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The manufacture of blue cheese from pasteurized milk

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THE MANUFACTURE OF BLUE CHEESE FROM PASTEURIZED MILK

by

Carlton Edwin Parmelee

A Thesis Submitted to the Graduate Faculty
for the degree of

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Approved:

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INTRODUCTION

The blue cheese industry in the United States is now one of considerable importance. The investigational work which formed the basis for the industry was started just prior to 1914 when Thom, Matheson, and Currie (38) reported a method for the manufacture of blue cheese from cow's milk. Since that time the industry has grown steadily and has been aided in its growth by the development of improved methods for the manufacture and curing of blue cheese. Blue cheese manufacture in the United States has been encouraged by the high prices of imported Roquefort cheese and the impossibility of importations during the war years.

During the past four years the Dominion of Canada and at least six states of the United States have passed laws requiring that all cheese sold within their boundaries must be made from pasteurized milk, must be exposed to temperatures equivalent to pasteurization, or must be held for a period ranging from 60 to 120 days, depending upon the particular law. These laws were passed in an effort to prevent the recurrence of the cheese-borne epidemics that have been reported in recent years. A summary of the epidemics up to 1946 and the state laws enacted in an attempt to prevent further outbreaks has been presented by Fabian (10). Of

21 cheese-borne epidemics reported in the United States from 1936 to 1944, only one was traced to domestic Roquefort-type cheese.

The normal curing period for good blue cheese at present is longer than 60 days. However, during periods when demand far exceeds supply, some blue cheese has been sold after a curing period of only 14 days. With the present method of manufacture of blue cheese from homogenized raw milk, the curing process is so accelerated that the cheese normally is marketed before it is 120 days old.

In addition to the public health aspect, the pasteurization of milk for blue cheese is desirable for the control of certain microbial defects such as gasiness and unclean flavors. These defects are especially prevalent during the summer months, and some cheese plants are forced to quit making blue cheese during these months for that reason.

The main difference between raw milk blue cheese and pasteurized milk blue cheese from the standpoint of the normal ripening process is the absence of active milk lipase in the latter. The lipase is inactivated by pasteurization. In the raw milk cheese the lipase hydrolyzes the butter fat to glycerol and fatty acids. The lower molecular weight fatty acids and the compounds formed from them by the action of the mold are responsible for the characteristic peppery flavor of good blue cheese.

After preliminary studies of factors which influence flavor development in blue cheese, the studies reported were made to help the industry in the development of methods for making good blue cheese from pasteurized milk. An attempt was made to find microbial sources of lipase which could be substituted for the normal milk lipase in making blue cheese from pasteurized milk.

REVIEW OF LITERATURE

The manufacture of blue-veined cheese has been a well-established industry in Europe for many years. Marre (31) in 1906 published a popular text book on the history and methods of manufacture of French Roquefort cheese. The first work done in the United States with blue-veined cheese was published by Thom, Matheson, and Currie (38) in 1914. They reported a procedure for the manufacture of blue cheese from cows' milk and concluded that such cheese could approximate but could not duplicate the flavor and texture of imported Roquefort cheese. After continued investigation, Matheson (32), in 1921, published a detailed procedure for the manufacture of blue cheese from raw cows' milk.

Hall and Phillips (17) in 1925 reported a method for the manufacture of Roquefort-type cheese from goats' milk. This cheese was reported to have duplicated more nearly the imported cheese than did that made from cows' milk. In 1935, Goss, Nielsen, and Mortensen (16) introduced their method for the manufacture of Iowa Blue Cheese. This method involves ripening high quality milk to an acidity of 0.19 to 0.20 percent, after the addition of 1.5 to 2.0 percent lactic culture, setting the milk at 84°F. with rennet at the rate of 90 c.c. per 1000 pounds, curdling for 1.75 to 2.0 hours,

and cutting the curd with 0.5 inch curd knives. The curd is left undisturbed for 15 minutes and then is stirred gently every few minutes for thirty minutes. Fifty minutes after cutting, the temperature is raised 2 to 3°F. by returning a pail of whey, previously removed and heated to 170 to 180°F. with steam. About 15 minutes later, or when the whey acidity has reached 0.18 to 0.19 percent, some of the whey is drained off and one-half of the curd is transferred to the drain rack. After draining for 15 to 20 minutes, the curd is hooped; mold powder is added to each layer as the curd is placed in the hoop. Later workers have modified this procedure somewhat.

Lane and Hammer (27) in 1936, introduced the method for the manufacture of blue cheese from homogenized raw milk. Since that time, homogenization of raw milk for blue cheese has been accepted widely by the industry in the United States. By this procedure, the curing period has been reduced considerably and the flavor and color of the cheese have been improved.

In 1938, Irvine (22) reported that the addition of 0.5 to 1.0 g. of lipase enzyme preparation per 100 pounds of raw milk accelerated fat hydrolysis but always gave a bitter flavor in blue cheese. The flavor was not improved by the addition of the lipase. The lipase used was identified as steapsin in another paper (34).

