetic acid was explained by Loeb (36). He showed that the solubility of casein in certain acids is directly proportional to the swelling of the casein molecule, and that this characteristic is dependent upon the Donnan equilibrium. A decreasing solubility is shown in solutions of the following acids in the order named: phosphoric, hydrochloric, nitric, sulphuric, and trichloracetic.

Phosphotungstic acid, as a precipitant for proteins and protein decomposition products, was used in 1902 by Van Slyke and Hart (65) to separate peptones from a water extract of cheddar cheese. He concluded that phosphotungstic acid completely precipitates peptones and some amino-acids. Van Slyke (59) later found that the addition of phosphotungstic acid to a protein hydrolysate separates the amino-acids into two fractions; the "bases" (histidine, arginine, lysine, and cystine) which are precipitated, and the other acids (primary acids) which are not precipitated. Domogalla (9) and others used phosphotungstic acid to precipitate proteins, peptones, and diamino-acids from certain lake waters. Kelly (34) also used phosphotungstic acid for the separation of various protein intermediate products from extracts of cheddar cheese.

In 1919, Folin and Wu (16) developed a method for removing the proteins from blood using sodium tungstate. This method was later modified by Haden (21).

Picric acid was employed to precipitate proteins and protein intermediate products by Folin (14) in the determination of ammonia in blood, and later by Greenwald (20) to estimate the inorganic constituents of blood and of other physiological materials.

In 1912, Van Slyke and Myer (62) used 95 per cent ethyl alcohol to precipitate proteins and some protein intermediate products in order to determine the amino-acid content

of blood. Both ethyl and methyl alcohols were employed by Wolf (74) to precipitate proteins and intermediate products in the estimation of non-protein and urea nitrogen in blood. He found that ethyl alcohol precipitates slightly more protein material than methyl alcohol. Ethyl alcohol was used by Welker and Williamson (72) to crystallize hemoglobin, while Dennis and Minot (8) demonstrated the use of ethyl alcohol, methyl alcohol, copper sulphate, and copper acetate in separating proteins and protein intermediate products for the determination of the non-protein nitrogenous constituents of milk. Allen (2) also used ethyl alcohol to precipitate all of the nitrogen compounds, with the exception of amino-acids, from water extracts of cheddar cheese.

Less commonly used precipitants are aluminum hydroxide, mercuric chloride, and phosphoric acid. Aluminum hydroxide was employed by Welker and Marsh (71) in the determination of lactose in milk. Gettler and Baker (17) used mercuric chloride to separate proteins in the chemical and physical analysis of blood, while Folin (13) precipitated blood proteins with phosphoric acid to determine the presence of creatinine and creatine.

PART 3. Enzymatic, Chemical, and Bacteriological Studies of Cheddar Cheese Ripening

The development of the characteristic flavor in cheddar cheese during ripening and the agents which produce this flavor have been the subject of considerable chemical and bacteriological research.

Among the early investigators were Babcock, Russell, Vivian, and Hastings (4), and Babcock, Russell, and Vivian (3) who, in 1899 and 1900, determined the proteolytic changes produced by galactase and rennet during the ripening of cheddar cheese. Rogers (48) noted the presence of enzymes in partially ripened and ripened cheddar cheese, while Van Slyke and Hart (67) concluded that the use of more than a normal amount of rennet in the manufacture of cheese increases the amount of soluble nitrogen products during the ripening period. Later investigations by Barthel, Sandberg, and Haglund (6) demonstrated the existence of active rennet in the serum of several varieties of hard and semi-hard cheeses. They found that the serum obtained from well ripened cheeses contained more rennet than the serum obtained from cheeses during the early stages of ripening.

In 1910, Suzuki, Hastings, and Hart (57) were unable to isolate, from cheddar cheese, enzymes which produced

lactic acid or volatile fatty acids from lactose, and concluded that these acids, normally found in cheese, are not formed by enzyme action, but rather by bacteria. In studying the effect of enzymes upon cheese proteins, Van Slyke, Harding, and Hart (69) made cheese with milk to which four or five per cent of chloroform had been added. In the chloroformed cheese they found less soluble nitrogen than in normal cheese, and relatively large amounts of albumoses and peptones in proportion to amides. From their experiments these investigators concluded not only that enzymes are necessary in the proper ripening of cheese, but that bacteria are indispensable in obtaining normal protein breakdown. Evans, Hastings, and Hart (12) also used chloroformed milk to study the effect of enzymes upon cheese ripening. They were unable to find any volatile acids in the chloroformed cheese, from which they concluded that inherent milk enzymes are not capable of producing these acids in any appreciable quantities. Price (44), working on the asumption that some inherent milk enzymes important in cheese ripening are destroyed by pasteurization, added small quantities of well ripened cheese to pasteurized milk used for cheese making. He found that the inoculated milk produced cheese having disagreeable and unnatural flavors.

As early as 1902, Van Slyke and Hart (66) concluded that

the first step in the ripening of cheddar cheese is probably a peptic digestion of unsaturated para-casein lactate. They noted that as cheese ripens, there is a decrease in the unsaturated para-casein lactate content with a corresponding increase in water soluble nitrogen products. Later, Van Slyke and Hart (68), in studying the individual proteolytic compounds from cheese, obtained relatively large amounts of para-nuclein from young cheese; small amounts of lysatine, histidine, and lysine from middle-aged cheese; and traces of putresine from old cheese. As a result of their investigations, they believed that the flavor found in well ripened cheddar cheese is due in a large part to the formation of some of these products, along with the conversion of primary amino compounds to secondary amino compounds. Conditions affecting the rate or proteolytic decomposition in cheddar cheese were also studied by Van Slyke and Hart (67). In cheddar cheese ripening studies, Van Slyke and Bosworth (64) determined some of the first chemical changes taking place during ripening. They found that insoluble protein, as represented in fresh curd, rapidly changed into protein soluble in warm, dilute salt solution. This brine soluble protein soon changed to a protein which was insoluble in the salt solution, but soluble in water.

In working with flavoring materials from cheddar cheese,

Suzuki, Hastings, and Hart (57) discovered that fatty acids, alcohols, and esters are important flavor contributants. They found that up to the age of about three months, cheese contained acetic and propionic acids in increasing amounts; that after about three months these acids decreased, while butyric and caprioic acids increased. By distilling portions of ground cheese with steam, these investigators obtained a "flavor solution" which had a typical cheese aroma and contained alcohols and esters.

In 1896, Russell (49) made a study of the numbers of bacteria in cheddar cheese at different ripening periods. The periods included: (a), period of initial decline, in which there is a marked falling off in numbers of bacteria for a few days after the manufacture of the cheese; (b), period of increase, in which there occurs a rapid increase in growth until there may be millions of bacteria per gram of cheese; and (c), period of final decline, in which there is apparent a gradual diminution in the numbers of bacteria until a point is reached where relatively few living bacteria remain. Russell considered that the lactic acid bacteria are the most important group in the ripening of cheese, since the greatest growth period of these organisms coincides with the change in the physical condition of the curd and the breaking down of the proteins.

The only group of bacteria constantly found in large numbers in cheddar cheese by Harding and Prucha (25) was the Bacterium lactis acidi group, while Hastings, Evans, and Hart (28) were able to find, in all cheddar cheese which they studied, large numbers of bacteria of both the Bacterium lactis acidi and Bacillus bulgaricus groups. Evans, Hastings, and Hart (12) found a rapid increase in numbers of the Bacillus bulgaricus group following a sharp decrease in the numbers of Bacterium lactis acidi organisms. These same investigators also isolated small numbers of chromogenic cocci and liquifying bacteria from cheddar cheese. When several types of cocci, isolated from cheddar cheese were grown in milk by Hart, Hastings, Flint, and Evans (27), large quantities of volatile acids, especially acetic acid, were produced, while several strains of Bacterium casei isolated from cheddar cheese produced both acetic and propionic acids in milk. One type of coccus was found to form comparatively large amounts of alcohols and esters which, according to these and other investigators, are bodies that contribute in large degree to the flavor of cheddar cheese.

Evans, Hastings, and Hart (12) studied the groups of bacteria which might be concerned in the production of cheddar cheese flavor. These included; first, the Bacterium lactis acidi group; second, the Bacterium casei group;

third, the Streptococcus group; and fourth, the Micrococcus group. These investigators concluded that organisms of the Bacterium casei group are responsible for the pungent taste that develops late in the ripening periods of both raw milk and pasteurized milk cheese, but that the addition of culture composed of Bacterium casei organisms to pasteurized milk used for cheese making, produces sour cheese. Evans, Hastings, and Hart (12) were unable to obtain any cheddar cheese flavor when cultures composed of Bacterium lactis acidi organisms alone were used in the manufacture of pasteurized milk cheese, but when cultures made from certain streptococci isolated from raw milk cheese were added, in addition to the lactic culture an increase in cheese flavor was noted. Evans (11) later isolated two strains of cocci from cheddar cheese, Streptococcus x and Streptococcus kefir, which produced comparatively large amounts of acetic acid when grown in milk. An improvement in flavor and a hastening of the protein breakdown occurred in cheese made from pasteurized milk inoculated with these organisms.

In studies on the types of bacteria in commercial cheddar cheese, Hucker (32) found that the better grades of cheese contained a flora differing widely from that of the poorer grades. Streptococcus lactis and Lactobacillus types predominated in the better grades of cheese, while the poorer grades contained many spore-forming, Gram negative rods.

The proportion of cocci other than Streptococcus lactis did not vary in cheese of differing qualities.

In bacteriological and chemical studies of cheddar cheese made from raw and pasteurized milk, Moir (40) showed that the pasteurization of milk for cheese making reduces the amounts of ultimate protein decomposition products which form in cheese during the ripening. Further investigations of pasteurized milk cheddar cheese by Moir (41) indicated that pasteurization modifies milk so that the lactic acid flora is able to develop more rapidly in pasteurized milk than in raw milk. One of the results of the altered acid development in pasteurized milk cheese is the production of cheese having abnormally low pH values, which modify the course of the ripening and flavor production by bacteria.

In 1926, Hucker and Marquardt (33) studied the effect of several streptococci upon the flavor of cheddar cheese made from pasteurized milk. They concluded that Streptococcus paracitrovorus, when added to pasteurized milk, improves the flavor of the cheese, while Streptococcus citrovorus has no effect upon the flavor. Cheese made with cultures of Streptococcus lactis was found to be similar to that made with commercial cultures, while certain strains of proteolytic streptococci, when used as cultures, produced bitter, unpalatable cheese. Similar researchers by Hansen, Bendixen, and Theophilus (24) indicated that cheese made with

Streptococcus citrovorus or Streptococcus paracitrovorus alone as starters, becomes bitter and has a poor body, while cheese made with Streptococcus lactis as starter, lacks flavor but has a good body and texture. Whitehead (73) found that representative strains of organisms of the colon group, when inoculated into milk immediately before starting the process of cheese manufacture, have a deleterious influence on the flavor of cheddar cheese, even when the inoculation is not sufficient to produce gas holes in the cheese.

In determining the chemical changes which take place in cheddar cheese during ripening, Kelly (34) found that the proteins in cheese made with strains of Streptococcus lactic or Streptococcus cremoris as cultures, undergo changes similar to those found in cheese made with commercial cheese cultures. Kelly (35) later concluded that acid production is the chief function of cheese cultures, and that the culture has little direct effect upon the flavor and aroma of the cheese at the time of marketing.

METHODS

PART 1. Manufacture, Ripening, and Scoring of Cheese

a. Source and treatment of milk

The milk used for making the experimental cheese came from three dairies and varied in quality. Milk designated as milk A came from the Iowa State College Holstein herd. It contained about 3.4 per cent fat and was produced under good sanitary conditions; the bacterial counts were usually under 20,000 per cc., according to the standard plate method. Milk designated as milk B or milk C came from mixed herds within a few miles of the Iowa State College. It contained from 3.7 to 4.0 per cent fat and was produced under average sanitary conditions; the bacterial counts were from 100,000 to 500,000 per cc., according to the standard plate method.

In each series of cheese, 381 pounds of milk were used. The milk was thoroughly mixed in a steam-jacketed pasteurizing vat equipped with a mechanical agitator. When one raw milk cheese and two pasteurized milk cheese were made, 127 pounds of milk were drawn from the vat, and the remaining milk was heated to 145° F. for 30 minutes, after which it was cooled and divided into two equal portions. When all pasteurized

milk cheese was made, the 381 pounds of milk were pasteurized, and divided into three equal portions. In a few cases, when cheese was made from raw milk and also from 90 per cent pasteurized milk plus 10 per cent raw milk, 139.7 pounds of milk were drawn from the pasteurizing vat prior to heating, 12.7 pounds of which were later added to 114.3 pounds of pasteurized milk. Each portion of milk was placed into a 40 gallon cheese vat.

b. Manufacture and ripening of cheese

The portions of milk in the cheese vats were inoculated with one and one-half or two per cent of a commercial cheese culture. The commercial cultures used were of the type that produce acid rapidly, and at the time of their inoculation into the milk, they contained from 0.8 to 0.9 per cent acid, calculated as lactic acid. In addition to the commercial culture, some of the vats of milk were inoculated with various percentages of a milk culture of a test organism. In experiments in which three vats of cheese were made from pasteurized milk, two of the portions of milk were inoculated with different strains of test organisms. In the experiments in which one vat of cheese was made from raw milk, and two vats of cheese from pasteurized milk, one of the two portions of pasteurized milk was inoculated with a strain of a test organism or 10 per cent raw milk.

After the temperature of the milk had been adjusted to 86 degrees F., commercial cheese color was added at the rate of one ounce per 1,000 pounds of milk, and commercial rennet at the rate of three ounces per 1,000 pounds of milk. About seven minutes after the addition of the rennet, the milk began to coagulate, and 20 to 25 minutes later, the curd was ready to cut; three-sixteenth inch knives were used for this purpose.

The curd was cooked at 104° F. until the acidity of the whey reached 0.15 to 0.16 per cent acid and the desired firmness of curd was obtained. After dipping, the curd was cheddared until 0.5 to 0.6 acidity in the whey was reached or until the curd produced one-half to three-quarter inch threads on the hot iron. After milling, the curd was forked for about one-half hour and three per cent cheese salt added. At least three-quarters of an hour was required to completely dissolve the salt, after which time the curd was rinsed with scalding water and then placed in the hoops.

The cheese was pressed for about 18 hours under continuous pressure and then placed in a curing room at 35 to 39° F. One longhorn cheese weighing about 12 pounds was obtained from an experimental vat of 127 pounds of milk.

Each vat of milk, regardless of the treatment prior to the addition of rennet, was made into the best cheese possible. To obtain cheese of high quality from both raw and pasteurized

milk, a different time schedule for the various processes of manufacture was necessary with the cheese in a series.

c. Preparation of special cultures

Special cultures of bacteria were prepared from flasks of sterile milk by inoculating each flask with a test organism. The organisms used included several strains of Lactobacillus casei, and one strain each of Aerobacter oxytocum, Streptococcus liquefaciens, Streptococcus paracitrovorus, and an unidentified Micrococcus. An abundant growth of the test organisms in the cultures was assurred before their inoculation into the pasteurized milk used for cheese making. The cultures of L. casei, A. oxytocum, and S. liquefaciens, were incubated at 98° F. for about 48 hours, prior to using, while the cultures of S. paracitrovorus and the Micrococcus were placed at room temperature for eight days. All of the strains of organisms used for special cultures were isolated from dairy products at the Dairy Industry department of Iowa State College.

d. Examination and scoring of cheese

The cheese was examined and scored for flavor at regular intervals during ripening by Professor E. F. Goss of the Dairy Industry department of Iowa State College, on the basis

of a perfect score of 45. The ripening periods of the experimental cheese did not exceed four months, since by far the greater proportion of commercial cheddar cheese is ripened only a few months before marketing. The experimental cheese varied in type and intensity of flavor to such an extent that numerical scores alone did not give sufficient information regarding the flavor of the cheese; therefore, a description of the flavor of each cheese at several periods during the ripening is included along with the numerical flavor score.

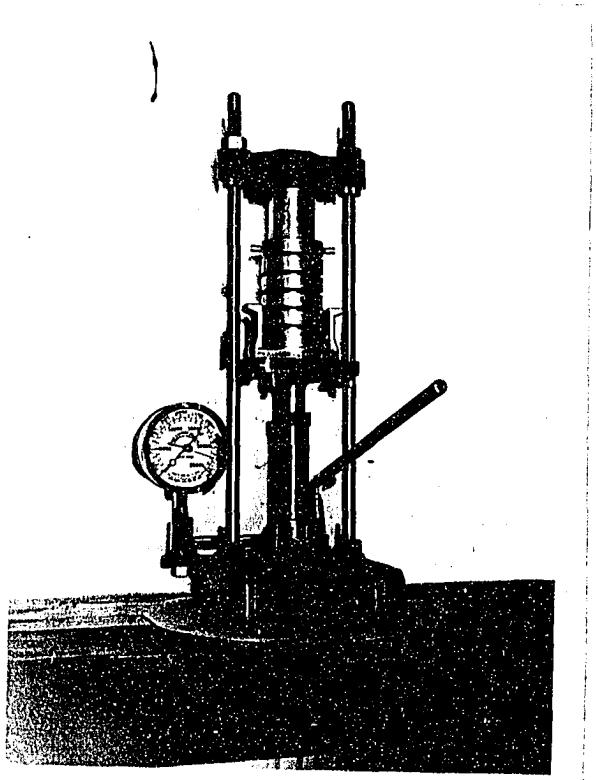
PART 2. Studies on the Nitrogen Distribution in Cheese by Chemical Analysis of Cheese Juice

a. Method of obtaining cheese juice*

The method used to obtain cheese juice was that developed by Barthel, Sandberg, and Haglund (5). It consists essentially of submitting a mixture of finely divided cheese and sand to relatively high pressures in a hydraulic laboratory press.