Investigations carried on at the University of Minnesota on the use of the commercial enzyme steapsin to improve the flavor of blue cheese were reported in 1939 by Coulter and Combs (5). These investigations showed that steapsin would give about the same amount of flavor in 5 months curing that had been obtained previously by curing for 12 months. However, a slightly bitter flavor which was objectionable to the connoisseur of blue cheese was associated with the cheese containing steapsin.

The manufacture of blue cheese from pasteurized milk was mentioned by Goss, Nielsen, and Mortensen (16) in 1935. They indicated that cheese made from pasteurized milk did not develop as much surface growth of bacteria and molds, did not have as much flavor, and did not become as sweet during curing as did the raw milk cheese.

In 1938, Lane and Hammer (28) reported the manufacture of some experimental pasteurized milk blue cheese. In each of three lots of cheese, one was made from unhomogenized raw milk, one from homogenized raw milk, and one from homogenized pasteurized milk. The total volatile acidities and fat acidities of the cheese made from homogenized raw milk generally were higher than those of the cheese from homogenized pasteurized milk, and those of the cheese from homogenized pasteurized milk generally were higher than those of the cheese from unhomogenized raw milk. The flavor and

color of the homogenized raw milk cheese were superior to those of the other two types of cheese.

Coulter and Combs (5) reported the manufacture of several lots of blue cheese from pasteurized milk to which 2 grams of steapsin per 100 pounds of milk had been added. These lots of cheese were not inoculated with mold and were not pierced. They had the rank flavor of butyric acid to the exclusion of all other flavors up to 15 months of curing time. The volatile acid values were very high on all these lots of cheese.

Studies of various kinds on the lipolytic activity of microorganisms have been made by a number of investigators. Only a few of the papers most directly applicable will be reviewed. Hussong (20), in the study of an organism which was isolated from two samples of rancid butter, linked the rancidity to the ability of the organism to hydrolyze fat. The organism was found to exist in three colony types, each of which had been described and named by two other workers. The organism was named Pseudomonas fragi. Variant colony types, which were unable to split fat, could be secured from parent cultures having marked fat-splitting ability. The S-type colonies from 4 cultures all were able to split fat. The O- and R- type colonies varied in their ability to hydrolyze fat. Salt in butter largely prevented the development of this organism.

Collins (4) isolated and identified a number of lipolytic bacteria and studied their action on some simple tri-glycerides and some natural fats. The organisms studied were Pseudomonas acidiconcoquens, the normal type and 4 varieties of Pseudomonas fluorescens, Achromobacter lipolyticum, Bacterium viscosum, and Achromobacter conii. A. lipolyticum was observed to hydrolyze small quantities of tri-butyrin dispersed in nile-blue sulfate agar plates. The odor of butyric acid was observed and the hydrolysis was proven by the disappearance of the fat globules and the detection of volatile fatty acids with moistened litmus paper. All other organisms studied were inhibited by the concentrations of tri-butyrin that were used. A.lipolyticum produced rancidity in butterfat, corn oil, and tri-butyrin and tallowiness in olive oil and tri-olein.

Long (29) isolated and studied several species of lipolytic organisms from various dairy products. A large number of cultures of P. fragi were isolated and studied. The variations in cultures noted by Hussong (20) were confirmed. The majority of freshly isolated cultures were of the S-type, were actively lipolytic, and were the least inclined to vary if carried through rapid transfers in litmus milk. Variations in cultures were induced by long holding of the cultures at low temperatures (5 - 10°C.) between transfers. The O-type colonies generally did not

hydrolyze fat and the R-type colonies varied in their action on fat.

Twenty-one cultures isolated and characterized by Long (29) were found to be the same as the organism described by Evans as Bacillus abortus var. lipolyticus and later as Bacterium lipolyticus. The name Alcaligenes lipolyticus was proposed for this organism. All of the cultures hydrolyzed cottonseed oil in agar plates. Eleven cultures were inoculated into sterile cream, and the cream was churned. All eleven samples of unsalted butter were rancid after 5 days at 21°C. The organism was found to utilize sodium acetate, sodium butyrate, and sodium oleate as sources of carbon.

Eleven cultures of a yeast that was both proteolytic and lipolytic were isolated from samples of normal and abnormal dairy products (29). The cultures were identified as Mycotorula lipolytica, which had been characterized by Harrison (19) in 1928. All of the cultures hydrolyzed fat actively on plates, caused putrid defects in cream, and caused rancid or cheesy defects in butter. The organism is characterized by its ability to bring about rapid and complete digestion of milk and its ability to grow vigorously on most ordinary laboratory media.

Long (29) has shown that the ability of an organism to hydrolyze tri-propionin and tri-butyrin is not an indication of the ability of that same organism to hydrolyze natural

fats, such as butterfat or cottonseed oil.

Fouts (11, 12), in studying the effect of the growth of organisms on the acidity of the fat in cream and butter, found that Penicillium roqueforti, Oospora lactis, M. lipolytica, A. lipolyticum, A. lipolyticus, and P. fluorescens but not P. fragi, caused increases in the acid numbers of the fat when inoculated into sterilized cream. O. lactis and all the species of bacteria studied were inhibited somewhat by the growth of butter culture organisms in the cream, but M. lipolytica showed increased growth in the presence of butter culture organisms. Lipolysis was extensive with all organisms, even in high acid cream. M. lipolytica caused lipolysis in cream with an acidity of 2.08 percent. Fouts (13) found that M. lipolytica could utilize sodium butyrate, calcium butyrate, calcium caproate or calcium caprylate as the sole source of carbon. A. lipolyticus could utilize all of these but calcium caproate and A. lipolyticum could utilize only calcium caprylate and that to but a small degree.

The production of lipolytic enzymes by molds and the effects of these enzymes have been recognized for some time. As early as 1910, Dox (9) reported that Laxa had noted that butter inoculated with Penicillium glaucum developed considerable acidity. In 1914 Currie (6) concluded that during the ripening of Roquefort cheese, considerable fat

is hydrolyzed by the water-soluble lipase produced by P. roqueforti. The characteristic peppery flavor and burning effect of the cheese on the tongue and palate were considered due to the caproic, caprylic, and capric acids and their readily hydrolyzable salts that had accumulated. He noted that P. roqueforti will grow in Czapek's medium when sucrose is replaced by pure butterfat, tri-butyrin, ethyl butyrate, glycerin, butyric acid, or ammonium butyrate as the source of carbon. Thus the mold not only has the power to hydrolyze fats but also is able to utilize their components. P. roqueforti was grown on 100 c.c. of Czapek's solution in which sugar was replaced by 3.0 g. of fresh filtered milk fat. The acid number of the fat showed that more than half of it had been hydrolyzed after 50 days incubation at 23°C., but only very small amounts of soluble and volatile acids had accumulated, indicating that they had been utilized by the mold. In cheese, it is believed that the enzyme diffuses beyond the feeding zone of the mold and hence free fatty acids accumulate.

Hammer and Bryant (18) succeeded in isolating a compound which they believed was one of the flavor constituents of blue cheese. The material was first isolated from 600 ml. of milk to which had been added 0.3 ml. of n-caprylic acid and some mold spores. This medium had the odor of blue cheese but showed no mold growth after several days

at room temperature. The material was isolated from it by steam distillation, ether extraction from the distillate, and evaporation of the ether. The material was identified as methyl-n-amyl ketone.

Stokoe (36) and Davies (8) explain the formation of methyl ketones by molds as being the second step in an abnormal oxidation of fatty acids. The first step in the oxidation of fatty acids by molds is the oxidation of the beta carbon atom to form a beta keto acid. The second step in a normal oxidation is the splitting of the molecule to form acetic acid and another acid with two less carbon atoms than in the original acid. The second step in the abnormal oxidation is the removal of carbon dioxide from the beta keto acid to form a methyl ketone. This abnormal oxidation is apparent when the mold is growing under poor nutritional conditions or when fatty acids build up to a point at which they impair the respiration of the mold.

Kirsh (24) reported that the water-soluble lipase of Penicillium oxalicum is highly non-specific, as it brings about almost the same amount of hydrolysis of esters, triglycerides of low molecular weight, and a variety of emulsified oils. The maximum activity of this lipase is at pH 5.0 at 37 to 40°C.

Naylor, Smith, and Collins (33) obtained maximum esterase production by P. roqueforti on Czapek's medium in

which the sodium nitrate was replaced by 0.10 N Ammonium chloride, 0.50 c.c. of ethyl butyrate was added per 1000 c.c., and the reaction was adjusted to pH 4.5.

Thibodeau and Macy (37) have reported an extensive study of the enzyme activity of P. roqueforti. They found that this mold does not grow readily in a medium with an oxidation-reduction potential of over 400 millivolts. The addition of 0.1 percent agar to Czapek's solution reduced the oxidation-reduction potential below 400 millivolts. The addition of peptone or milk further reduces it. These authors found that the presence of sugar in a medium tended to reduce the production of lipase by P. roqueforti. Maximum lipase was produced in Czapek's medium without sugar, but with 3.0 g. peptone, and 3.0 g. butterfat per liter. The optimum activity of this lipase is over the pH range from 5.3 to 7.5, when the substrate is a 3.0 percent butter oil emulsion in the presence of an acetate buffer. The production of lipase varies widely from strain to strain, and seems to be at a maximum as soon as the cultures have attained the stage of full sporulation. Sodium chloride in concentrations existing in blue cheese does not retard the action of either the lipase or the protease of the mold.

METHODS

Manufacture of Blue Cheese

The method employed for the manufacture of blue cheese was that of Goss, Nielsen, and Mortensen (16), as modified by Lane and Hammer (28) with some modifications for use with pasteurized milk. The milk used in lots 5 through 71 was normal mixed herd milk that was not standardized. That used in lots 72 through 147 was mixed herd milk that was standardized at 3.5 percent butterfat before homogenization. The milk that was pasteurized was heated at 143 to 147° F. for 30 minutes, cooled to about 130° F. homogenized at 1700 pounds pressure, and then cooled to 40 to 45° F. The milk was placed in experimental cheese vats, heated to 89 to 90° F. and 1.0 percent lactic culture was added.