To extract the juice from cheddar cheese, 400 grams of cheese were first cut into thin shreds with a small grater of the type commonly used by housewives to grate soap. The shreds thus obtained were mixed by hand on parchment paper with 800 grams of fine sea sand. In order to obtain good results it was important to procure not only the proper ratio between the weights of the cheese and the sand, but also the correct

*The development of the method used to obtain cheese juice, so that it is applicable to cheddar cheese, is discussed under RESULTS, Part 1.



Hydraulic laboratory press

size of sand particles; with the sand used, about 80 per cent of the volume did not exceed 0.3 mm. in diameter. The sand gave a certain stability to the mixture, and at the same time acted as a filter by allowing the juice to escape during the pressing.

A hydraulic laboratory press with an iron cylinder attachment was used to extract the juice. (see illus.) It was 36 inches high, weighed 115 pounds, and exerted any force up to 20,000 pounds per square inch. For each extraction the hollow press cylinder attachment (two and one-quarter inches in diameter) was entirely covered on the inside with a closely woven linen cloth, and the mixture of cheese and sand placed into the cylinder between felt filter pads. The cylinder was set on an iron plate at the base of the press, and as the pressure was slowly applied by the pump handle, the cheese liquid was forced out of the cylinder, through clearance spaces, on to a grooved outlet around the outer edge of the plate, from where it dropped into a beaker. The cheese liquid, containing the cheese juice, began to flow from the cylinder when the pressure reached about 3,000 pounds per square inch. The pressure was slowly increased until the desired amount of liquid was obtained, although it was rarely necessary to use pressures exceeding 10,000 pounds. The hard cylindrical cake remaining in the press cylinder was discarded.

The extracted liquid was composed of amber cheese oil and of a viscous fluid resembling rich cream or partially melted butter. The creamy fluid, which contained the cheese juice, readily separated from the oil when the mixture was placed into a separatory funnel. Cheese juice practically free from fat, was then obtained by filtering the creamy fluid through paper. It was brownish-yellow in color and opalescent.

b. Chemical analysis of cheese juice

Chemical analyses of the juice of each cheese were made after approximately one, five, ten, and fifteen weeks of ripening. The analyses included determinations of total nitrogen, amino nitrogen, and various fractions of proteins and protein decomposition products which were soluble or insoluble in trichloracetic acid, ethyl alcohol, phosphotungstic acid, and tungstic acid. The procedures used for the quantitative determinations of the various nitrogen forms in cheese juice were as follows:

Total nitrogen

One cc. of juice was analyzed by the Kjeldahl method.

Amino nitrogen

One cc. of juice was analyzed by the Van Slyke method (60).

Trichloracetic acid soluble and insoluble nitrogenous fractions

One cc. of juice was treated with 44 cc. of water and 5 cc. of a 20 per cent aqueous solution of trichloracetic acid. After standing 8 to 10 hours at room temperature, the mixture was filtered and the precipitate washed with a trichloracetic acid solution containing 45 cc. of water and 5 cc. of 20 per cent aqueous trichloracetic acid. The solution used for washing the precipitate contained the same concentration of reagent as that used for the precipitation.* The filtrate and the precipitate were analyzed separately for nitrogen by the Kjeldahl method.

Ethyl alcohol soluble and insoluble nitrogenous fractions

One cc. of juice was treated with 9 cc. of water and 85 cc. of 95 per cent ethyl alcohol. After standing 8 to 10 hours at room temperature, the mixture was filtered and the precipitate washed with an ethyl alcohol solution containing 10 cc. of water and 85 cc. of 95 per cent ethyl alcohol. The nitrogen content of the precipitate and the filtrate were determined by the Kjeldahl method.

Phosphotungstic acid soluble and insoluble nitrogenous fractions

One cc. of juice was treated with 49 cc. of water, 15 cc. of 15 per cent (by volume) sulphuric acid, and

*This relationship was maintained in washing the various precipitates.

10 cc. of 10 per cent aqueous sodium tungstate. After standing 8 to 10 hours at room temperature, the mixture was filtered and the precipitate washed with a tungstic acid solution containing 50 cc. of water, 15 cc. of 25 per cent sulphuric acid, and 10 cc. of 10 per cent aqueous sodium tungstate. The filtrate and the precipitate were analyzed separately for nitrogen by the Kjeldahl method.

RESULTS

PART 1. The Development of the Method Used to Obtain Juice From Cheddar Cheese

In securing material to be used in chemical studies on the products of protein decomposition in cheese, the method of Barthel, Sandberg, and Haglund (5), which consists of subjecting a mixture of sand and finely divided cheese to relatively high pressures, appeared to have such a definite advantage over the method of extracting cheese with water that its used was attempted.

The press employed was secured from the Carver Company of New York City. It was 36 inches high, weighed 115 pounds, and gave any desired pressure up to approximately 20,000 pounds per square inch. The pressure exerted was registered on a meter so that the operator could note at any time the exact force being applied to the test material. Four loose attachments were used in the extraction of cheese juice. These consisted of, (a) a flat metal plate, 8 inches square and 1 inch thick, having a grooved outlet around the outer edge, (b) a hollow metal cylinder, 7.5 inches high, 4.5 inches in diameter, and containing a number of small holes which

allowed the escape of liquid squeezed from the test material, (c) a solid metal cylindrical plunger, 7.5 inches high, which exactly fitted into the hollow portion of the iron cylinder, and (d) a solid metal base for the hollow metal cylinder.

In preparing and operating the press for the squeezing of juice from cheese, the hollow press cylinder was first placed on the iron base. A tightly fitting felt pad was then put into the bottom of the press cylinder and the inner sides of the cylinder were covered with cloth. The mixture of sand and cheese was transferred to the cylinder, another felt pad was put on top of the mixture, and the plunger was set on top of the upper felt pad. The complete setup was then placed on the flat metal plate which rested on the ram of the press. Pressure was applied by the pump handle, which slowly forced up the ram so that the top of the solid metal plunger came in contact with the rigid top of the press, thereby exerting a force on the test material.

To prepare a satisfactory cheddar cheese and sand mixture, it was necessary to cut or shred the cheese. The trials with a small meat grinder were not successful, since the pieces of cheese matted together as they came from the grinder, and did not form small masses of cheese such as are needed for best results. A small grater, of the type commonly used by housewives to grate soap, produced thin, flaky shreds of about one inch in length which held their individual shapes. The shreds

obtained in this manner were of satisfactory size and shape to be mixed with sand readily. Barthel, Sandberg, and Haglund (5), pointed out the importance of the size of the sand used in the cheese and sand mixture and showed that the sand added stability to the mixture and also acted as a filter by allowing the cheese liquid to escape during the pressing. The size of the sand was also important in obtaining juice from cheddar cheese. At first a coarse sand was employed but this type of sand caused the cheese and sand mixture to push out through the cloth when pressure was applied. Although better success was obtained with sand of a smaller size, the best results were secured when a very fine sand was used; about 80 per cent of the sand passed through a sieve containing openings 0.3 mm. square.

After the cheese and the sand had been satisfactorily prepared for mixing, it was important to obtain the correct proportion between them. Barthel, Sandberg, and Haglund (5), suggested a ratio of one part of cheese to two parts of sand by weight, for obtaining maximum amounts of juice from several varieties of cheese. When a mixture of one part of cheddar cheese to three parts of sand by weight were used, little or no juice was obtained. A mixture of one part of cheese and one part of sand was also unsatisfactory, as the cheese and sand came out through the cloth during the pressing. The most satisfactory proportions by weight proved to be one part of

cheese to two parts of sand; in the actual operation 400 grams of cheese and 800 grams of sand were employed. The cheese and sand were mixed by hand on parchment paper; the sand adhered to the cheese particles and formed a coating on the cheese. The resulting mixture felt moist and was greyish yellow in color.

The type of cloth used to line the inside of the hollow cylinder was given considerable attention. Three types were tried, - muslin, canvas, and linen. A muslin lining proved unsatisfactory since it allowed the escape of the cheese and sand mixture during the pressing. Although the canvas did not have this fault, it absorbed too much juice, and therefore was not practical. The most satisfactory cloth tried was a heavy, high quality linen. This was cut into pieces measuring six and one-half inches by twelve inches. After the hollow press cylinder was set on its base, one of the cloth pieces was folded around the inside of the hollow cylinder. Some overlapping was allowed in order to make certain that no part of the inside of the cylinder would be left uncovered. The cloth was not allowed to wrinkle, for folds thus formed permitted the escape of the cheese and sand mixture when pressure was applied. The circular felt pads, one of which was placed against the base supporting the cylinder and the other of which was placed on top of the cheese and sand mixture, were necessary to prevent the escape of the cheese and sand mixture

during the pressing. After the cloth and lower pad were properly adjusted, the cheese was placed in the cylinder and packed with the end of a test tube.

In order to obtain satisfactory results, it was necessary to apply the pressure slowly and evenly. In the early trials the pressure was applied too quickly (a pressure of 10,000 to 12,000 pounds being reached in the course of an hour) and as a result all oil and no juice was obtained in some cases. An attempt was made to overcome this difficulty by operating the press in a cooler at a temperature of 39° F. Under these conditions oil alone was obtained. In some cases, when high pressures were quickly applied, the cheese and sand mixture was forced through the press cloth and no juice was secured. Best results with ripened cheese were obtained by applying the pressure very slowly. During the first hour the pressure was increased from 0 to 4,000 pounds per square inch; during the second and third hours it was increased from 4,000 to a maximum of 12,000 pounds per square inch. Liquid began to flow through the clearance holes in the cylinder when a pressure of about 2,000 pounds had been reached. Oil usually came first followed by a mixture of oil, cheese juice, and a yellow viscous liquid which was later found to be composed of cheese juice and solid protein material. It was necessary to increase the pressure gradually and not make rapid changes in the pressure, for in the latter case, little or no juice

was obtained. The liquid secured from the cheese did not drain well so that it was removed from the plate and placed in a beaker several times during the pressing period. If the liquid was allowed to remain on the plate for a considerable period, evaporation took place which affected the results of subsequent analyses of the cheese juice. Once in the beaker, the oil in the juice formed a layer over the top of the liquid and prevented evaporation.

In the attempts to improve the procedure used in securing the cheese juice it was found that the juice was obtained from the cheese more easily if the cheese were allowed to stand several hours at room temperature before shredding was begun. Usually when cheese directly from the cooler was pressed, the cheese shreds pushed out through the cloth. The rigidity of the cold cheese probably facilitated its escape through the cloth.

Two methods were used in separating the cheese juice and the viscous yellow liquid containing some of the cheese juice, from the oil. The first method consisted of centrifuging the liquid squeezed from the press for several minutes. After the centrifuging, the oil formed a clear layer at the top of the centrifuge tube, while some cheese juice and some viscous yellow liquid formed the bottom layer. This method of separating the cheese juice from the oil was not entirely successful when particles of protein were present in the

mixture, since during centrifuging the particles were thrown to the bottom of the centrifuge tube with such force that they formed a hard mass, which prevented the removal of the juice through the stop-cock. The second and better method for separating the cheese juice and viscous yellow liquid from the oil, consisted of gravimetric separation in a 200 cc. separatory funnel. After the liquid squeezed from the cheese was placed in the separatory funnel, 20 to 60 minutes were required for the complete separation of the oil. In some cases an emulsion was formed by the oil and viscous yellow liquid, and this prevented separation. The difficulty was overcome by placing the funnel containing the material from the cheese in a water bath at 95° F. and shaking lightly for several minutes. To obtain the pure cheese juice, the bottom layer of the material in the separatory funnel was filtered through a fluted filter paper in a long-stemmed funnel. Evaporation of juice during the filtration was prevented by covering the filter paper with a watch glass, and by arranging the apparatus so that the funnel stem entered a corked collection tube through a hole in the cork. About two hours were required to complete the filtration. The heavy yellow precipitate was discarded when the filtration was complete. The collection tube containing the juice was placed in ice water until the juice was analyzed. The pure juice was brownish yellow and opalescent; it tasted very salty and had

a characteristic cheddar cheese odor. A greyish white precipitate formed when a small quantity of juice was diluted with water.

The age of the cheese proved to be an important factor in determining the yields of juice, and the time required to obtain the juice. Very young cheese, that is cheese up to two weeks old, yielded juice very readily. Only about 5,000 pounds pressure in the course of one hour were necessary to obtain large amounts. The fluid obtained was opalescent and brownish yellow in appearance, and contained only traces of oil. About 25 cc. of pure juice per 400 grams of cheese were obtained from the very young cheese. The juice from older cheese was mixed with considerable oil when it came from the press. Juice was secured from cheese one to two months old only with considerable difficulty, and about three hours were generally required to obtain relatively small amounts, for example about 10 cc. per 400 grams of cheese. However, at about three months of age and thereafter, juice was again obtained without difficulty. Cheese aged from three to five months yielded about 20 cc. of juice per 400 grams of cheese.

The moisture content and quality of the cheese were also factors in determining the yields of juice. Amounts of juice larger than normal were generally obtained from high moisture cheese without difficulty. Amounts larger than normal were

also obtained from cheese having certain defects, such as bitterness, sourness, and a weak body.

During the development of the method used to obtain the juice from cheddar cheese, trials were made with Roquefort and Swiss types of cheese. By employing the same methods used with cheddar cheese, little difficulty was encountered in obtaining relatively high yields of juice from them. Upon the application of pressures up to 10,000 pounds per square inch during the course of one hour, 25 to 35 cc. of juice per 400 grams of Roquefort type cheese and 20 to 25 cc. of juice per 400 grams of Swiss type cheese were obtained. Little or no oil was present in the liquid pressed from these cheeses.

The results of certain experiments carried out during the development of a suitable procedure for securing juice from cheddar cheese, suggested several interesting facts. First, the large quantities of oil pressed from ripened cheddar cheese as compared to the very small amounts pressed from the Roquefort and Swiss types of cheese, suggested that the fat in cheddar cheese exists in a more loosely combined state than in some other types of cheese. Second, the influence of the age of the cheese on the yields of cheese juice suggested that the moisture in cheddar cheese is more closely combined with the cheese body at certain periods during the ripening than at other periods. Third, the

precipitation of some brine soluble protein material, which occurred directly after the addition of water to small samples of pure cheese juice, suggested that inaccurate results may be obtained when water extraction methods are employed in quantitative studies of various soluble nitrogenous products in cheddar cheese.

PART 2. The Effect of Raw Milk or Various Bacteria on the Ripening of Cheddar Cheese made from Pasteurized Milk

a. The effect of adding 10 per cent raw milk to pasteurized milk used for making cheddar cheese on the nitrogen distribution in the cheese and on the flavor

The effect of adding 10 per cent raw milk to pasteurized milk used for making cheddar cheese on the nitrogen distribution in the cheese and on the flavor was studied with three series of cheese. Each series consisted of three cheese, manufactured at the same time from equal portions of a single lot of milk; one cheese was made from raw milk, one from pasteurized milk, and one from 90 per cent pasteurized milk and 10 per cent raw milk. The results of the chemical analyses of cheese juice and of the scoring of the cheese for flavor, are given in Table I.

A steady breaking down of the proteins was shown in all of the cheese by increases in the various nitrogenous fractions

TABLE I

THE EFFECT OF ADDING 10 PER CENT RAW MILK TO PASTEURI
ON THE NITROGEN DISTRIBUTION IN THE C

Cheese number	Milk used	Age in days	% H_2O in cheese	Total Nitrogen	cc. of 0.1 normal acid equiv. to nitrogen of Nitrogen fractionated into sol. and: Trichloracetic acid Sol.	Trichloracetic acid Insol.	Ethyl alcohol Sol.	Ethyl alcohol Insol.	Pho tungs Sol.
Series 1. Milk from source A. inoculated wi									
1-1	Raw	3	40.2	4.4	3.1	1.3	1.5	3.0	1.3
1-2	Pasteurized	3	40.8	3.7	2.6	1.2	1.3	2.5	1.3
1-3	Past.+10%raw	3	40.6	3.7	2.8	1.0	1.3	2.5	1.1
1-1	"	37		8.2	6.0	2.3	3.4	4.7	2.1
1-2	"	37		6.2	4.0	2.3	3.0	3.3	2.3
1-3	"	37		7.4	5.1	2.3	3.1	4.5	2.1
1-1	"	78		11.3	8.0	3.3	5.4	5.9	3.4
1-2	"	78		9.0	6.7	2.5	4.3	4.7	3.0
1-3	"	78		10.3	7.6	2.8	4.7	5.8	2.9
1-1	"	112		14.8	9.9	4.8	5.8	8.9	4.7
1-2	"	112		12.0	7.6	4.4	5.2	6.8	4.0
1-3	"	112		15.3	9.7	5.4	5.3	10.0	4.5
Series 2. Milk from source B. inoculated v									
2-1	Raw	6	39.2	4.2	2.1	1.9	1.4	2.7	1.2
2-2	Pasteurized	6	39.8	3.9	2.3	1.6	1.4	2.6	0.9
2-3	Past.+10%raw	6	40.0	4.2	2.7	1.6	1.5	2.7	1.0
2-1	"	52		13.0	7.8	5.2	5.2	8.0	3.0
2-2	"	52		11.6	6.2	5.6	4.6	7.2	2.4
2-3	"	52		12.6	7.6	5.0	4.6	7.8	3.2
2-1	"	82		16.1	10.0	6.2	6.1	10.2	4.6
2-2	"	82		13.8	7.9	5.9	5.0	8.7	3.3
2-3	"	82		15.0	9.5	5.6	5.4	9.7	4.1
2-1	"	112		18.1	11.3	6.7	6.2	12.0	5.4
2-2	"	112		15.4	9.6	6.0	5.6	9.8	4.3
2-3	"	112		17.2	10.8	6.4	6.0	11.0	5.0
Series 3. Milk from source A. inoculated v									
3-1	Raw	5	39.0	3.5	2.3	1.1	1.4	2.1	1.0
3-2	Pasteurized	5	39.6	3.3	2.1	1.2	1.2	1.9	0.8
3-3	Past.+10%raw	5	39.1	3.3	2.2	1.0	1.4	1.8	1.0
3-1	"	53		8.7	6.5	2.3	3.8	4.8	2.1
3-2	"	53		7.9	5.3	2.7	3.4	4.4	1.8
3-3	"	53		8.3	6.2	2.2	3.5	5.0	1.9

LE I

PASTEURIZED MILK USED FOR MAKING CHEDDAR CHEESE
IN THE CHEESE AND ON THE FLAVOR

rogen of 1 cc. of cheese juice : sol. and insol. portions with -:: Phospho- :: Tungstic :: *Amino :Flavor : Remarks on									
	::tungstic acid:: acid :: :score of:								
1.:: Sol.	:Insol.:: Sol.	:Insol.::	Nitrogen:	cheese : cheese flavor					
ulated with 2% of commercial cheese culture G									
0 ::	1.3 :	3.2 ::	1.2 :	3.1 ::	1.12 :	:			
5 ::	1.3 :	2.6 ::	1.1 :	2.8 ::	1.04 :	:			
5 ::	1.1 :	2.8 ::	0.9 :	2.8 ::	0.99 :	:			
7 ::	2.1 :	6.0 ::	2.1 :	6.2 ::	1.99 :	38	:Fair, sl. lacking		
3 ::	2.3 :	4.0 ::	1.8 :	4.5 ::	1.56 :	37½	:Poor, very lacking		
5 ::	2.1 :	5.5 ::	1.7 :	5.9 ::	1.68 :	39	:Good, characteristic		
9 ::	3.4 :	9.7 ::	3.1 :	8.2 ::	2.15 :	39½	:V.good, v. typical		
7 ::	3.0 :	6.1 ::	3.1 :	6.0 ::	1.88 :	38	:Fair, lacking		
8 ::	2.9 :	7.3 ::	3.2 :	7.0 ::	2.03 :	39½	:V.good, full cheddar		
9 ::	4.7 :	10.0 ::	4.0 :	10.8 ::	3.37 :	39	:Good, typical cheddar		
8 ::	4.0 :	8.2 ::	3.4 :	8.6 ::	2.74 :	38	:Fair, lacking		
0 ::	4.5 :	11.0 ::	3.5 :	12.0 ::	3.05 :	38½	:Good, typical cheddar		
culated with 1½% of commercial cheese culture G.									
7 ::	1.2 :	3.0 ::	1.2 :	3.0 ::	0.78 :	:			
6 ::	0.9 :	3.1 ::	0.7 :	3.1 ::	0.72 :	:			
7 ::	1.0 :	3.3 ::	0.9 :	3.4 ::	0.85 :	:			
0 ::	3.0 :	9.8 ::	3.8 :	9.0 ::	1.80 :	39	:Good, characteristic		
2 ::	2.4 :	9.2 ::	2.8 :	8.6 ::	1.54 :	38	:Fair, very lacking		
8 ::	3.2 :	9.4 ::	3.0 :	9.6 ::	1.69 :	39½	:V.good, typical cheddar		
2 ::	4.6 :	11.4 ::	4.0 :	12.3 ::	2.83 :	38½	:Good, typical cheddar		
7 ::	3.3 :	10.5 ::	3.1 :	10.7 ::	2.30 :	39	:Good, sl. lacking		
7 ::	4.1 :	10.9 ::	3.6 :	11.6 ::	2.64 :	39½	:V.good, typical cheddar		
0 ::	5.4 :	12.6 ::	4.4 :	13.7 ::	3.66 :	40	:V.good, typical cheddar		
8 ::	4.3 :	11.2 ::	3.6 :	11.8 ::	3.08 :	39	:Good, sl. lacking		
0 ::	5.0 :	12.2 ::	4.0 :	13.2 ::	3.36 :	39½	:V.good, typical cheddar		
oculated with 2% of commercial cheese culture NG									
1 ::	1.0 :	2.5 ::	1.2 :	2.3 ::	0.73 :	:			
9 ::	0.8 :	2.4 ::	1.1 :	2.2 ::	0.66 :	:			
8 ::	1.0 :	2.5 ::	1.1 :	2.3 ::	0.66 :	:			
8 ::	2.1 :	6.6 ::	2.4 :	6.4 ::	1.86 :	37½	:Poor, sour, sl. acid		
4 ::	1.8 :	6.0 ::	2.0 :	5.7 ::	1.40 :	39	:Good, sl. lacking		
0 ::	1.9 :	6.4 ::	2.1 :	6.3 ::	1.58 :	39	:Good, typical cheddar		

Cheese number	Milk used	Age in days	% H_2O in cheese	Total Nitrogen	Trichloracetic acid Sol.	Trichloracetic acid Insol.	Ethyl alcohol Sol.	Ethyl alcohol Insol.	Ph tung Sol
Series 1. Milk from source A. inoculated w									
1-1	Raw	3	40.2	4.4	3.1	1.3	1.5	3.0	1.
1-2	Pasteurized	3	40.8	3.7	2.6	1.2	1.3	2.5	1.
1-3	Past.+10%raw	3	40.6	3.7	2.8	1.0	1.3	2.5	1.
1-1	"	37		8.2	6.0	2.3	3.4	4.7	2.
1-2	"	37		6.2	4.0	2.3	3.0	3.3	2.
1-3	"	37		7.4	5.1	2.3	3.1	4.5	2.
1-1	"	78		11.3	8.0	3.3	5.4	5.9	3.
1-2	"	78		9.0	6.7	2.5	4.3	4.7	3.
1-3	"	78		10.3	7.6	2.8	4.7	5.8	2.
1-1	"	112		14.8	9.9	4.8	5.8	8.9	4.
1-2	"	112		12.0	7.6	4.4	5.2	6.8	4.
1-3	"	112		15.3	9.7	5.4	5.5	10.0	4.
Series 2. Milk from source B. inoculated									
2-1	Raw	6	39.2	4.2	2.1	1.9	1.4	2.7	1.
2-2	Pasteurized	6	39.8	3.9	2.3	1.6	1.4	2.6	0.
2-3	Past.+10%raw	6	40.0	4.2	2.7	1.6	1.5	2.7	1.
2-1	"	52		13.0	7.8	5.2	5.2	8.0	3.
2-2	"	52		11.6	6.2	5.6	4.6	7.2	2
2-3	"	52		12.6	7.6	5.0	4.6	7.8	3
2-1	"	82		16.1	10.0	6.2	6.1	10.2	4
2-2	"	82		13.8	7.9	5.9	5.0	8.7	3
2-3	"	82		15.0	9.5	5.6	5.4	9.7	4
2-1	"	112		18.1	11.3	6.7	6.2	12.0	5
2-2	"	112		15.4	9.6	6.0	5.6	9.8	4
2-3	"	112		17.2	10.8	6.4	6.0	11.0	5
Series 3. Milk from source A. inoculated									
3-1	Raw	5	39.0	3.5	2.3	1.1	1.4	2.1	1
3-2	Pasteurized	5	39.6	3.3	2.1	1.2	1.2	1.9	0
3-3	Past.+10%raw	5	39.1	3.3	2.2	1.0	1.4	1.8	1
3-1	"	53		8.7	6.5	2.3	3.8	4.8	2
3-2	"	53		7.9	5.3	2.7	3.4	4.4	1
3-3	"	53		8.3	6.2	2.2	3.5	5.0	1
3-1	"	82		15.6	11.4	4.0	5.0	10.3	3
3-2	"	82		10.9	7.3	3.7	4.2	6.7	2
3-3	"	82		14.3	10.2	4.1	4.8	9.6	3
3-1	"	110		17.0	12.3	4.6	6.1	10.7	4
3-2	"	110		12.8	8.2	4.5	4.8	7.9	3
3-3	"	110		16.4	11.4	5.1	5.6	10.8	4

*Calculated from milligrams of amino nitrogen (amino nitrogen determined

Phospho-tungstic acid: Sol.	Phospho-tungstic acid: Insol.	Tungstic acid: Sol.	Tungstic acid: Insol.	*Amino Nitrogen	Flavor score of cheese		Remarks on cheese flavor
ulated with 2% of commercial cheese culture G							
0	1.3	3.2	1.2	3.1	1.12		
5	1.3	2.6	1.1	2.8	1.04		
5	1.1	2.8	0.9	2.8	0.99		
7	2.1	6.0	2.1	6.2	1.99	38	Fair, sl. lacking
3	2.3	4.0	1.8	4.5	1.56	37½	Poor, very lacking
5	2.1	5.5	1.7	5.9	1.68	39	Good, characteristic
9	3.4	9.7	3.1	8.2	2.15	39½	V.good, v. typical
7	3.0	6.1	3.1	6.0	1.88	38	Fair, lacking
8	2.9	7.3	3.2	7.0	2.03	39½	V.good, full cheddar
9	4.7	10.0	4.0	10.8	3.37	39	Good, typical cheddar
8	4.0	8.2	3.4	8.6	2.74	38	Fair, lacking
0	4.5	11.0	3.5	12.0	3.05	38½	Good, typical cheddar
culated with 1½% of commercial cheese culture G.							
7	1.2	3.0	1.2	3.0	0.78		
6	0.9	3.1	0.7	3.1	0.72		
7	1.0	3.3	0.9	3.4	0.85		
0	3.0	9.8	3.8	9.0	1.80	39	Good, characteristic
2	2.4	9.2	2.8	8.6	1.54	38	Fair, very lacking
8	3.2	9.4	3.0	9.6	1.69	39½	V.good, typical cheddar
2	4.6	11.4	4.0	12.3	2.83	38½	Good, typical cheddar
7	3.3	10.5	3.1	10.7	2.30	39	Good, sl. lacking
7	4.1	10.9	3.6	11.6	2.64	39½	V.good, typical cheddar
0	5.4	12.6	4.4	13.7	3.66	40	V.good, typical cheddar
8	4.3	11.2	3.6	11.8	3.08	39	Good, sl. lacking
0	5.0	12.2	4.0	13.2	3.36	39½	V.good, typical cheddar
culated with 2% of commercial cheese culture NG							
1	1.0	2.5	1.2	2.3	0.73		
9	0.8	2.4	1.1	2.2	0.66		
8	1.0	2.5	1.1	2.3	0.66		
8	2.1	6.6	2.4	6.4	1.86	37½	Poor, sour, sl. acid
4	1.8	6.0	2.0	5.7	1.40	39	Good, sl. lacking
0	1.9	6.4	2.1	6.3	1.58	39	Good, typical cheddar
3	3.9	11.7	3.2	12.2	2.30	38	Fair, typical cheddar
7	2.4	8.4	2.5	8.5	1.80	38½	Fair, lacking
6	3.5	11.0	3.1	11.3	2.15	39	Good, typical cheddar
7	4.6	12.2	3.6	13.4	3.03	38½	Fair, typical cheddar
9	3.4	9.4	3.3	9.3	2.24	37½	Poor, lacking, bitter
8	4.0	12.3	3.7	12.5	2.73	39½	V.good, typical cheddar

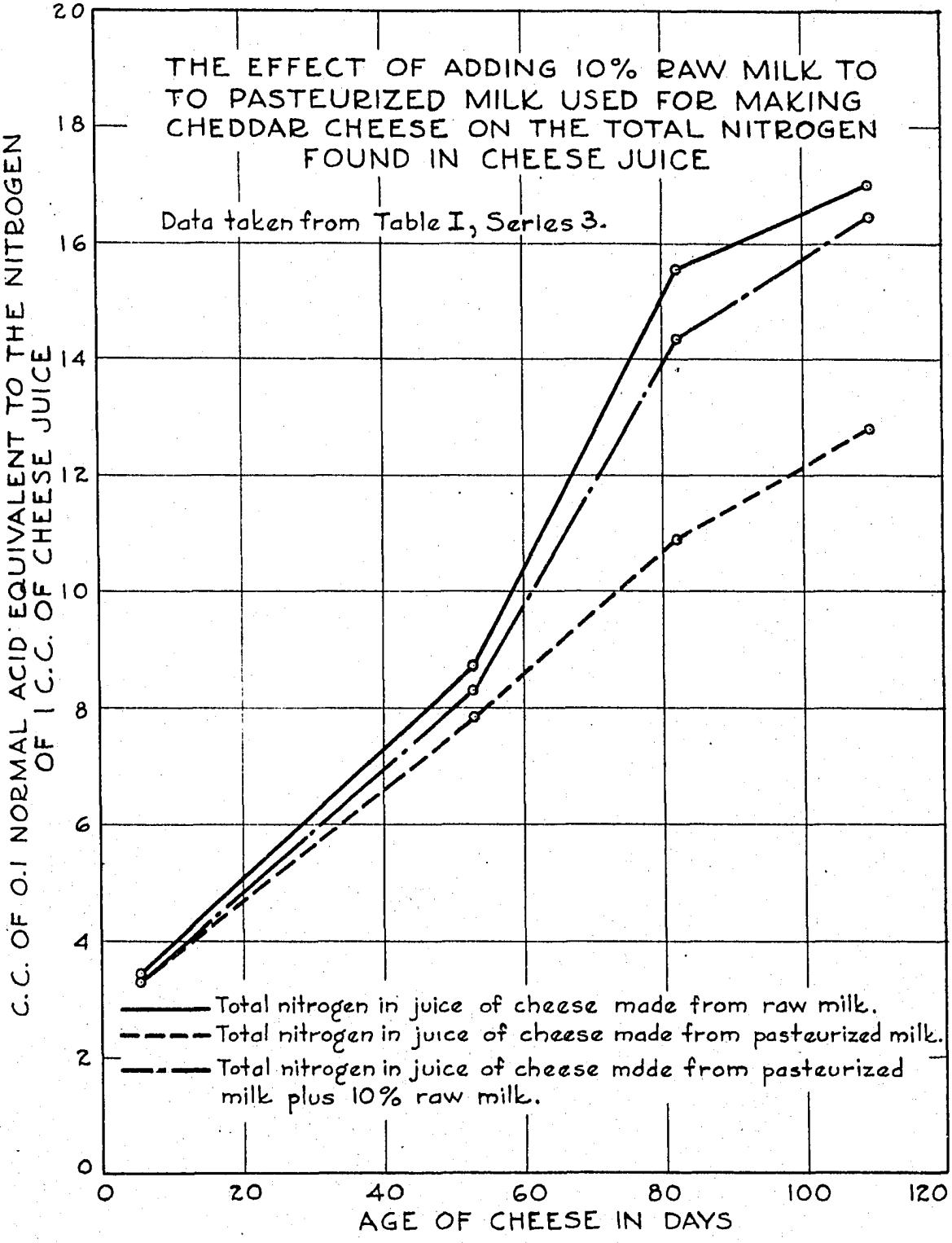
rmined by the Van Slyke gasometric method).

determined. The cheese made from raw milk and from pasteurized milk plus 10 per cent raw milk, showed a more rapid and extensive breakdown than the cheese made from pasteurized milk.

During the early stages of ripening there was very little difference in the amounts of total nitrogen in the juices of the three types of cheese, although slightly higher amounts were regularly found in the juice of cheese made from raw milk, than in that from the other types of cheese. After about one month of ripening, the amounts of total nitrogen in the juices of the three types of cheese showed greater variations than were shown during the early stages of ripening. The amounts of total nitrogen in the juices of the cheese made from raw milk and pasteurized milk plus 10 per cent raw milk, were about the same, and were regularly larger than the amounts in the juices of cheese made from pasteurized milk. With longer ripening, still larger variations in the amounts of total nitrogen in the juices of the three types of cheese were shown; after about four months, the amounts of nitrogen in the juices of cheese made from raw milk and from pasteurized milk plus 10 per cent raw milk, were decidedly larger than the amounts in the juices of cheese made from pasteurized milk.