Four vats were used, and in every trial but one, four lots of cheese were made at one time. The manufacturing schedules for each of the four vats were staggered by 15 minute intervals. The milk was ripened for 30 minutes, which gave an acidity increase of 0.010 to 0.015 percent, and then was set with rennet. In lots 4 to 51, rennet was used at rate of 90 ml. per 1000 pounds of milk and in all other lots it was used at the rate of 130 ml. per 1000 pounds. The curd was cut with 0.5 inch curd knives, 70 minutes after the ad-

dition of the rennet. The acidity of the whey generally was 0.11 to 0.14 percent just after cutting. The curd was allowed to stand for about 30 minutes and then was stirred every 20 to 30 minutes until firm enough to hoop. Heat was applied to the jacket of the vat at the stirring times, whenever necessary to keep the temperature at 90° F. inside the vat. The curd usually was firm enough to hoop in 2.0 or 2.5 hours from the time it was cut, and the whey acidity usually had increased 0.04 to 0.06 percent.

When the curd was ready to hoop, the whey was drained from the vat, a mixture of 1.0 percent salt and 0.01 percent mold powder, based upon the weight of cheese expected, was mixed into the curd, which then was placed in the hoops. The cheese was turned within 5 minutes and then about every 30 minutes for 2 to 3 hours. The following morning it was removed from the hoops and weighed, and the salting was started. Dry salt was rubbed on the outside of the cheese each day for about 5 days until 5 percent salt had been used. On the seventh day, the cheese in lots 4 to 119 were dipped in paraffin and pierced through the paraffin about 50 times with a 0.095 inch diameter wire needle. The cheese in lots 120 to 147 were not dipped in paraffin before being pierced. The cheese was cured at 48 to 52° F. in a humidity of 90 to 95 percent saturation.

In lots 60 to 71 and 108 to 147, calcium chloride was added to the milk before setting, at the rate of 0.015 percent.

Part of the manufacturing data, and the fat, moisture, and total chlorides contents of the various lots of cheese are presented in Appendix A.

Analyses of Blue Cheese

Portions of the cheese were removed from the curing room for analyses at regular intervals. Fat, moisture, and total chlorides determinations were made when the cheese was 4 weeks old. Lots 5 to 20 were scored at 4 to 5 weeks and 17 to 18 weeks, lots 21 to 28 were scored at 4 weeks and were discarded, lots 29 to 71 were scored at 4 and 12 weeks, and lots 72 to 147 were scored at 4, 8, and 12 weeks. The total volatile acidities were determined on all lots of cheese at approximately these same times. The fat acidities were determined on lots 5 to 55 at the times they were scored, and on lots 56 to 60 at 4 weeks only.

Total nitrogen values, nitrogen fractions soluble and insoluble in phosphotungstic acid and in trichloroacetic acid were determined on lots 5 to 71 at 4 weeks, and on lots 5 to 63 at 12 weeks. Amino nitrogen determinations were made on lots 5 to 28 and 56 to 71 at 4 weeks and on lots 5 to 20 and 29 to 63 at 12 weeks.

Fat

The fat content of the cheese was determined by the method of the Association of Official Agricultural Chemists (1)

modified to make it suitable for use with the Mojonnier tester. The modifications include the use of 5 ml. of water instead of 9 ml., the use of glass beads instead of sand to prevent bumping, transfer to a Mojonnier fat extraction flask instead of a Rohrig tube, and the use of 10 ml. of alcohol with the 25 ml. of ethyl ether and 25 ml. of petroleum ether. The ether-fat mixture was poured into a weighed Mojonnier fat dish after being centrifuged 30 turns in 30 seconds. The second extraction was made with 25 ml. of ethyl ether and 25 ml. of petroleum ether and was poured into the same dish. After the ether was evaporated, the dish and fat were dried 5 minutes at 100° C. under 20 inches vacuum, cooled in the Mojonnier dessicator 7 minutes, and weighed.

Moisture

The moisture content of the cheese was determined by covering the bottom of a Mojonnier moisture dish with oven-dried sand, weighing in 5 g. of finely divided cheese, and drying in an atmospheric oven at 100° C. for 24 hours. The moisture determinations on part of the cheese were made by drying a 10 g. sample of cheese in a Brabender Simi-automatic Moisture Tester at 142° C. until the weights at 10 minute intervals became constant. There was good agreement between these two methods.

Total chlorides

The total chlorides content of the cheese was determined by the method of the Association of Official Agricultural Chemists (1) with one modification. In the case of blue cheese, 50 ml. of 0.1 N silver nitrate must be used instead of 25 ml.

Total volatile acidity

The total volatile acidity of the cheese was determined by the method of Lane and Hammer (28).

Fat acidity

The fat acidities were measured by the method of Breazeale and Bird (2). The fat for the fat acidity determinations was obtained and purified by the method of Lane and Hammer (28).

Protein degradation

In making analyses to determine the amount of protein degradation, the cheese first had to be emulsified and made into a uniform suspension. The method used for making the suspension was a modification of that used by Knudsen and Sørensen (25). In the first part of the work, the cheese suspension was made by emulsifying 25 g. of cheese in 200 ml. of boiling 2.0 percent sodium citrate solution by agitation in an Eskimo Whiz-mix model 515 J B. for 5 minutes at high speed. The mixture was kept alkaline to brom thymol blue by

adding alkali when necessary. This solution was transferred quantitatively to a 250 ml. volumetric flask, cooled to 20° C., and made to volume with distilled water. Five milliliters (equivalent to 0.5 g. of cheese) of this suspension were used in each precipitation. Some difficulty was encountered with churning of the fat due to the cooling of the suspension in the Whiz-mix. To prevent this difficulty in later studies, 25 g. of cheese were emulsified in 400 ml. of 2.0 percent sodium citrate solution and then made up to 500 ml. This amount of material held the heat well enough to prevent the difficulty with churning of the fat. Ten milliliters (equivalent to 0.5 g. of cheese) of this solution then were used in each precipitation. Preliminary studies on this method of preparing the suspension, using completely dispersible peptone added to milk, indicated the citrate did not influence the nitrogen partition.

Total nitrogen

The total nitrogen was determined in an amount of the above cheese suspension equivalent to 0.5 g. of cheese by the Kjeldahl-Gunning-Arnold method of the Association of Official Agricultural Chemists (1). Copper sulfate was used as the catalyst, and the mixed methyl red-methylene blue indicator of Johnson and Green (23) was used in the titration.

Nitrogen fractions soluble and insoluble in phosphotungstic acid

The nitrogen fractions soluble and insoluble in phosphotungstic acid were determined by the method of Lane and Hammer (26), with slight modification. An amount of the above cheese suspension equivalent to 0.5 g. of cheese was treated with 50 ml. water, 15 ml. 25 percent aqueous sulfuric acid, and 5 ml. 20 percent aqueous phosphotungstic acid for 16 to 18 hours at room temperature. The solution then was filtered through a Whatman no. 12, 12.5 cm. fluted filter paper, the precipitate was washed three times with a solution of the same concentration as that used in the precipitation, and the total nitrogen was determined in the filtrate and in the precipitate.

Nitrogen fractions soluble and insoluble in trichloroacetic acid

The nitrogen fractions soluble and insoluble in trichloroacetic acid were determined by a modification of the method of Lane and Hammer (26). An amount of the cheese suspension equivalent to 0.5 g. of cheese was treated with 45 ml. water, and 5 ml. 20 percent aqueous trichloroacetic acid for 16 to 18 hours at room temperature. The solution was filtered through the same type of paper, the precipitate was washed in the same way, and the total nitrogen was determined in the filtrate and in the precipitate in the

same way as for the phosphotungstic acid separation.

Amino nitrogen

The amino nitrogen values were determined by placing an amount of the cheese suspension equivalent to 1.0 g. of cheese in a 25 ml. volumetric flask, adding seven drops of glacial acetic acid to precipitate the casein, making to volume, filtering, and running a Van Slyke amino nitrogen determination (41) on the filtrate. The values were expressed as milligrams of amino nitrogen per gram of cheese.

Tests of Molds

Lipolytic activity

The lipolytic activity of P. roqueforti cultures was studied by the nile-blue sulfate technique developed by Turner (39, 40), Hussong (20), and Long and Hammer (30). Czapek's solution (7) with 3.0 percent sucrose and 1.5 percent agar added (hereafter referred to as Czapek's agar) was used in studies of both the lipolytic and the proteolytic activity of the mold.

Proteolytic activity

The proteolytic activity was determined by the use of 10 percent of sterile milk in the medium as suggested by Freudenreich (15) and reported by Frazier and Rupp (14).

Cheese Scoring

In judging the cheese, three scores were placed upon each lot, namely: Mold score, flavor score, and defect score. Each score had a range of 0 to 10, in which 10 was the most perfect score and 0 was the lowest possible score. This should be remembered when considering the defect scores, because the higher the defect score, the less serious was the defect. In determining the mold score, the amount of mold as well as the distribution of the mold in the cheese was considered. The flavor score is an evaluation of all the positive flavor characteristics in which the cheese resembled a good raw milk blue cheese or Roquefort cheese. The defect score is an evaluation of all the defect flavors which were detected in the cheese.

Propagation of Stock Cultures

The stock cultures of bacteria and mycotorula were carried by inoculation in tubes of litmus milk, incubation at 30° C. until growth was apparent and storage in a refrigerator at 1 to 3° C. until used as inoculating material. The cultures were transferred at least once each month.

The stock cultures of the molds were carried as streaks on Czapek's agar slants. The cultures were incubated at 21° C. until the slants were covered with mold and then were stored

at 1 to 3° C. The cultures were transferred at least once every two months.

The mold powder which was used in most of the cheese was prepared by the method of Hussong and Hammer (21).