Graph I illustrates the rates of increase of the total nitrogen in the juices of the three types of cheese. The data used in preparing the graph were obtained from Table I, Series 3. In the period from 5 to 55 days of ripening, the

GRAPH I


THE EFFECT OF ADDING 10% RAW MILK TO TO PASTEURIZED MILK USED FOR MAKING CHEDDAR CHEESE ON THE TOTAL NITROGEN FOUND IN CHEESE JUICE
Data taken from Table I, Series 3.
C.C. OF 0.1 NORMAL ACID EQUIVALENT TO THE NITROGEN OF 1 C.C. OF CHEESE JUICE
0
2
4
6
8
10
12
14
16
18
20
0
20
40
60
80
100
120
AGE OF CHEESE IN DAYS
Total nitrogen in juice of cheese made from raw milk.
Total nitrogen in juice of cheese made from pasteurized milk.
Total nitrogen in juice of cheese mdde from pasteurized milk plus 10% raw milk.

rates of increase in the total nitrogen in the juices of the three types of cheese were about the same. In the period from 50 to 82 days, the rates of increase became more rapid in the juices of cheese made from raw milk and from pasteurized milk plus 10 per cent raw milk, while there was very little change in the rate of increase in the juice of cheese made from pasteurized milk. A decided falling off in the rates of increase of total nitrogen was characteristic of the juices of all the cheese after 82 days of ripening.

The amounts of amino nitrogen and the nitrogen which was soluble or insoluble in trichloracetic acid, ethyl alcohol, phosphotungstic acid, and tungstic acid, were very similar in the juices of the cheese made from raw milk and from pasteurized milk plus 10 per cent raw milk; in practically all cases the amounts were larger than those in the juice of cheese made from pasteurized milk, especially in the fractions of nitrogen which were soluble in trichloracetic acid. However, in several cases the fractions of nitrogen which were insoluble in trichloracetic acid were larger in the juice of cheese made from pasteurized milk than in the juices of the other types of cheese.

The flavor scores of the cheese made from raw milk and from pasteurized milk plus 10 per cent raw milk were, in all cases, similar and relatively high compared to the scores of

the cheese made from pasteurized milk. A characteristic cheddar flavor was regularly shown by the cheese made from raw milk and from pasteurized milk plus 10 per cent raw milk after about two months of ripening, while cheese made from pasteurized milk was characterized by having a flat flavor and a tough rubbery body. No pronounced defects were shown by the cheese made from raw milk and from pasteurized milk plus 10 per cent raw milk, while the cheese made from pasteurized milk in Series 3 became bitter at about two months of ripening.

The effect of adding 10 per cent raw milk to pasteurized milk used for making cheddar cheese on the amounts and percentages of various forms of nitrogen in the juice of ripened cheese is summarized in Table II. Various fractions of nitrogen in the cheese juices were arranged in the order of their increasing complexity, and the amount and percentage of nitrogen in each fraction calculated. In the juices of the three types of cheese, no variations which pointed in a definite direction were shown in the amounts and percentages of the fractions classed as nitrogen soluble in tungstic acid but not amino nitrogen, nitrogen soluble in phosphotungstic acid but not in tungstic acid, and nitrogen soluble in ethyl alcohol but not in phosphotungstic acid. However, a definite variation was shown in the amounts and percentages of the fractions classed as nitrogen soluble in trichloracetic acid but not in ethyl alcohol, and nitrogen insoluble in

TABLE II

THE EFFECT OF ADDING 10 PER CENT RAW MILK TO PASTEURIZED M
AMOUNTS AND PERCENTAGES OF VARIOUS FORMS OF NITROGE

Milk used			
Raw	Past.	Pasteurized +10% raw	Various fractions of nitrogen
cc. of 0.1 normal acid equiv. to the nitrogen fractions of 1 cc. of cheese juice			
Series 1. Cheese ripened 112 days made from source A.			
3.4	2.7	3.1	Amino N.
0.6	0.7	0.4	N. soluble in tungstic acid bu
0.7	0.6	1.0	N. soluble in phosphotungstic acid b
1.1	1.2	0.8	N. soluble in ethyl alcohol but not
4.1	2.4	4.4	N. soluble in trichloracetic acid bu
4.8	4.4	5.4	N. insoluble in trichlor
14.7	12.0	15.1	Total N.
Series 2. Cheese ripened 112 days made from source B.			
3.7	3.1	3.4	Amino N.
0.7	0.5	0.6	N. soluble in tungstic acid bu
1.0	0.7	1.0	N. soluble in phosphotungstic acid b
0.8	1.3	1.0	N. soluble in ethyl alcohol but not
5.1	4.0	4.8	N. soluble in trichloracetic acid bu
6.7	6.0	6.4	N. insoluble in trichlor
18.0	15.6	17.2	Total N.
Series 3. Cheese ripened 110 days made from source A.			
3.0	2.2	2.7	Amino N.
0.6	1.1	1.0	N. soluble in tungstic acid bu
1.0	0.1	0.3	N. soluble in phosphotungstic acid b
1.5	1.5	1.6	N. soluble in ethyl alcohol but not
6.2	3.4	5.8	N. soluble in trichloracetic acid bu
4.6	4.5	5.1	N. insoluble in trichlor
16.9	12.8	16.5	Total N.

ABLE II

STEURIZED MILK USED FOR MAKING CHEDDAR CHEESE ON THE
OF NITROGEN IN THE JUICE OF RIPENED CHEESE

f nitrogen in cheese juice	Milk used		
	Raw	Past.	Pasteurized + 10% raw
	% of total nitrogen made up of various fractions		
source A. milk inoculated with 2% commercial culture G.			
Amino N.	24	22	20
tic acid but not amino nitrogen	4	6	4
stic acid but not in tungstic acid	5	5	6
ol but not in phosphotungstic acid	7	10	5
tic acid but not in ethyl alcohol	28	20	29
in trichloracetic acid	32	37	36
Total N.			
source B. milk inoculated with 1½% commercial culture G.			
Amino N.	20	20	20
tic acid but not amino nitrogen	4	3	3
stic acid but not in tungstic acid	6	4	5
ol but not in phosphotungstic acid	4	8	5
tic acid but not in ethyl alcohol	28	25	28
in trichloracetic acid	38	40	39
Total N.			
source A. milk inoculated with 2% commercial culture NG			
Amino N.	18	17	16
tic acid but not amino nitrogen	4	8	6
stic acid but not in tungstic acid	6	1	2
ol but not in phosphotungstic acid	9	10	10
tic acid but not in ethyl alcohol	36	28	35
in trichloracetic acid	27	36	31
Total N.			

trichloracetic acid. The juices of cheese made from raw milk and from pasteurized milk plus 10 per cent raw milk, regularly contained larger amounts and percentages of the fraction which was soluble in trichloracetic acid but not in ethyl alcohol, and smaller amounts and percentages of the fraction which was insoluble in trichloracetic acid, than the juice from cheese made with pasteurized milk.

b. The effect of adding L. casei 1 to pasteurized milk used for making cheddar cheese on the nitrogen distribution in the cheese and on the flavor

The effect of adding L. casei 1 to pasteurized milk used for making cheddar cheese on the nitrogen distribution in the cheese and on the flavor was studied with three series of cheese. Three cheese manufactured at the same time from equal portions of the same lot of milk were included in each series; one cheese being made from raw milk, one from pasteurized milk, and one from pasteurized milk plus 1.5 per cent of a milk culture of L. casei 1. The data obtained in the chemical analyses of the cheese juice and in the examination of cheese for flavor, are given in Table III.

In all of the cheese there was a steady breaking down of the proteins as indicated by increases in the various nitrogenous fractions determined. A more rapid and extensive

TABLE XII

THE EFFECT OF ADDING L. CASEI 1. TO PASTEURIZED
ON THE NITROGEN DISTRIBUTION IN THE

Cheese number	Milk used	Age in days	% H_2O in cheese	Total Nitrogen	cc. of 0.1 normal acid equiv. to nitrogen of 1 Nitrogen fractionated into sol. and Trichlor-acetic acid Sol.	Trichlor-acetic acid Insol.	Ethyl alcohol Sol.	Ethyl alcohol Insol.	Phosphotungs Sol.
Series 1. Milk from source B. inoculated with									
4-1	Raw	2	39.2	3.8	2.2	1.5	0.7	3.1	0.0
4-2	Past.	2	39.6	4.0	2.4	1.7	0.8	3.1	0.7
4-3	Past.+1½% L.casei 1.	2	39.7	4.5	2.6	2.0	0.9	3.6	0.9
4-1	"	29		8.3	5.7	2.5	3.2	5.1	2.0
4-2	"	29		7.5	4.4	3.3	2.7	5.0	1.9
4-3	"	29		8.6	4.9	3.6	3.8	5.6	2.0
4-1	"	64		13.8	9.0	4.8	6.2	8.0	4.2
4-2	"	64		13.0	7.9	5.3	4.0	9.1	2.8
4-3	"	64		13.6	8.9	4.6	5.0	8.4	5.2
4-1	"	108		16.4	10.4	6.0	6.6	9.7	6.0
4-2	"	108		15.3	8.5	7.0	6.0	9.1	5.1
4-3	"	108		16.1	10.2	6.2	6.6	9.6	5.9
Series 2. Milk from source C. inoculated with									
5-1	Raw	2	39.1	4.5	3.0	1.5	1.7	2.8	1.2
5-2	Past.	2	39.4	4.3	3.2	1.2	1.5	2.8	1.0
5-3	Past.+1½% L.casei 1.	2	40.0	4.6	3.2	1.5	1.7	2.8	1.1
5-1	"	38		7.8	5.4	2.5	3.2	4.5	2.6
5-2	"	38		7.4	4.8	2.8	3.5	4.0	2.0
5-3	"	38		8.6	6.0	2.6	3.4	5.0	2.3
5-1	"	76		14.8	9.4	5.4	6.4	8.2	5.2
5-2	"	76		13.2	8.0	5.0	6.0	7.1	4.0
5-3	"	76		13.8	8.0	5.8	5.2	8.4	4.2
5-1	"	116		16.2	10.0	6.2	6.6	9.6	5.8
5-2	"	116		14.2	8.4	5.8	6.2	8.0	5.0
5-3	"	116		15.4	9.6	5.6	5.7	9.4	4.8
Series 3. Milk from source A. inoculated with									
6-1	Raw	2	37.9	3.3	3.0	1.3	1.2	2.3	0.9
6-2	Past.	2	38.3	3.0	1.6	1.4	1.1	2.0	1.1
6-3	Past.+1½% L.casei 1.	2	38.5	3.8	2.7	1.2	1.4	2.5	1.0
6-1	"	35		6.2	4.2	1.8	2.5	4.0	1.6

LE III

STEURIZED MILK USED FOR MAKING CHEDDAR CHEESE
N IN THE CHEESE AND ON THE FLAVOR.

gen of 1 cc. of cheese juice							
sol. and insol. portions with-							
Phospho-	Tungstic		*Amino	Flavor		Remarks on
tungstic acid:	acid			score of:		
Sol.	Insol.	Sol.	Insol.	Nitrogen:	cheese		cheese flavor
ed with 2% of commercial cheese culture G.							
0.6	3.1	0.9	2.7	0.87			
0.7	3.2	1.0	2.9	0.82			
0.0	3.7	0.7	2.8	0.82			
2.0	6.5	2.0	6.4	1.71	40		V. good, characteristic
1.8	5.7	1.7	5.8	1.32	39		Good, lacking
2.0	6.8	1.9	6.8	1.82	40		V. good, distinct, buttery
4.2	9.8	4.0	9.6	2.64	40⅝		V. good, typical cheddar
2.3	10.6	3.2	9.7	2.48	39		Good, slightly lacking
3.3	10.5	3.0	9.6	3.14	40½		V. good, distinct buttery
6.0	10.4	5.6	10.6	4.05	40		V. good, typical cheddar
5.1	10.0	4.9	10.2	3.38	39½		Good, slightly lacking
5.3	10.2	4.8	11.2	3.81	38⅝		Good, sour, buttery
ed with 2% commercial cheese culture G.							
1.2	3.2	1.3	3.0	0.75			
1.0	3.4	1.3	2.8	0.54			
1.1	3.5	1.2	3.4	0.64			
2.6	5.3	1.6	6.1	1.56	39⅝		V. good, slightly lacking
2.0	5.6	1.5	6.1	1.46	38		Fair, lacking
2.3	6.6	1.7	6.8	1.50	39		Good, buttery
5.2	9.4	2.6	12.2	1.95	40⅝		V. good, typical cheddar
4.0	9.0	2.5	10.5	1.68	39		Good, lacking
4.3	9.8	2.2	11.6	1.78	39		Good, very buttery
5.8	10.6	3.6	12.6	2.99	40		V. good, full, cheddar
5.0	8.2	2.5	11.9	2.18	39		Good, slightly lacking
4.8	12.6	3.2	12.2	2.62	39½		V. good, very buttery
ed with 2% commercial cheese culture G.							
0.9	2.2	1.0	2.2	0.67			
1.1	2.1	0.9	2.2	0.67			
1.0	2.8	1.1	2.7	0.74			
1.9	4.3	1.9	4.1	1.34	38		Good, slightly lacking

Series 1. Milk from source B. inoculated with 2%

4-1	Raw	2	39.2	3.8	2.2	1.5	0.7	3.1	0.6
4-2	Past.	2	39.6	4.0	2.4	1.7	0.8	3.1	0.7
4-3	Past.+1% L.casei 1	2	39.7	4.5	2.6	2.0	0.9	3.6	0.9
4-1	"	29		8.3	5.7	2.5	3.2	5.1	2.0
4-2	"	29		7.5	4.4	3.3	2.7	5.0	1.8
4-3	"	29		8.6	4.9	3.6	3.8	5.6	2.0
4-1	"	64		13.8	9.0	4.8	6.2	8.0	4.2
4-2	"	64		13.0	7.9	5.3	4.6	9.1	2.3
4-3	"	64		13.6	8.9	4.6	5.0	8.4	5.3
4-1	"	108		16.4	10.4	6.0	6.6	9.7	6.0
4-2	"	108		15.3	8.5	7.0	6.0	9.1	5.1
4-3	"	108		16.1	10.2	6.2	6.6	9.6	5.8

Series 2. Milk from source C. inoculated with 2%

5-1	Raw	2	39.1	4.5	3.0	1.5	1.7	2.8	1.2
5-2	Past.	2	39.4	4.3	3.2	1.2	1.5	2.9	1.0
5-3	Past.+1% L.casei 1	2	40.0	4.6	3.2	1.5	1.7	2.8	1.1
5-1	"	38		7.8	5.4	2.5	3.2	4.5	2.6
5-2	"	38		7.4	4.8	2.8	3.5	4.0	2.0
5-3	"	38		8.6	6.0	2.6	3.4	5.0	2.3
5-1	"	76		14.6	9.4	5.4	6.4	8.2	5.2
5-2	"	76		13.2	8.0	5.0	6.0	7.1	4.0
5-3	"	76		13.8	8.0	5.8	5.2	8.4	4.3
5-1	"	116		16.2	10.0	6.2	6.6	9.6	5.8
5-2	"	116		14.2	8.4	5.8	6.2	9.0	5.0
5-3	"	116		15.4	9.6	5.6	5.7	9.4	4.8

Series 3. Milk from source A. inoculated with 2%

6-1	Raw	2	37.0	3.3	2.0	1.3	1.2	2.3	0.9
6-2	Past.	2	38.3	3.0	1.6	1.4	1.1	2.0	1.1
6-3	Past.+1% L.casei 1	2	38.5	3.8	2.7	1.2	1.4	2.5	1.0
6-1	"	35		6.2	4.2	1.8	2.5	3.6	1.9
6-2	"	35		5.9	4.2	1.8	2.6	3.4	1.7
6-3	"	35		6.9	4.7	2.3	2.9	3.9	1.5
6-1	"	73		13.1	7.8	5.2	5.0	7.6	5.0
6-2	"	73		11.6	7.2	4.2	5.2	6.2	5.2
6-3	"	73		13.8	8.2	5.6	5.4	8.4	4.0
6-1	"	106		15.8	11.2	4.8	6.8	8.8	6.4
6-2	"	106		13.5	9.0	4.7	6.0	7.5	5.6
6-3	"	106		16.1	11.4	4.6	6.3	9.6	6.4

*Calculated from milligrams of amino nitrogen (amino nitrogen determined by

od with 2% of commercial cheese culture C.

::	0.6 :	3.1 ::	0.9 :	2.7 ::	0.87 :		:	
::	0.7 :	3.2 ::	1.0 :	2.9 ::	0.82 :		:	
::	0.9 :	3.7 ::	0.7 :	2.8 ::	0.82 :		:	
::	:	::	:	::	:		:	
::	2.0 :	6.5 ::	3.0 :	6.4 ::	1.71 :	40	:V. good, characteristic	
::	1.8 :	5.7 ::	1.7 :	5.8 ::	1.32 :	39	:Good, lacking	
::	2.0 :	6.8 ::	1.9 :	6.8 ::	1.82 :	40	:V. good, distinct, buttery	
::	4.2 :	9.8 ::	4.0 :	9.6 ::	2.64 :	40½	:V. good, typical cheddar	
::	2.3 :	10.6 ::	3.2 :	9.7 ::	2.48 :	39	:Good, slightly lacking	
::	3.3 :	10.5 ::	3.0 :	9.6 ::	3.14 :	40½	:V. good, distinct buttery	
::	6.0 :	10.4 ::	5.6 :	10.6 ::	4.05 :	40	:V. good, typical cheddar	
::	5.1 :	10.0 ::	4.9 :	10.2 ::	3.38 :	39½	:Good, slightly lacking	
::	5.9 :	10.2 ::	4.8 :	11.2 ::	3.81 :	38½	:Good, sour, buttery	

ed with 2% commercial cheese culture C.