EXPERIMENTAL

Addition of Fatty Acids to Milk

The contribution of the lower molecular weight fatty acids to the flavor of blue cheese has been stressed by many investigators. In order to learn more about the part which these fatty acids contribute to the flavor of the cheese, two series of trials were made in which varying amounts of some of the lower molecular weight fatty acids were added to the lots of milk which were made into cheese. In the first series, each of three lots of cheese was made from 100 pounds of milk. This series consisted of a control lot with no fatty acid added, one lot with 2.0 g. of caprylic acid added, and one lot with 10.0 g. of caprylic acid added. The second series consisted of a control lot; a second lot with 0.4 g. caproic, 0.2 g. caprylic, and 0.4 g. capric acid added; a third lot with the above amounts of acids plus 0.8 g. lauric acid added; and a fourth lot with the amounts of acids added to the second lot plus 0.6 g. butyric and 0.8 g. lauric acid added. Each lot in the second series was made from 115 pounds of milk. In all cases, the fatty acids were added to 100 g. of melted butterfat, which then was homogenized into one quart of milk and

added to the cheese milk before setting with rennet. The same amount of butterfat alone homogenized into milk was added to the control lots. The results are presented in Table 1.

The cheese with added fatty acids showed definite improvement in flavor score over the control cheese; however, none was typical of good blue cheese, lacking the smoothness and roundness of flavor desired. The flavor scores of the cheese with added fatty acids, with the exception of lot 31, remained constant or increased from the fourth to the twelfth week of curing. The fat acidities and total volatile acidities increased considerably from the fourth to the twelfth week.

The mold scores at 4 weeks are lowest in the cheese with the largest amounts of added fatty acids and at 12 weeks are highest with the exception of lot 31, which indicates that the fatty acids may be toxic to the mold until the mold is established and has started to utilize the acids in its growth. In the case of lot 31, there was so much added caprylic acid that it may have been toxic during the entire 12 weeks. The flavor of the caprylic acid was pronounced in the cheese at 12 weeks.

Use of Bacterial and Yeast Cultures

The production of lipase by various microorganisms

Tabl

Effect of the Addition of Low Molecular Weight Fatty Acids on the Score

Lot No.	Grams of Acid Added to 100 g. of Butter Fat *					Mold Score		Defect Score	
	Butyric	Caproic	Caprylic	Capric	Lauric	4 wks.	12 wks.	4 wks.	12 wks
29	-	-	-	-	-	6.0	4.5	3.0	6.5
30	-	-	2.0	-	-	5.0	5.5	7.0	7.0
31	-	-	10.0	-	-	4.0	3.5	5.0	4.0
52	-	-	-	-	-	6.5	5.0	5.0	2.5
53	-	0.4	0.2	0.4	-	5.0	6.0	4.0	3.5
54	-	0.4	0.2	0.4	0.8	4.5	7.5	5.5	5.5
55	0.6	0.4	0.2	0.4	0.8	4.5	7.0	8.0	4.5

* The 100 g. of melted butter fat was homogenized into one quart of mi

able 1

re, Total Volatile Acidity and Fat Acidity of Pasteurized Milk Blue Cheese

re	Flavor Score		Total Volatile Acidity		Fat Acidity		Defect Comments
ks.	4 wks.	12 wks.	4 wks.	12 wks.	4 wks.	12 wks.	
5	3.0	4.0	8.00	18.40	4.26	20.00	Flat, yeasty, nutty.
0	6.0	6.0	7.45	15.50	4.18	8.45	Flat, nutty
0	6.0	4.5	15.30	18.90	5.25	14.72	Musty, caprylic acid.
5	4.5	3.5	7.00	13.90	2.65	37.30	Nutty, unnatural.
5	3.5	5.0	7.32	12.70	4.00	29.18	Sl. nutty, unnatural.
5	4.5	7.0	6.40	20.60	3.17	29.98	Sl. nutty, unnatural.
5	6.0	6.0	6.00	14.20	2.74	18.27	Sl. nutty, unnatural, uncl

milk, and added to the cheese milk.

has been investigated by several workers, as pointed out previously. So far as can be determined from the literature, no one has investigated the possibility of substituting the lipase produced by these microorganisms for the normal milk lipase in the manufacture of blue cheese from pasteurized milk. To determine whether this was possible, a series of experiments was set up using four lipolytic organisms: M. lipolytica, A. lipolyticus, A. lipolyticum, and P. fragi. With each organism four lots of cheese were made: A control lot with 555 g. of sterilized cream containing 18 percent fat; one lot with 555 g. of culture grown for 48 hours at 30°C. in sterilized cream containing 18 percent fat and then sterilized by autoclaving; one lot with 555 g. of the culture grown for 24 hours at 30°C. in cream containing 18 percent fat; and one lot with 555 g. of the culture grown for 48 hours at 30°C. in cream containing 18 percent fat. The 555 g. of cream containing 18 percent fat added to each 100 pound lot of milk was comparable in fat content to the 100 g. of fat added in the preceding experiment. The cream was sterilized at 15 pounds pressure for 25 minutes and cooled to room temperature before inoculation. The results are presented in Table 2.

In the experiments with the M. lipolytica culture the flavor and defect scores were erratic at 4 weeks and were low at 12 weeks; however, there was a tendency for

Effect of the Addition of Various Cultures of Four Lipolytic Organis

Lot No.	Culture Used	30°C. Culture		Mold Score		Defect Sc	
		age-hrs.	Treatment	4 wks.	12 wks.	4 wks.	12
32	-	-	-	2.5	1.5	4.5	1
33	M. lipolytica (846)	48	Sterilized	5.0	2.0	7.0	1
34	M. lipolytica (846)	24	-	2.0	2.5	4.5	4
35	M. lipolytica (846)	48	-	4.5	3.5	3.0	5
44	-	-	-	5.5	5.0	7.0	4
45	A. lipolyticus (852)	48	Sterilized	5.0	7.0	6.0	3
46	A. lipolyticus (852)	24	-	7.0	5.5	7.0	3
47	A. lipolyticus (852)	48	-	7.5	7.5	7.0	2
36	-	-	-	3.0	3.5	4.0	7
37	A. lipolyticum (12)	48	Sterilized	2.5	5.0	3.0	8
38	A. lipolyticum (12)	24	-	5.0	6.0	6.5	8
39	A. lipolyticum (12)	48	-	8.0	4.5	8.0	5
40	-	-	-	5.0	6.0	6.0	6
41	P. fragi (9)	48	Sterilized	5.5	7.0	5.0	4
42	P. fragi (9)	24	-	3.5	5.0	8.5	2
43	P. fragi (9)	48	-	3.0	6.0	9.0	5

* Defect Comments are for cheese 12 weeks old.

ble 2

in 555 g. of 18 Percent Cream to Pasteurized Milk

Flavor Score		Total Volatile Acidity		Fat Acidity		Defect Comments*
4 wks.	12 wks.	4 wks.	12 wks.	4 wks.	12 wks.	
1.5	2.0	7.80	7.60	9.05	2.68	Sour, yeasty, musty
5.0	2.0	5.00	12.00	4.03	7.58	Musty, yeasty.(invasion)
4.0	4.0	7.00	13.50	7.45	8.68	Musty, sl. sour, yeasty
3.5	3.0	11.40	26.00	18.00	23.40	Excessive fatty acids
4.0	5.5	5.80	15.00	4.58	8.85	Musty, yeasty
5.0	6.0	6.00	15.30	2.59	18.63	Unnatural, musty, yeasty
5.5	6.5	6.30	16.80	2.40	26.30	Unnatural, musty, yeasty
6.0	7.0	5.60	16.30	2.52	10.12	Unnatural, sour, musty, yee
3.0	5.0	7.00	13.36	5.74	9.23	Yeasty, sl. unclean, green
3.0	7.0	8.00	15.60	6.12	26.38	Sl. musty, sl. unclean
5.0	7.5	7.70	15.00	5.26	10.33	Sl. musty, sl. unclean
4.5	6.0	7.00	20.60	5.88	16.58	Very musty, sl. unclean
2.5	5.0	6.90	15.00	1.40	8.18	Sour, musty
3.0	4.0	6.70	15.30	4.82	17.48	Musty, cheddar
4.5	4.0	7.00	16.80	5.14	11.98	Musty, unclean
6.0	5.5	6.70	16.30	4.50	14.22	Musty, unclean

these scores to be higher in the cheese with the added Mycotorula culture. The mold scores followed much the same pattern. The fat acidities and total volatile acidities definitely were increased by the addition of the Mycotorula, although the addition of 555 g. of the active 48 hour culture resulted in too much fat hydrolysis, as indicated by the defect comments. Although the sterilized 48 hour culture increased the total volatile acidity and fat acidity values over those of the control cheese at 12 weeks, it did not improve the flavor to any extent.

With A. lipolyticus the 4 week values showed a slight improvement in mold, defect, and flavor score over those of the control. The total volatile acidities were erratic and the fat acidities showed a slight decrease. The scores at 12 weeks showed a decrease in the defect score which counterbalanced all of the increase in flavor score. The total volatile acidities showed no marked increase and the fat acidities were erratic. All cheese in this series were criticized for being yeasty and musty, and those with the lipolytic bacteria added were unnatural in flavor.

The cheese made with A. lipolyticum showed much the same trends as those made with A. lipolyticus, and were criticized for being musty and unclean in flavor. Those made with P. fragi gave no indication of definite improvement by any of the analyses made, and were criticized for

being musty and unclean in flavor.

These experiments indicated that at least the Mycotorula culture and possibly some of the other cultures had possibilities for use in blue cheese. The kind of culture and amount used might not have been right to give the desired result. Accordingly a series of experiments was set up in which different amounts of a culture of each organism grown for 48 hours at 30°C. in sterile homogenized milk were added to each of three lots of milk for cheese. The amounts of culture used and the results obtained are presented in Table 3.