::	1.2 :	3.2 ::	1.3 :	3.0 ::	0.75 :		:	
::	1.0 :	3.4 ::	1.3 :	2.8 ::	0.54 :		:	
::	1.1 :	3.5 ::	1.2 :	3.4 ::	0.64 :		:	
::	:	::	:	::	:		:	
::	2.6 :	5.3 ::	1.6 :	6.1 ::	1.56 :	39½	:V. good, slightly lacking	
::	2.0 :	5.6 ::	1.5 :	6.1 ::	1.46 :	38	:Fair, lacking	
::	2.3 :	6.6 ::	1.7 :	6.8 ::	1.50 :	39	:Good, buttery	
::	5.2 :	9.4 ::	2.6 :	12.2 ::	1.95 :	40½	:V. good, typical cheddar	
::	4.0 :	9.0 ::	2.5 :	10.5 ::	1.68 :	39	:Good, lacking	
::	4.3 :	9.8 ::	2.2 :	11.6 ::	1.78 :	39	:Good, very buttery	
::	5.8 :	10.6 ::	3.6 :	12.6 ::	2.99 :	40	:V. good, full, cheddar	
::	5.0 :	9.2 ::	2.5 :	11.9 ::	2.18 :	39	:Good, slightly lacking	
::	4.8 :	12.6 ::	3.2 :	12.2 ::	2.62 :	39½	:V. good, very buttery	

od with 2% commercial cheese culture C.

::	0.9 :	2.2 ::	1.0 :	2.2 ::	0.67 :		:	
::	1.1 :	3.1 ::	0.9 :	2.2 ::	0.67 :		:	
::	1.0 :	2.8 ::	1.1 :	2.7 ::	0.74 :		:	
::	:	::	:	::	:		:	
::	1.9 :	4.3 ::	1.9 :	4.1 ::	1.34 :	38	:Good, slightly lacking	
::	1.7 :	4.0 ::	2.0 :	4.1 ::	1.27 :	37½	:Poor, no cheddar	
::	1.5 :	5.3 ::	1.7 :	5.1 ::	1.47 :	39½	:V. good, rich, buttery	
::	5.0 :	8.0 ::	3.8 :	9.2 ::	2.03 :	38½	:Good, slightly lacking	
::	5.2 :	6.4 ::	3.6 :	7.8 ::	1.88 :	38	:Fair, lacking	
::	4.0 :	9.8 ::	3.4 :	10.2 ::	2.12 :	39½	:V. good, very buttery	
::	6.4 :	9.2 ::	5.0 :	10.6 ::	2.30 :	39	:Good, typical cheddar	
::	5.6 :	7.8 ::	4.6 :	9.2 ::	2.40 :	38	:Fair, lacking	
::	5.4 :	10.7 ::	4.8 :	11.5 ::	3.04 :	37	:Poor,sour, sl. buttery	

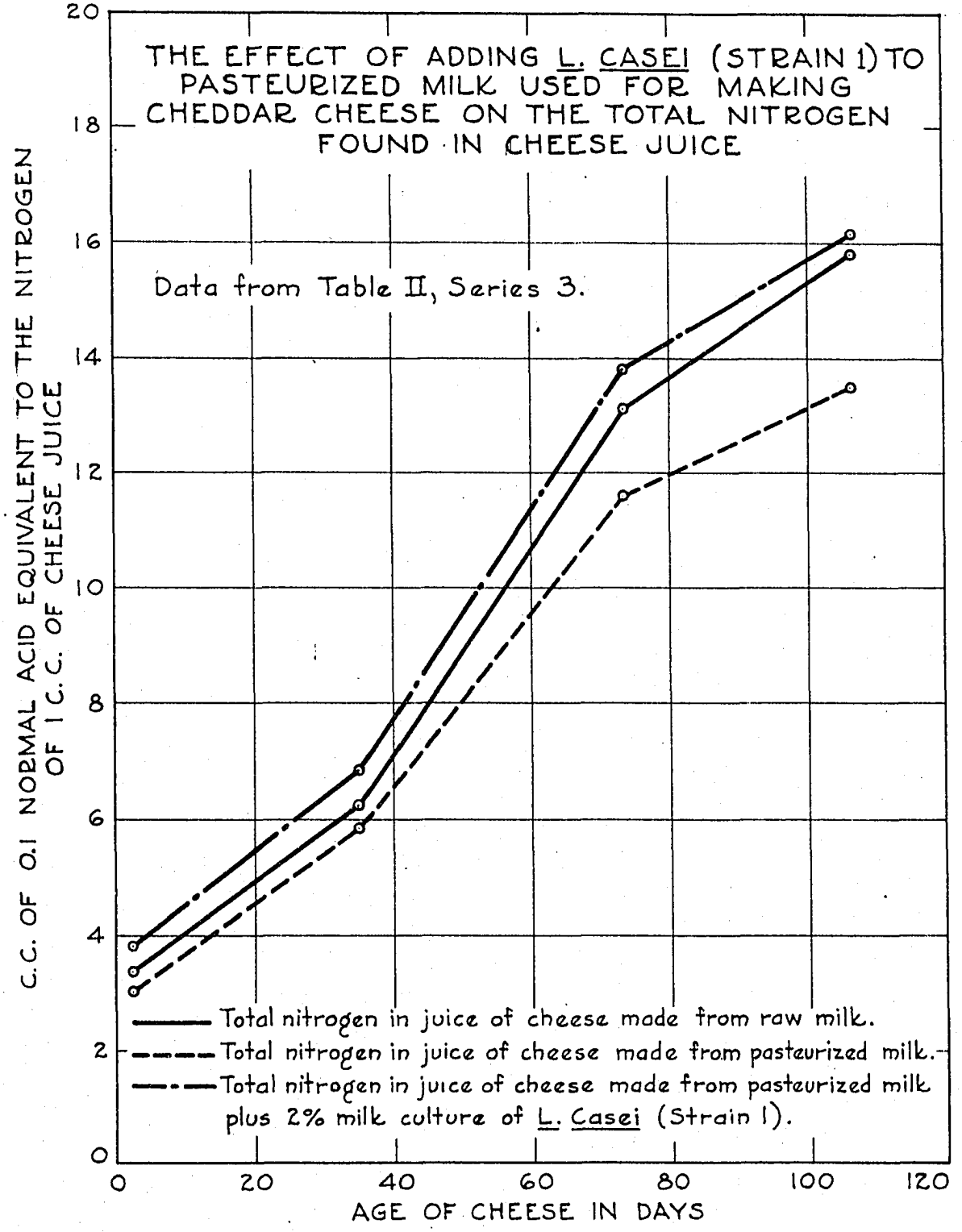
termined by the Van Slyke gasometric method).

breakdown occurred in the cheese made from raw milk and from pasteurized milk plus L. casei 1 than in the cheese made from pasteurized milk.

There were only slight variations in the amounts of total nitrogen in the juices of the three types of cheese during the early stages of ripening, although the amounts in the juices of cheese made from raw milk and from pasteurized milk plus L. casei 1 were regularly slightly larger than the amounts in the juice of cheese made from pasteurized milk. After about one month of ripening the three types of cheese varied slightly more in the amounts of total nitrogen in the juices than during the early stages of ripening. The amounts in the juices of the cheese made from raw milk and from pasteurized milk plus L. casei 1 were regularly larger than the amounts in the juice of cheese made with pasteurized milk. Still greater variations in the amounts of total nitrogen in the cheese juices were shown after longer ripening; after about four months, the amounts in the juices of cheese made from raw milk and from pasteurized milk plus L. casei 1 were decidedly larger than the amounts in the juice of cheese made from pasteurized milk.

The rates of increase of the total nitrogen in the juices of the three types of cheese during ripening are illustrated in Graph II. The graph was prepared from data given in Table III, Series 1. The rates of increase in the

GRAPH II


THE EFFECT OF ADDING L. CASEI (STRAIN 1) TO PASTEURIZED MILK USED FOR MAKING CHEDDAR CHEESE ON THE TOTAL NITROGEN FOUND IN CHEESE JUICE
Data from Table II, Series 3.
C.C. OF 0.1 NORMAL ACID EQUIVALENT TO THE NITROGEN OF 1 C.C. OF CHEESE JUICE
AGE OF CHEESE IN DAYS
0
2
4
6
8
10
12
14
16
18
20
0
20
40
60
80
100
120
Total nitrogen in juice of cheese made from raw milk.
Total nitrogen in juice of cheese made from pasteurized milk.
Total nitrogen in juice of cheese made from pasteurized milk plus 2% milk culture of L. Casei (Strain 1).

total nitrogen in the juices of the three types of cheese were essentially the same during the period from 2 to 35 days. During the period from 35 to 73 days, there was an increase in the rate in the juices of all the cheese, although in the case of the cheese made from pasteurized milk, the increase was not so pronounced as with the other types of cheese. During the period from 73 to 106 days, a decided falling off in the rate of increase in total nitrogen was shown in the juices of all of the cheese.

The amounts of amino nitrogen and the nitrogen soluble or insoluble in trichloracetic acid, ethyl alcohol, phosphotungstic acid, and tungstic acid, were essentially the same in the juices of the cheese made from raw milk and from pasteurized milk plus L. casei 1, and usually these amounts were larger than those in the juice of cheese made from pasteurized milk, especially in the fractions of nitrogen which were soluble in trichloracetic acid. However, in a number of cases the fractions of nitrogen which were insoluble in trichloracetic acid were larger in the juice of cheese made from pasteurized milk than in juices of the other types of cheese.

The flavor scores of the cheese made from raw milk and from pasteurized milk plus L. casei 1 were essentially the same and were higher than the scores of the cheese made with pasteurized milk. A characteristic cheddar flavor was

regularly shown by the cheese made from raw milk after about two months of ripening. The cheese made from pasteurized milk plus L. casei 1 were consistently characterized by having a rich, pleasing, buttery flavor after about one month of ripening. The buttery flavor differed distinctly from the characteristic flavor normally found in cheddar cheese, but was very desirable. After three to four months of ripening the buttery flavor became less pronounced and a sour flavor usually developed in the cheese. The cheese made from pasteurized milk was generally characterized by having a flat flavor and a tough, rubbery body.

A summary of the effect of adding L. casei 1 to pasteurized milk used for making cheddar cheese on the amounts and percentages of various forms of nitrogen in the juice of ripened cheese is given in Table IV. In the juices of the three types of cheese, there were no consistent variations in the amounts and percentages of the fractions classed as nitrogen soluble in tungstic acid but not amino nitrogen, nitrogen soluble in phosphotungstic acid but not in tungstic acid, and nitrogen soluble in ethyl alcohol but not in phosphotungstic acid. There were, however, consistent variations in the amounts and percentages of fractions classed as nitrogen soluble in trichloracetic acid but not in ethyl alcohol, and nitrogen insoluble in trichloracetic acid. The

TABLE IV

THE EFFECT OF ADDING L. CASEI 1. TO PASTEURIZED MILK USE
AND PERCENTAGES OF VARIOUS FORMS OF NITROGEN IN TH

Milk used			Various fractions of nitrogen
Raw	Past.	Pasteurized + 1½% L.casei 1 culture	
cc. of 0.1 normal acid equiv. to the nitrogen fractions of 1 cc. of cheese juice			
Series 1. Cheese ripened 108 days made from Source B. m			
4.0	3.4	3.8	Amino N.
1.6	1.5	1.0	N. soluble in tungstic acid but
0.4	0.2	1.0	N. soluble in phosphotungstic acid b
0.6	0.9	0.8	N. soluble in ethyl alcohol but not
3.8	2.5	3.6	N. soluble in trichloracetic acid bu
6.0	7.0	6.2	N. insoluble in trichlor
16.4	15.5	16.4	Total N.
Series 2. Cheese ripened 116 days made from source C. m			
3.0	2.2	2.6	Amino N.
0.6	0.3	0.6	N. soluble in tungstic acid but
2.2	2.5	1.6	N. soluble in phosphotungstic acid b
0.8	1.2	0.9	N. soluble in ethyl alcohol but not
3.4	2.2	3.9	N. soluble in trichloracetic acid bu
6.2	5.8	5.6	N. insoluble in trichlor
16.2	14.2	15.2	Total N.
Series 3. Cheese ripened 106 days made from source A. m			
2.8	2.4	3.0	Amino N.
2.2	2.2	1.8	N. soluble in tungstic acid but
1.4	1.0	0.6	N. soluble in phosphotungstic acid b
0.4	0.4	0.9	N. soluble in ethyl alcohol but not
4.4	3.0	5.3	N. soluble in trichloracetic acid bu
4.8	4.7	4.6	N. insoluble in trichlor
16.0	13.7	16.2	Total N.

TABLE IV

IZED MILK USED FOR MAKING CHEDDAR CHEESE ON THE AMOUNTS
ITROGEN IN THE JUICE OF RIPENED CHEESE

of nitrogen in cheese juice	Milk used		
	Raw	Past.	Pasteurized + 1% L.casei 1 culture
	% of total nitrogen made up of various fractions		
om Source B. milk inoculated with 2% commercial culture G			
Amino N.	24	22	23
gstic acid but not amino nitrogen	9	10	6
ungstic acid but not in tungstic acid	2	1	6
cohol but not in phosphotungstic acid	3	6	5
acetic acid but not in ethyl alcohol	23	16	22
le in trichloracetic acid	39	45	38
Total N.			
om source C. milk inoculated with 2% commercial culture G.			
Amino N.	18	16	17
gstic acid but not amino nitrogen	4	2	4
ungstic acid but not in tungstic acid	14	18	11
cohol but not in phosphotungstic acid	5	8	6
acetic acid but not in ethyl alcohol	21	15	25
le in trichloracetic acid	38	41	37
Total N.			
om source A. milk inoculated with 2% commercial culture G.			
Amino N.	18	18	19
gstic acid but not amino nitrogen	14	16	11
ungstic acid but not in tungstic acid	8	7	4
cohol but not in phosphotungstic acid	2	3	5
acetic acid but not in ethyl alcohol	28	22	33
le in trichloracetic acid	30	34	28
Total N.			

juices of cheese made from raw milk and from pasteurized milk plus L. casei 1, regularly contained larger amounts and percentages of nitrogen which was soluble in trichloracetic acid but not in ethyl alcohol, and smaller amounts and percentages of nitrogen which was insoluble in trichloracetic acid, than the juice of cheese made from pasteurized milk.

c. The effect of adding several strains of L. casei to pasteurized milk used for making cheddar cheese on the nitrogen distribution in the cheese and on the flavor

The effect of adding several strains of L. casei to pasteurized milk used for making cheddar cheese on the nitrogen distribution in the cheese and on the flavor was studied with three series of cheese. Each of the series consisted of three cheese, manufactured at the same time from equal portions of a single lot of milk; one was made from pasteurized milk, and two were made from pasteurized milk inoculated with 1.5 per cent of a milk culture of a strain of L. casei. Table V presents the results secured in the chemical analyses of cheese juice and in the scoring of the cheese for flavor.

Increases in the various nitrogenous fractions showed that there was a steady breaking down of the proteins in all of the cheese during the ripening period. Throughout the

TABLE V

THE EFFECT OF ADDING SEVERAL STRAINS OF L. CASEI T
CHEDDAR CHEESE ON THE NITROGEN DISTRIBUTION IN

Cheese number	Milk used	Age in days	% H_2O in cheese	cc. of 0.1 normal acid equiv. to nitrogen of 1						
				Total Nitrogen	Nitrogen fractionated into sol. and					
					Trichloracetic acid		Ethyl alcohol		Phos tungs	
					Sol.	Insol.	Sol.	Insol.	Sol.	
Series 1. Milk from source B. inoculated with										
7-1	Past.	8	38.0	5.0	3.2	1.7	2.0	3.2	1.0	
	Past.+1½%									
7-2	L.casei 0	8	38.4	6.1	3.8	2.2	2.2	3.8	1.6	
	Past.+1½%									
7-3	L.casei 2	8	38.3	5.7	3.4	2.4	2.0	3.4	1.2	
7-1		49		7.9	4.9	3.0	3.2	4.8	2.2	
7-2	"	49		9.1	6.3	2.7	3.7	5.2	2.8	
7-3		49		8.7	5.9	2.7	3.2	5.4	2.1	
7-1		79		11.8	7.1	4.9	4.4	7.5	2.4	
7-2	"	79		12.5	8.3	4.0	5.0	7.4	3.2	
7-3		79		12.4	8.5	3.7	5.4	7.1	3.6	
7-1		110		15.9	9.4	6.4	5.4	10.2	3.9	
7-2	"	110		17.3	11.2	6.0	6.8	10.4	5.1	
7-3		110		18.4	11.8	6.6	7.3	10.9	5.4	
Series 2. Milk from source A. inoculated with										
8-1	Past.	7	39.2	5.6	3.5	2.3	2.3	3.3	2.2	
	Past.+1½%									
8-2	L.casei 3	7	39.8	5.4	3.2	2.1	2.1	3.2	1.8	
	Past.+1½%									
8-3	L.casei 4	7	40.0	5.7	3.6	1.9	2.4	3.2	2.3	
8-1		47		8.2	5.0	3.0	2.6	5.5	2.2	
8-2	"	47		8.7	6.1	2.5	3.2	5.3	2.1	
8-3		47		9.0	5.6	3.3	3.6	5.5	2.5	
8-1		80		13.2	9.0	4.3	4.0	9.1	3.0	
8-2	"	80		13.7	9.3	4.4	4.8	9.0	3.4	
8-3		80		14.4	9.8	4.6	5.2	9.2	4.0	
8-1		111		14.6	9.3	5.3	4.2	10.4	3.4	
8-2	"	111		15.0	10.0	4.9	4.7	10.2	3.8	
8-3		111		16.8	12.0	4.7	5.7	10.6	4.7	
Series 3. Milk from source A. inoculated with										
9-1	Past.	6	37.6	5.0	3.1	1.8	2.0	3.1	2.2	
	Past.+1½%									

BLE V

. CASEI TO PASTEURIZED MILK USED FOR MAKING
BUTTON IN THE CHEESE AND ON THE FLAVOR

ogen of 1 cc. of cheese juice sol. and insol. portions with-							
Phospho-tungstic acid		Tungstic acid		*Amino Nitrogen	Flavor score of cheese		Remarks on cheese flavor
1. Sol.	Insol.	Sol.	Insol.				
ted with 1⅓% of commercial cheese culture NG.							
2	1.0	4.2	0.9	4.1	0.78		
8	1.6	4.6	1.8	4.3	0.94		
4	1.2	4.4	1.4	4.1	0.87		
8	2.2	5.8	2.0	5.8	1.34	38½	Fair, slightly flat
2	2.8	6.2	2.3	6.8	1.47	37	Poor, sour, lacking
4	2.1	6.5	2.0	6.4	1.41	38	Fair, sl. sour, lacking
5	2.4	9.5	2.8	9.0	1.61	38	Fair, sour, flat
4	3.2	9.2	3.5	9.1	1.70	38	Fair, sour, cheddar
1	3.6	9.1	3.5	8.8	1.66	38½	Good, sl. sour, typical
2	3.9	11.7	3.0	12.8	2.23	38	Fair, sl. lacking
4	5.1	12.2	4.3	13.1	3.06	40	V. good, sl. sour, typical
9	5.4	15.0	5.0	13.6	3.24	38½	Good, sl. sour, typical
ted with 1⅓% of commercial cheese culture G.							
3	2.2	3.5	1.4	4.1	0.85		
2	1.8	3.6	1.6	3.6	0.72		
2	2.3	3.6	1.8	4.0	0.83		
5	2.2	6.1	2.0	6.3	1.40	39	Good, sl. lacking
3	2.1	6.6	1.8	7.0	1.48	38½	Good, sl. lacking, sour
5	2.5	6.5	2.4	6.8	1.56	40	V. good, cheddar, buttery
1	3.0	10.1	2.6	10.6	2.06	38	Fair, sour, sl. lacking
0	3.4	10.3	2.9	11.0	2.17	38	Fair, sour, sl. lacking
2	4.0	10.5	3.4	11.1	2.74	39½	V.good, v. cheddar, buttery
4	3.4	11.2	3.2	11.4	2.81	37½	Poor, sour, bitter
2	3.8	11.3	3.4	11.7	2.96	38	Fair, sl. sour, bitter
6	4.7	12.0	3.9	12.8	3.44	39½	V. good, rich, v. buttery
ted with 1⅓% of commercial cheese culture G.							
1	2.2	2.8	1.2	3.9	0.78		

7-1	Past.	8	38.0	5.0	3.2	1.7	2.0	3.2	1.0
	Past.+1⅛%								
7-2	L.casei 0	8	38.4	6.1	3.8	2.2	2.2	3.8	1.6
	Past.+1⅛%								
7-3	L.casei 2	8	38.3	5.7	3.4	2.4	2.0	3.4	1.2
7-1	"	49		7.9	4.9	3.0	3.2	4.8	2.2
7-2	"	49		9.1	6.3	2.7	3.7	5.2	2.8
7-3	"	49		8.7	5.9	2.7	3.2	5.4	2.1
7-1	"	79		11.8	7.1	4.9	4.4	7.5	2.4
7-2	"	79		12.5	8.3	4.0	5.0	7.4	3.2
7-3	"	79		12.4	8.5	3.7	5.4	7.1	3.6
7-1	"	110		15.9	9.4	6.4	5.4	10.2	3.9
7-2	"	110		17.3	11.2	6.0	6.8	10.4	5.1
7-3	"	110		18.4	11.8	6.6	7.3	10.9	5.4

Series 2. Milk from source A. inoculated with 1

8-1	Past.	7	39.2	5.6	3.5	2.3	2.3	3.3	2.2
	Past.+1⅛%								
8-2	L.casei 3	7	39.8	5.4	3.2	2.1	2.1	3.2	1.8
	Past.+1⅛%								
8-3	L.casei 4	7	40.0	5.7	3.6	1.9	2.4	3.2	2.3
8-1	"	47		8.2	5.0	3.0	2.6	5.5	2.2
8-2	"	47		8.7	6.1	2.5	3.2	5.3	2.1
8-3	"	47		9.0	5.6	3.3	3.6	5.5	2.5
8-1	"	80		13.2	9.0	4.3	4.0	9.1	3.0
8-2	"	80		13.7	9.3	4.4	4.8	9.0	3.4
8-3	"	80		14.4	9.8	4.6	5.2	9.2	4.0
8-1	"	111		14.6	9.3	5.3	4.2	10.4	3.4
8-2	"	111		15.0	10.0	4.9	4.7	10.2	3.8
8-3	"	111		16.8	12.0	4.7	5.7	10.6	4.7

Series 3. Milk from source A. inoculated with 1

9-1	Past.	6	37.6	5.0	3.1	1.8	2.0	3.1	2.2
	Past.+1⅛%								
9-2	L.casei 5	6	38.0	4.5	2.5	2.0	1.8	2.6	1.9
	Past.+1⅛%								
9-3	L.casei 6	6	38.2	4.4	2.4	2.0	1.5	2.9	1.3
9-1	"	40		11.2	7.6	3.6	3.8	7.4	3.4
9-2	"	40		11.6	7.8	3.6	5.2	6.6	3.0
9-3	"	40		11.4	7.5	3.8	4.0	7.3	3.4
9-1	"	71		13.3	8.6	4.6	5.2	7.8	3.6
9-2	"	71		12.9	8.5	4.4	4.0	8.2	3.4
9-3	"	71		14.4	9.9	4.6	5.6	8.8	4.5
9-1	"	93		14.4	9.6	4.0	5.8	8.5	3.9
9-2	"	93		14.5	9.9	4.6	5.0	9.6	3.7
9-3	"	93		17.6	13.1	4.4	6.8	10.7	5.4

*Calculated from milligrams of amino nitrogen (amino nitrogen determined

.2	1.0	4.2	0.9	4.1	0.78				
.8	1.6	4.6	1.8	4.3	0.94				
.4	1.2	4.4	1.4	4.1	0.87				
.8	2.2	5.8	2.0	5.8	1.34		38⅜		Fair, slightly flat
.2	2.8	6.2	2.3	6.8	1.47		37		Poor, sour, lacking
.4	2.1	6.5	2.0	6.4	1.41		38		Fair, sl. sour, lacking
.5	2.4	9.5	2.8	9.0	1.61		38		Fair, sour, flat
.4	3.2	9.2	3.5	9.1	1.70		38		Fair, sour, cheddar
.1	3.6	9.1	3.5	8.8	1.66		38½		Good, sl. sour, typical
.2	3.9	11.7	3.0	12.8	2.23		38		Fair, sl. lacking
.4	5.1	12.2	4.3	13.1	3.06		40		V. good, sl. sour, typical
.9	5.4	13.0	5.0	13.6	3.24		38½		Good, sl. sour, typical

ated with 1⅓% of commercial cheese culture G.

.3	2.2	3.5	1.4	4.1	0.85				
.2	1.8	3.6	1.6	3.6	0.72				
.2	2.3	3.6	1.8	4.0	0.83				
.5	2.2	6.1	2.0	6.3	1.40		39		Good, sl. lacking
.3	2.1	6.6	1.8	7.0	1.48		38½		Good, sl. lacking, sour
.5	2.5	6.5	2.4	6.8	1.56		40		V. good, cheddar, buttery
.1	3.0	10.1	2.6	10.6	2.06		38		Fair, sour, sl. lacking
.0	3.4	10.3	2.9	11.0	2.17		38		Fair, sour, sl. lacking
.2	4.0	10.5	3.4	11.1	2.74		39½		V.good, v. cheddar, buttery
.4	3.4	11.2	3.2	11.4	2.81		37⅝		Poor, sour, bitter
.2	3.8	11.3	3.4	11.7	2.96		38		Fair, sl. sour, bitter
.6	4.7	12.0	3.9	12.8	3.44		39½		V. good, rich, v. buttery

ated with 1⅓% of commercial cheese culture G.

.1	2.2	2.8	1.2	3.9	0.78				
.6	1.9	2.6	0.9	3.5	0.70				
.9	1.3	3.3	1.2	3.2	0.78				
.4	3.4	7.9	2.6	8.8	1.55		39		Good, lacking
.6	3.0	8.6	2.6	8.8	1.48		39⅛		Good, lacking
.3	3.4	7.8	2.7	8.6	1.59		39⅛		Good, sl.lacking
.8	3.6	9.5	3.2	10.0	2.11		38		Fair, lacking
.2	3.4	9.4	2.6	10.1	2.01		38½		Fair, lacking
.8	4.5	10.0	3.8	10.7	2.21		39⅛		Good, typical cheddar
.5	3.9	10.6	3.6	10.8	2.58		39		Good, sl.lacking
.6	3.7	10.9	3.2	11.4	2.36		39		Good, sl. lacking
.7	5.4	12.3	4.3	13.2	3.52		39⅛		V. good, v. cheddar

etermined by the Van Slyke gasometric method).

ripening, the cheese containing L. casei strains 0, 2, 4, or 6, showed a more rapid and extensive breakdown than the control cheese, while the cheese containing L. casei strains 3 or 5 showed about the same rate and extent of the protein breakdown as the control cheese.

During the early stages of ripening, the amounts of total nitrogen in the juices of the cheese containing L. casei strains 0, 2, 4 or 6, were invariably slightly larger than the amounts in the juices of the control cheese. With longer ripening, larger differences between the amounts of nitrogen in the juices of the cheese containing L. casei strains 0, 2, 4, or 6, and the amounts in the juices of the control cheese developed; after about four months, the amounts in the juices of the cheese containing L. casei strains 0, 2, 4, or 6, were about the same, and were in all cases decidedly larger than the amounts in the juices of the control cheese. Throughout the ripening, the amounts of total nitrogen in the juices of cheese containing L. casei strains 3 or 5 were regularly about the same as the amounts in the juices of the control cheese.

At each determination, the amounts of amino nitrogen and the nitrogen soluble or insoluble in trichloracetic acid, ethyl alcohol, phosphotungstic acid, and tungstic acid, were very similar in the juices of the cheese containing L. casei strains 0, 2, 4, or 6. In practically all cases

the amounts were larger than those in the juices of the cheese containing L. casei strains 3 or 5, and of the control cheese, especially in the fraction of nitrogen which was soluble in trichloracetic acid. However, in a few cases the fraction of nitrogen which was insoluble in trichloracetic acid was larger in the juices of cheese containing L. casei strains 3 or 5 than in the juices of the cheese containing L. casei strains 0, 2, 4, and 6. At each determination, the amounts of the various nitrogenous fractions were about the same in the juices of the cheese containing the L. casei strains 3 or 5, and of the control cheese.

The flavor scores of the cheese containing L. casei strains 0, 2, 4, or 6, were very similar and, in practically all cases, relatively high compared to the scores of the cheese containing L. casei strains 3 or 5, and of the control cheese. Although most of the cheese containing L. casei strains 0, 2, 4, or 6, developed a slightly sour flavor during the early stages of ripening, a characteristic cheddar flavor regularly developed after about two months of ripening, at which time the sour flavor usually disappeared. The cheese containing L. casei strains 0 and 4 were characterized by having a rich, buttery flavor in addition to the characteristic cheddar flavor. The buttery flavor, which appeared early in the ripening period, had decreased somewhat in intensity after about three months of ripening, and a sour flavor usually

developed in the cheese. The flavor scores of the cheese containing L. casei strains 3 or 5 and of the control cheese were regularly about the same, and these cheese were characterized by a lack of typical cheddar flavor; in some cases a bitter flavor was evident.

Table VI summarizes the effect of adding each of the several strains of L. casei to pasteurized milk used for making cheddar cheese on the amounts and percentages of various forms of nitrogen in the juice of ripened cheese. In all of the cheese juices, no constant variations were shown in the amounts and percentages of the fractions classed as nitrogen soluble in tungstic acid but not amino nitrogen, nitrogen soluble in phosphotungstic acid but not in tungstic acid, and nitrogen soluble in ethyl alcohol but not in phosphotungstic acid. However, consistent variations were shown in the amounts and percentages of the fractions classed as nitrogen soluble in trichloracetic acid but not in ethyl alcohol, and nitrogen insoluble in trichloracetic acid. In practically all cases, the juices of cheese containing strains of L. casei showed a greater amount and percentage of the fraction which was soluble in trichloracetic acid but not in ethyl alcohol, and a smaller amount and percentage of the fraction which was insoluble in trichloracetic acid, than the juices of the control cheese. The large amount and percentage of the fraction which was soluble in trichloracetic

TABLE VI

THE EFFECT OF ADDING SEVERAL STRAINS OF L. CASEI TO PASTEU
AMOUNTS AND PERCENTAGES OF VARIOUS FORMS OF NITROGI

Milk used			
Past.	Past. + 1⅛% L. casei culs.	Past. + 1⅛ % L. casei culs.	Various fractions of nitrog
cc. of 0.1 normal acid equiv. to the nitrogen fractions of 1 cc. of cheese juice			
Series 1. Cheese ripened 110 days made from source I			
	a.*	b.*	
22	3.0	3.2	Amino N
0.8	1.3	1.8	N. soluble in tungstic acid
0.9	0.8	0.4	N. soluble in phosphotungstic acid
1.5	1.7	1.9	N. soluble in ethyl alcohol but no
4.0	4.4	4.5	N. soluble in trichloracetic acid
6.4	6.0	6.6	N. insoluble in trichlor
15.8	17.2	18.4	Total N
Series 2. Cheese ripened 111 days made with source A			
	c.*	d.*	
2.8	3.0	3.4	Amino N
0.4	0.4	0.5	N. soluble in tungstic acid
0.2	0.4	0.8	N. soluble in phosphotungstic acid
0.8	0.9	1.0	N. soluble in ethyl alcohol but no
5.1	5.3	6.3	N. soluble in trichloracetic acid
5.3	4.9	4.7	N. insoluble in trichl
14.4	14.9	16.7	Total N
Series 3. Cheese ripened 93 days made with source A.			
	e.*	f.*	
2.6	2.4	3.5	Amino N
1.0	0.8	0.8	N. soluble in tungstic acid
0.3	0.5	1.1	N. soluble in phosphotungstic acid
1.9	1.3	1.4	N. soluble in ethyl alcohol but no
3.8	4.9	6.3	N. soluble in trichloracetic acid
5.0	4.6	4.4	N. insoluble in trichl
14.6	14.5	17.5	Total N

*a = Strain 0 b. = Strain 2 c. = Strain 5 d. = Strain 4 e.

ABLE VI

TO PASTEURIZED MILK USED FOR MAKING CHEDDAR CHEESE ON THE
OF NITROGEN IN THE JUICE OF RIPENED CHEESE

of nitrogen in cheese juice	Milk used		
	Past.	Past. + 1½% L. casei culs.	Past. + 1½% L. casei culs.
	% of total nitrogen made up of various fractions		

n source B. milk inoculated with 1½% commercial culture G

		a.*	b.*
Amino N.	14	17	17
stic acid but not amino nitrogen	5	8	10
gstic acid but not in tungstic acid	6	5	2
nol but not in phosphotungstic acid	10	9	10
stic acid but not in ethyl alcohol	25	26	25
n trichloracetic acid	40	35	36
Total N.			

n source A. milk inoculated with 2% commercial culture G.

		c.*	d.*
Amino N.	19	20	20
stic acid but not amino nitrogen	3	3	3
gstic acid but not in tungstic acid	1	3	4
nol but not in phosphotungstic acid	5	6	6
stic acid but not in ethyl alcohol	35	35	39
in trichloracetic acid	37	33	28
Total N.			

source A. milk inoculated with 2% commercial culture G.

		e*	f.*
Amino N.	18	17	20
stic acid but not amino nitrogen	7	6	5
gstic acid but not in tungstic acid	2	3	6
nol but not in phosphotungstic acid	13	9	8
stic acid but not in ethyl alcohol	26	34	36
in trichloracetic acid	34	31	25
Total N.			

In 4 e. = Strain 5 f. = Strain 6

acid but not in ethyl alcohol, and the small amount and percentage of the fraction insoluble in trichloracetic acid, was especially evident in the cheese of higher quality, namely, the cheese which contained L. casei strains 0, 2, 4, or 6.

d. The effect of adding Aerobacter oxytocum or Streptococcus liquefaciens to pasteurized milk used for making cheddar cheese on the nitrogen distribution in the cheese and on the flavor.

The effect of adding A. oxytocum or S. liquefaciens to pasteurized milk used for making cheddar cheese on the nitrogen distribution in the cheese and on the flavor was studied with two series of cheese. Each series consisted of three cheese, manufactured at the same time from equal portions of a single lot of milk; one was made from pasteurized milk, one from pasteurized milk plus A. oxytocum, and one from pasteurized milk plus S. liquefaciens . In the first series, the milk was inoculated with 1 per cent of a milk culture of the test organisms and in the second series with 2 per cent. Table VII gives the results obtained in the chemical analyses of cheese juice, and in the scoring of the cheese for flavor.

A steady breaking down of the proteins in all the cheese was indicated by increases in the various nitrogenous

TABLE VII

THE EFFECT OF ADDING A. OXYTOCUM OR S. LIQUEFACIENS
CHEDDAR CHEESE ON THE NITROGEN DISTRIBUTION

Cheese number	Milk used	Age in days	% H_2O in cheese	cc. of 0.1 normal acid equiv. to nitrogen: Total Nitrogen	Nitrogen fractionated into sol. Trichloracetic acid Sol.	Trichloracetic acid Insol.	Ethyl alcohol Sol.	Ethyl alcohol Insol.	t
				Series 1. Milk from source C. inoculated					
10-1	Pasteurized	7	40.2	5.8	3.6	2.2	2.3	3.3	
	Past. + 1% Aerobacter								
10-2	oxytocum	7	39.8	5.8	3.8	2.0	2.0	3.8	
	Past. + 1% Streptococcus								
10-3	liquefaciens	7	39.4	6.5	4.5	2.1	2.3	4.0	
10-1	"	38		9.1	6.0	3.0	2.9	6.1	
10-2	"	38		9.1	5.6	3.4	3.1	5.9	
10-3	"	38		12.5	8.8	3.6	4.0	8.4	
10-1	"	75		10.5	7.5	2.8	3.7	6.7	
10-2	"	75		10.8	7.2	3.4	3.8	6.9	
10-3	"	75		14.9	11.0	3.8	5.6	9.3	
				Series 2. Milk from source B. inoculated					
11-1	Pasteurized	7	40.8	5.3	2.9	2.2	1.6	3.5	
	Past. + 2% Aerobacter								
11-2	oxytocum	7	40.2	5.7	3.2	2.4	1.5	4.1	
	Past. + 2% Streptococcus								
11-3	liquefaciens	7	40.6	17.4	15.0	2.4	7.4	9.8	
11-1	"	39		7.9	5.0	2.9	3.6	4.4	
11-2	"	39		10.1	5.6	4.4	3.2	6.7	
11-3	"	39		24.0	21.3	2.8	10.4	13.5	
11-1	"	70		9.6	5.9	3.8	4.2	5.4	
11-2	"	70		11.8	6.2	5.6	4.3	7.4	
11-3	"	70		27.4	24.4	3.0	13.4	14.0	

*Calculated from milligrams of amino nitrogen (amino nitrogen determined

ABLE VII

QUEFACIENS TO PASTEURIZED MILK USED FOR MAKING
RIBUTION IN THE CHEESE AND ON THE FLAVOR

nitrogen of 1 cc. of cheese juice into sol. and insol. portions with-								
yl	Phospho-		Tungstic		*Amino	Flavor		Remarks on
hol	tungstic acid		acid			score of		
Insol.	Sol.	Insol.	Sol.	Insol.	Nitrogen	cheese		cheese flavor
noculated with 1½% of commercial cheese culture C.								
3.3	1.6	4.3	1.3	4.7	1.11			
3.8	1.4	4.3	1.4	4.6	1.00			
4.0	2.0	4.6	1.6	5.0	1.33			
6.1	2.4	6.6	2.0	7.1	1.95	38	Fair, lacking	
5.9	2.3	6.8	2.2	7.0	1.73	37	Poor, unclean	
8.4	3.3	9.2	2.9	9.6	2.44	38½	Good, typical cheddar	
6.7	3.2	7.4	3.1	7.5	2.93	38	Fair, lacking	
6.9	3.1	7.6	3.0	7.9	2.77	37½	Poor, unclean	
9.3	4.2	10.7	3.9	11.1	3.79	39	Good, typical cheddar	
noculated with 2% of commercial cheese culture C.								
3.5	1.7	3.5	1.6	3.7	1.21			
4.1	1.8	4.1	1.6	4.2	1.06			
9.8	3.0	14.3	2.6	14.6	2.66			
4.4	3.0	6.0	2.6	5.2	2.41	38	Fair, lacking	
6.7	2.6	7.4	2.3	7.8	2.19	35	Poor, v. unclean	
13.5	6.8	17.4	6.5	17.6	6.36	32	V. poor, v. bitter	
5.4	3.6	6.1	5.4	6.3	3.14	38½	Fair, lacking	
7.4	3.3	8.4	3.0	9.0	2.44	36	Poor, unclean, fermented	
14.0	8.4	19.2	8.0	19.7	7.58	30	V. poor, v. bitter	

etermined by the Van Slyke gasometric method).

fractions. Throughout the ripening, the juices of cheese made from milk containing 1 per cent of the A. oxytocum culture showed about the same amounts of nitrogen in the various forms as the control cheese, while the juices of the cheese made from milk containing 2 per cent of the A. oxytocum culture showed amounts which varied from those of the control cheese. During the early stages of ripening, the amounts of total nitrogen in the juice of the cheese made from milk containing 2 per cent of the A. oxytocum culture were slightly higher than the amounts in the juice of the control cheese; while after about two months of ripening, the amounts were considerably higher in the juice of the cheese made with A. oxytocum. Although the amounts of total nitrogen were regularly higher in the juice of the cheese made from milk containing 2 per cent of the A. oxytocum culture than in the juice of the control cheese, the amounts of amino nitrogen in the juice of the cheese containing A. oxytocum were regularly lower, than the amounts in the juice of the control cheese. The fractions of nitrogen which were insoluble in trichloracetic acid, ethyl alcohol, phosphotungstic acid, and tungstic acid were, in all cases, considerably higher in the juice of the cheese made from milk containing 2 per cent of the A. oxytocum culture than in the juice of the control cheese, while the fractions soluble in ethyl alcohol, phosphotungstic acid, and tungstic acid were either about the

same or slightly lower in the juice of cheese made with A. oxytocum culture than in the juice of the control cheese.

Throughout the ripening, the amounts of total nitrogen in the juices of the cheese made from milk containing 1 per cent of the S. liquefaciens culture were consistently larger than the amounts in the juice of the control cheese. During the early stages of ripening, the amount of total nitrogen in the juice of cheese made from milk with 1 per cent of the S. liquefaciens culture was only slightly larger than the amount in the juice of the control cheese; while after about two months, the amount was considerably higher. The amounts of the various nitrogenous fractions were always larger in the juice of cheese made from milk plus 1 per cent of the S. liquefaciens culture than in the juice of the control cheese. The fraction which was soluble in trichloracetic acid, and the fractions which were insoluble in ethyl alcohol, phosphotungstic acid, and tungstic acid were especially large in the juice of the cheese made with S. liquefaciens.

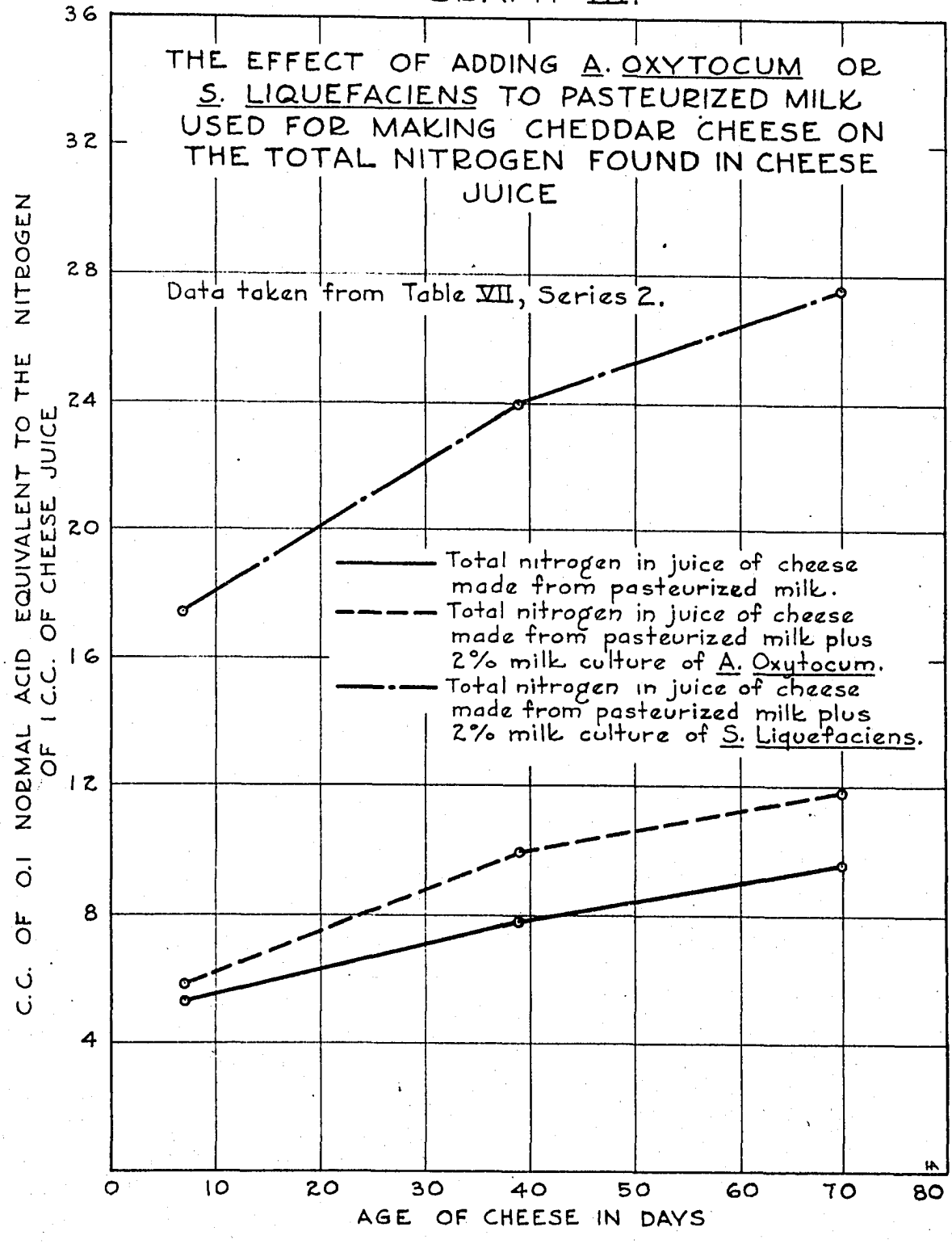
There were extremely large differences in the amounts of the various forms of nitrogen in the juice of the cheese made from milk containing 2 per cent of the S. liquefaciens culture, and in the juice of the control cheese. Throughout the ripening, the amounts of total nitrogen in the juice of the cheese with S. liquefaciens were about three times those in the juice of the control cheese. The amounts of the

various fractions of nitrogen were all regularly two to four times as large in the juice of cheese made with S. liquefaciens culture, as in the juice of the control cheese, except for the fraction insoluble in trichloracetic acid. This fraction was usually slightly smaller in the juice of cheese made with S. liquefaciens than in the juice of the control cheese.

Graph III illustrates the amounts and rates of increase in the total nitrogen of the juices of the three types of cheese. The data for the graph were obtained from Table VII, Series 2. The amounts of total nitrogen in the juice of the cheese made from milk plus 2 per cent A. oxytocum culture were noticeably larger than the amounts in the juices of the control cheese throughout the ripening, while the rate of increase in the total nitrogen of the cheese made with A. oxytocum was slightly more rapid than the rate of increase in the juice of the control cheese. The amounts of total nitrogen in the juice of the cheese made from milk plus 2 per cent S. liquefaciens were about three times those in the juice of the control cheese throughout the ripening, while the rate of increase in the total nigrogen was considerably more rapid in the juice of cheese made with S. liquefaciens than the increase in the juice of the control cheese.

The flavor scores of the cheese made from milk containing 1 per cent of the A. oxytocum culture were in all cases slightly lower than the scores of the control cheese, since an unclean

GRAPH III.


THE EFFECT OF ADDING A. OXYTOCUM OR S. LIQUEFACIENS TO PASTEURIZED MILK USED FOR MAKING CHEDDAR CHEESE ON THE TOTAL NITROGEN FOUND IN CHEESE JUICE
Data taken from Table VII, Series 2.
Total nitrogen in juice of cheese made from pasteurized milk.
Total nitrogen in juice of cheese made from pasteurized milk plus 2% milk culture of A. Oxytocum.
Total nitrogen in juice of cheese made from pasteurized milk plus 2% milk culture of S. Liquefaciens.
C.C. OF 0.1 NORMAL ACID EQUIVALENT TO THE NITROGEN OF 1 C.C. OF CHEESE JUICE
36
32
28
24
20
16
12
8
4
0
10
20
30
40
50
60
70
80
AGE OF CHEESE IN DAYS

flavor was regularly present in the cheese during the entire ripening period. The flavor scores of the cheese made from milk containing 2 per cent of the A. oxytocum culture were invariably much lower than the scores of the control cheese; the cheese was characterized by having an unclean, fermented flavor, and a gassy body.

The flavor scores of the cheese made from milk containing 1 per cent of the S. liquefaciens culture were regularly higher than the scores of the control cheese. A typical cheddar flavor developed in the cheese made with S. liquefaciens after about one month of ripening; after two months, the cheddar flavor became more pronounced, and was accompanied by a rapid breakdown of the cheese body. The flavor scores of the cheese made from milk plus 2 per cent of the S. liquefaciens culture were decidedly low throughout the ripening. The cheese had an extremely bitter flavor, and a very soft, pasty body.

The effect of adding A. oxytocum or S. liquefaciens to pasteurized milk used for making cheddar cheese on the amounts and percentages of the various forms of nitrogen in the juice of ripened cheese is summarized in Table VIII. In the juices of the three types of cheese, there was no consistency in the variations in the amounts and percentages of the nitrogen soluble in tungstic acid but not amino nitrogen, nitrogen soluble in phosphotungstic acid but not in tungstic

TABLE VIII

THE EFFECT OF ADDING A. OXYTOCUM OR S. LIQUEFACIENS TO PAST
AMOUNTS AND PERCENTAGES OF VARIOUS FORMS OF N

Milk used			
Past.	Past. + A.oxytocum	Past. + S.liquefaciens	Various fractions of nitro
cc. of 0.1 normal acid equiv. to the nitrogen fractions of 1 cc. of cheese juice			
Series 1. Cheese ripened 75 days made from source C			
	a.*	b.*	
2.9	2.8	3.7	Amino N.
0.2	0.2	0.2	N. soluble in tungstic acid bu
0.1	0.1	0.3	N.soluble in phosphotungstic acid b
0.5	0.7	1.4	N.soluble in ethyl alcohol but not
3.8	3.4	5.4	N.soluble in trichloracetic acid bu
2.8	3.4	3.8	N. insoluble in trichlor
10.3	10.6	14.8	Total N.
Series 2. Cheese ripened 70 days made from source D			
	c.*	d.*	
3.1	2.4	7.6	Amino N.
0.3	0.6	0.4	N. soluble in tungstic acid bu
0.2	0.3	0.4	N.soluble in phosphotungstic acid b
0.6	1.0	5.0	N. soluble in ethyl alcohol but not
1.7	1.9	11.0	N. soluble in trichloracetic acid b
3.8	5.6	3.0	N. insoluble in trichlor
9.7	11.8	27.4	Total N.

*a. = 1% A. oxytocum culture b. = 1% S. liquefaciens culture c. = 2% A. o

TABLE VIII

ENS TO PASTEURIZED MILK USED FOR MAKING CHEDDAR CHEESE ON THE
FORMS OF NITROGEN IN THE JUICE OF RIPENED CHEESE

ons of nitrogen in cheese juice	Milk used		
	Past.	Past. + A.oxytocum	Past. + S.liquefaciens
	% of total nitrogen made up of various fractions		
rom source C. milk inoculated with 1½% commercial culture C.			
		a.*	b.*
Amino N.	28	26	25
tic acid but not amino nitrogen	2	2	1
gstic acid but not in tungstic acid	1	1	2
ol but not in phosphotungstic acid	5	7	9
tic acid but not in ethyl alcohol	36	32	37
in trichloracetic acid	28	32	26
Total N.			
rom source B. milk inoculated with 2% commercial culture C.			
		c.*	d.*
Amino N.	32	20	28
tic acid but not amino nitrogen	3	5	1
gstic acid but not in tungstic acid	2	3	1
hol but not in phosphotungstic acid	6	9	18
etic acid but not in ethyl alcohol	18	16	40
in trichloracetic acid	39	47	12
Total N.			

c. = 2% A. oxytocum culture d. = 2% S. liquefaciens culture

acid, and nitrogen soluble in ethyl alcohol but not in phosphotungstic acid. However, in the juices of the three types of cheese, there were consistent variations in the amounts and percentages of nitrogen soluble in trichloracetic acid but not in ethyl alcohol, and nitrogen insoluble in trichloracetic acid. The juices of cheese made with A. oxytocum regularly contained larger amounts and percentages of the fraction which was insoluble in trichloracetic acid, and smaller amounts and percentages of the fraction which was soluble in trichloracetic acid but not in ethyl alcohol, than the juices of the control cheese. In the case of the juices of cheese containing S. liquefaciens, the results were reversed. The juices of cheese made with S. liquefaciens, (especially in Series 2, where 2 per cent S. liquefaciens was added to the milk), contained much smaller amounts and percentages of the fraction which was insoluble in trichloracetic acid, and much larger amounts and percentages of the fraction which was soluble in trichloracetic acid but not in ethyl alcohol, than the juice of the control cheese.

e. The effect of adding a Micrococcus or S. paracitrovorus to pasteurized milk used for making cheddar cheese on the nitrogen distribution in the cheese and on the flavor

The effect of adding an unidentified Micrococcus or S. paracitrovorus to pasteurized milk used for making cheddar cheese on the nitrogen distribution in the cheese and on the flavor was studied with two series of cheese. Three cheese, manufactured at the same time from equal portions of a single lot of milk were included in each series; one cheese was made from pasteurized milk, one from pasteurized milk plus 2 per cent of a milk culture of the Micrococcus and one from pasteurized milk plus 2 per cent of a milk culture of S. paracitrovorus. The results of the chemical analyses of the cheese juice and of the scoring of the cheese for flavor, are given in Table IX.

Increases in the various nitrogenous fractions in all of the cheese during the ripening indicated a steady breaking down of the proteins. There was little variation in the amounts of total nitrogen in the juices of the three types of cheese during the early stages of ripening. However, after about two months of ripening, the juices of the cheese made from milk plus the Micrococcus or S. paracitrovorus regularly showed slightly larger amounts of total nitrogen than the juices of the control cheese.

TABLE IX

THE EFFECT OF ADDING A MICROCOCCUS OR S. PARACITRO
CHEDDAR CHEESE ON THE NITROGEN DISTRIBUTION

Cheese number	Milk used	Age in days	% H_2O in cheese	cc. of 0.1 normal acid equiv. to nitrog: Total Nitrogen	Nitrogen fractionated into s: Trichloracetic acid Sol.	Trichloracetic acid Insol.	Ethyl alcohol Sol.	Ethyl alcohol Insol.
Series 1. Milk from source A. inoculate								
12-1	Pasteurized	4	37.4	5.8	3.0	2.5	2.0	3.8
	Past. + 2%							
12-2	Micrococcus	4	38.0	5.4	3.3	1.8	1.9	3.6
	Past. + 2%							
12-3	S. paracitrovorus	4	38.0	5.8	3.2	2.3	2.1	3.8
12-1		33		7.4	4.8	2.6	2.6	4.8
12-2	"	33		7.8	5.6	2.1	2.7	4.9
12-3		33		7.6	5.1	2.5	2.6	4.8
12-1		62		9.2	7.0	2.2	3.8	5.4
12-2	"	62		10.1	7.8	2.2	4.2	6.0
12-3		62		10.0	7.7	2.3	4.0	6.2
Series 2. Milk from source B. inoculate								
13-1	Pasteurized	4	38.2	5.4	3.0	2.1	2.1	3.2
	Past. + 2%							
13-2	Micrococcus	4	38.6	5.1	2.9	2.0	2.2	3.0
	Past. + 2%							
13-3	S. paracitrovorus	4	39.0	4.7	2.8	1.8	1.5	3.2
13-1		27		8.8	5.4	3.6	3.2	5.6
13-2	"	27		8.4	5.2	3.2	2.6	5.9
13-3		27		9.1	5.4	3.6	2.9	6.0
13-1		65		10.3	6.6	3.8	4.5	5.7
13-2	"	65		11.3	8.0	3.4	4.6	6.6
13-3		65		11.6	7.6	3.9	4.7	6.8

*Calculated from milligrams of amino nitrogen (amino nigrogen determined

ABLE IX

. PARACITROVORUS TO PASTEURIZED MILK USED FOR MAKING
STRIBUTION IN THE CHEESE AND ON THE FLAVOR

. to nitrogen of 1 cc. of cheese juice									
ated into sol. and insol. portions with-									
Ethyl alcohol		Phospho-tungstic acid		Tungstic acid		*Amino Nitrogen	Flavor score of cheese		Remarks on cheese flavor
ol.	Insol.	Sol.	Insol.	Sol.	Insol.				
. inoculated with 2% of commercial cheese culture C.									
2.0	3.8	1.3	4.3	1.0	4.6	1.16			
1.9	3.6	1.6	3.6	0.8	4.6	1.00			
2.1	3.8	1.7	4.0	1.4	4.4	1.13			
2.6	4.8	1.8	5.8	1.0	6.5	1.53	38½		Fair, lacking
2.7	4.9	1.9	6.0	1.7	6.1	1.70	40		Good, sl. lacking
2.6	4.8	2.0	5.7	1.5	6.0	1.64	39		Good, pleasing, full
3.8	5.4	2.6	6.6	2.4	7.0	2.17	38½		Fair, lacking
4.2	6.0	2.9	7.3	2.8	7.4	2.58	39½		Good, typical cheddar
4.0	6.2	3.0	7.2	2.7	7.3	2.34	40		V. good, pleasing, ful
. inoculated with 2% of commercial cheese culture C.									
2.1	3.2	1.8	3.5	1.3	3.9	1.08			
2.2	3.0	2.0	3.2	1.4	3.7	1.02			
1.5	3.2	1.3	3.4	1.0	3.7	0.81			
3.2	5.6	2.6	6.4	2.0	6.8	1.56	39		Good, lacking
2.6	5.9	2.4	6.0	2.2	6.2	1.44	38		Fair, lacking
2.9	6.0	2.2	6.8	1.8	7.0	1.68	39		Good, pleasing, full
4.5	5.7	3.5	6.7	3.3	7.2	2.26	40		Good, sl. lacking
4.6	6.6	3.7	7.6	3.4	8.1	2.67	40½		V. good, typical
4.7	6.8	3.1	8.6	2.8	8.9	2.71	40½		V. good, mild, full

determined by the Van Slyke gasometric method).

The amounts of amino nitrogen in the juices of the three types of cheese were about the same during the early stages of ripening. After about two months, the amounts of amino nitrogen in the cheese made from milk plus the Micrococcus or S. paracitrovorus were in all cases slightly larger than the amounts in the juices of the control cheese.

The amounts of nitrogen in the fractions of cheese juice which were soluble in trichloracetic acid, insoluble in phosphotungstic acid, and insoluble in tungstic acid, were about the same in the cheese made from milk plus the Micrococcus and in the cheese made from milk plus S. paracitrovorus, and were invariably slightly larger than the amounts in the juices of the control cheese. The amounts of nitrogen in the fractions which were insoluble in trichloracetic acid, soluble or insoluble in ethyl alcohol, soluble in phosphotungstic acid, and soluble in tungstic acid, were very similar in the juices of the three types of cheese.

Little variation was shown in the flavor scores of the three types of cheese during the ripening, although the scores of the cheese made from milk plus the Micrococcus or S. paracitrovorus were usually slightly higher than the scores of the control cheese. The cheese made from milk plus the Micrococcus developed a more pronounced cheddar flavor than the control cheese after about two months of ripening. The cheese made from milk plus S. paracitrovorus developed

during the early stages of ripening, a mild, buttery flavor and aroma which was not present in the control cheese. The flavor and aroma persisted as long as the cheese was held.

The effect of adding the Micrococcus or S. paracitrovorus to pasteurized milk used for making cheddar cheese on the amounts and percentages of the various forms of nitrogen in the juice of ripened cheese is summarized in Table X. In the juices of the three types of cheese, the amounts and percentages of the various nitrogenous fractions were very similar, with the exception of the percentages of the fraction soluble in trichloracetic acid but not in ethyl alcohol, and the fraction insoluble in trichloracetic acid. Slightly larger percentages of the former fraction, and smaller percentages of the latter fraction, were regularly present in the juices of cheese made from milk plus the Micrococcus or S. paracitrovorus, as compared to the percentages of the same fractions in the juices of the control cheese.

TABLE X

THE EFFECT OF ADDING A MICROCOCCUS OR S. PARACITROVORUS TO
ON THE AMOUNTS AND PERCENTAGES OF VARIOUS FORMS OF N

Milk used			
Past.	Past. + 2% Micrococcus cult.	Past. + 2% S. paracitrovorus	Various fractions of nitroge
cc. of 0.1 normal acid equiv. to the nitrogen fractions of 1 cc. of cheese juice			
Series 1. Cheese ripened 62 days made from source A. m			
2.2	2.6	2.3	Amino N.
0.2	.2	0.4	N. soluble in tungstic acid b
0.2	.1	0.3	N. soluble in phosphotungstic acid
1.2	1.3	1.0	N. soluble in ethyl alcohol but not
3.2	3.6	3.7	N. soluble in trichloracetic acid b
2.2	2.2	2.3	N. insoluble in trichlo
9.2	10.0	10.0	Total N.
Series 2. Cheese ripened 65 days made from source B. m			
2.3	2.7	2.7	Amino N.
1.0	0.7	0.1	N. soluble in tungstic acid b
0.2	0.3	0.3	N. soluble in phosphotungstic acid
1.0	0.9	1.6	N. soluble in ethyl alcohol but not
2.1	3.4	2.9	N. soluble in trichloracetic acid b
3.8	3.4	3.9	N. insoluble in trichlo
10.4	11.4	11.5	Total N.

TABLE X

ROVORUS TO PASTEURIZED MILK USED FOR MAKING CHEDDAR CHEESE
FORMS OF NITROGEN IN THE JUICE OF RIPENED CHEESE

of nitrogen in cheese juice	Milk used		
	Past.	Past. + 2% Micrococcus cult.	Past. + 2% S. paracitrovorus
	% of total nitrogen made up of various fractions		
source A. milk inoculated with 2% commercial culture C.			
Amino N.	23	26	23
stic acid but not amino nitrogen	2	2	4
gstic acid but not in tungstic acid	2	1	3
hol but not in phosphotungstic acid	14	13	10
etic acid but not in ethyl alcohol	35	36	37
in trichloracetic acid	24	22	23
Total N.			
source B. milk inoculated with 2% commercial culture C.			
Amino N.	22	23	23
stic acid but not amino nitrogen	10	6	1
gstic acid but not in tungstic acid	2	3	3
hol but not in phosphotungstic acid	10	8	14
etic acid but not in ethyl alcohol	20	30	25
in trichloracetic acid	36	30	34
Total N.			

DISCUSSION OF RESULTS

The general belief that cheddar cheese made from pasteurized milk usually requires longer ripening and contains less typical cheddar flavor than cheese made from raw milk, is substantiated by the data obtained. The experimental cheese made from pasteurized milk regularly showed less rapid and extensive decomposition of the proteins and a less pronounced cheddar flavor than the cheese made from raw milk.

The results presented show that the addition of various organisms, either in the form of pure cultures or in the form of raw milk, definitely hastened the breakdown of the proteins and in most cases improved the flavor of cheddar cheese made from pasteurized milk. The addition of 10 per cent raw milk to pasteurized milk used for cheese making probably contributed, to the milk, various types of bacteria which are desirable from the standpoint of cheese ripening. The cheese made from pasteurized milk plus 10 per cent raw milk was very similar in nitrogen distribution and flavor to cheese made from raw milk. The application of this information should be of practical significance in commercial cheese factories where pasteurization of the greater part of the milk is necessary. For example, when the milk is received, a small percentage

of raw milk, known to be of high quality, could be set aside and later added to the pasteurized milk before the cheese making process was begun. In connection with the effect of adding raw to pasteurized milk to be made with cheddar cheese, it should be noted that Price (44) was unable to improve the quality of pasteurized milk cheese by the addition of small quantities of raw milk cheese to the milk.

The fact that each of several strains of L. casei, when added to pasteurized milk used for cheese making, hastened the ripening, intensified the typical cheddar flavor and in the case of a few strains regularly caused the development of an unusual, buttery flavor in the resulting cheese, suggests the important influence these organisms exert on the ripening of cheese. To account for the fact that the flavor scores of the cheese containing the buttery flavor were only slightly higher than the scores of the other cheese in the same series, it is necessary to realize that the buttery flavor was not characteristic of typical cheddar cheese. However, since the buttery flavor invariably developed in cheese containing certain L. casei strains, the production of special flavored cheese of the cheddar type by the use of these organisms may be of practical significance.

The fact that large numbers of A. oxytocum, when added to pasteurized milk used for cheese making, produced a deleterious effect on the body and the flavor of the cheese

was expected, since bacteria of the genus Aerobacter generally produce large quantities of gas and an unclean flavor in cheese and other dairy products. The large amounts of a nitrogenous fraction insoluble in trichloracetic acid, which were regularly found in the juices of cheese containing A. oxytocum, suggests that these organisms, under certain conditions, are capable of producing the more complex forms of soluble nitrogen from the proteins of cheese.

The relatively rapid, normal decomposition of cheese proteins that resulted from the addition of small numbers of S. liquefaciens to pasteurized milk used for making cheese, indicates that these organisms, if properly controlled, may be utilized in the production of well-ripened cheese in comparatively short periods of time. The rather sharp, characteristic cheddar flavor, which develops late in the ripening periods of high quality cheddar cheese, was quite evident in the cheese made from milk inoculated with small numbers of S. liquefaciens, after only a few months of ripening. The extremely bitter and soft bodied cheese which resulted from the inoculation of milk with large numbers of the same organism, demonstrated the deleterious effect of these bacteria in cheese when their numbers are not controlled.

The somewhat hastened protein breakdown and the slight improvement in the flavor of cheese resulting from the addition

of an unidentified Micrococcus to pasteurized milk used for cheese making, indicates that bacteria of this type may be of some importance from the standpoint of cheese ripening. Various investigators have reported that considerable numbers of micrococci are normally present in ripened cheese, but have not considered the organism a factor in cheese ripening. It is probable that certain types of micrococci, especially those types which are able to decompose proteins at relatively low temperatures, may be significant aids in the ripening process.

S. paracitrovorus would not be expected to have any considerable effect on the breakdown of the proteins in cheese, but since this organism is capable of fermenting citric acid with the formation of volatile acids, acetylmethylcarbinol and diacetyl, a desirable influence on the cheese flavor was anticipated when it was added to milk used for making cheese. The flavor produced by the addition of S. paracitrovorus was a mild, buttery flavor, which was desirable from the standpoint of bringing about full-flavored cheese after relatively short ripening periods. Hucker and Marquart (33) noted an improved cheese flavor resulting from the addition of S. paracitrovorus to the milk.

The fact that the juices of cheese made from raw milk, from pasteurized milk plus 10 per cent raw milk, and from pasteurized milk after the addition of desirable bacteria,

were all characterized by the presence of large amounts and percentages of a nitrogenous fraction which was soluble in trichloracetic acid but not in ethyl alcohol, suggests that the formation of relatively large amounts of a compound or compounds soluble in trichloracetic acid but not in ethyl alcohol, may be responsible, in part, for the characteristic flavor of high quality cheddar cheese.

CONCLUSIONS

1. Cheese juice or serum for analytical purposes was readily obtained from cheddar cheese by submitting mixtures of cheese and sand to relatively high pressures. The general procedure used was that developed by Barthel, Sandberg and Haglund (5); certain modifications were advantageous when the method was applied to cheddar cheese.
2. Cheddar cheese made from 90 per cent pasteurized milk and 10 per cent raw milk was very similar in nitrogen distribution and flavor to cheese made from raw milk. The cheese made from the mixture of pasteurized and raw milk or from raw milk showed a more rapid and extensive protein decomposition and a more typical cheddar flavor than cheese made from pasteurized milk.
3. Each of several strains of L. casei, when added to pasteurized milk used for making cheddar cheese, hastened the ripening and improved the flavor of the resulting cheese. Two of the strains used consistently produced an unusual, buttery flavor, which was very desirable.

4. A. oxytocum, when added to pasteurized milk used for making cheddar cheese, had a deleterious effect on the flavor and the body of cheese and produced more than a normal amount of nitrogenous compounds which were insoluble in trichloracetic acid.

5. S. liquefaciens, when added in small numbers to pasteurized milk used for making cheddar cheese, hastened the normal decomposition of cheese proteins and produced a pronounced cheddar flavor. The same organism, when added in large numbers, produced far more than the normal amounts of soluble nitrogen products and an extremely bitter flavor in the cheese.

6. An unidentified Micrococcus, when added to pasteurized milk used for making cheddar cheese, hastened somewhat the protein breakdown and slightly improved the flavor of the resulting cheese.

7. S. paracitrovorus, when added to pasteurized milk used for making cheddar cheese, had little effect on the breakdown of the proteins, but produced a mild, buttery flavor in the cheese during the early stages of ripening.

8. Cheese of high quality was regularly characterized by the presence, in the cheese juice, of relatively large amounts of a nitrogenous fraction which was soluble in trichloracetic acid but insoluble in ethyl alcohol, and also by the presence of relatively small amounts of a

fraction insoluble in trichloracetic acid. It appears that the formation of large amounts of a compound or compounds soluble in trichloracetic acid and insoluble in ethyl alcohol, may be responsible in part for the characteristic flavor of high quality cheddar cheese.

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