The results are very similar to those presented in Table 2, showing that M. lipolytica was the only organism of the four tested which increased the total volatile acidity and which greatly improved the flavor of the cheese. Of the different amounts of culture used, 1.0 percent gave the highest total volatile acidity and the highest flavor score. This study indicated that homogenized milk could be used for the culture as effectively as the 18 percent fat cream and would be much more economical. The fat acidity determinations were discontinued both because of the great difficulty of pressing enough fat from the cheese at the end of 4 weeks for analysis and because the values apparently were not as closely related to the flavor as were the total volatile acidities.

The two preceding trials indicated that M. lipolytica

Table 3

Effect of the Addition of Varying Amounts of Homogenized Milk Cultur

Lot No.	Culture Used	Culture %	Mold Score 4 wks.	Mold Score 12 wks.	Defect Score 4 wks.	Defect Score 12 wks.	Flavo 4 wks.
56	-	-	8.0	4.5	3.0	3.5	3.5
57	M. lipolytica (846)	0.1	6.0	7.0	8.0	4.5	6.0
58	M. lipolytica (846)	0.3	8.0	5.0	8.0	6.5	7.0
59	M. lipolytica (846)	1.0	6.5	6.5	7.5	7.5	6.5
60	-	-	3.0	6.5	2.0	6.0	2.0
61	A. lipolyticus (852)	0.1	5.0	7.0	3.0	6.0	3.0
62	A. lipolyticus (852)	0.3	3.0	4.0	3.5	6.0	3.5
63	A. lipolyticus (852)	1.0	3.0	4.5	4.0	6.0	4.0
64	-	-	7.0	6.5	3.0	3.5	4.0
65	A. lipolyticum (12)	0.1	8.0	7.5	7.0	5.0	5.5
66	A. lipolyticum (12)	0.3	4.5	7.5	5.0	6.0	3.0
67	A. lipolyticum (12)	1.0	6.0	6.5	4.5	4.0	3.0
68	-	-	3.0	7.0	2.5	3.5	3.0
69	P. fragi (9)	0.1	5.5	5.0	4.0	5.0	3.5
70	P. fragi (9)	0.3	3.5	4.5	5.5	4.5	5.0
71	P. fragi (9)	1.0	4.5	6.5	5.0	4.0	4.5

* Defect Comments are for cheese 12 weeks old.

Table 3

Homogenized Milk Culture of Four Lipolytic Organisms to Pasteurized Milk

Defect Score		Flavor Score		Total Volatile Acidity		Defect Comments*
4 wks.	12 wks.	4 wks.	12 wks.	4 wks.	12 wks.	
3.0	3.5	3.5	4.0	6.20	19.50	Sour, unclean
8.0	4.5	6.0	6.0	12.70	30.20	Unclean
8.0	6.5	7.0	7.0	8.60	35.50	Sl. unclean
7.5	7.5	6.5	8.0	17.60	40.90	Sl. unclean (invasion)
2.0	6.0	2.0	4.5	8.00	24.00	Sour, salty, lacking
3.0	6.0	3.0	5.0	7.40	19.00	Lacking
3.5	6.0	3.5	4.0	7.00	18.00	Sour, lacking
4.0	6.0	4.0	4.0	6.80	18.20	Sour, lacking
3.0	3.5	4.0	4.5	5.50	7.80	Unnatural
7.0	5.0	5.5	5.0	6.00	8.00	Unnatural
5.0	6.0	3.0	5.5	5.40	8.00	Unnatural
4.5	4.0	3.0	6.0	5.50	6.00	Unnatural, yeasty
2.5	3.5	3.0	3.5	6.60	8.50	Unnatural, sour, yeasty
4.0	5.0	3.5	5.0	7.10	7.70	Unnatural, sour, yeasty
5.5	4.5	5.0	4.0	5.90	6.60	Unnatural, sour
5.0	4.0	4.5	5.0	6.00	8.50	Unnatural, sour

old.

was the only organism of the group studied that improved the flavor of cheese; therefore, attention was concentrated upon this one organism. The question arose as to whether all strains of this organism isolated from various sources would have the same effect in cheese. A group of eleven strains was collected; some had been isolated from cream and butter by Chinn (3), some were isolated from various dairy products in class work and some were from the stock collection in the Dairy Industry Department at Iowa State College. A lot of cheese was made with each strain by preparing a 48 hour 30°C. culture of the organism in sterile homogenized milk and adding this to the cheese milk at the rate of 0.3 percent before the milk was set with rennet. Second and third trials were made with some of the strains which gave the best results in the first trials. All trials are reported in Table 4. Various trials with a single strain were not grouped in the table because in each case the three lots of cheese should be compared with the control lot of cheese for that group. In the first trial every Mycotorula strain, with one exception, gave cheese which was as good as or better than the control cheese from the standpoint of flavor and defect score at 8 and 12 weeks. Also every strain, with two exceptions, gave cheese which had a higher total volatile acidity at 8 and 12 weeks than did the control cheese. The five strains which increased the

Table 4

Effect of the Addition of Various Strains of Mycotorula lipolytica to

Lot No.	Strain No.	Mold Score 4 wks.	Mold Score 8 wks.	Mold Score 12 wks.	Defect Score 4 wks.	Defect Score 8 wks.	Defect Score 12 wks.	Flavor S 4 wks.	Flavor S 8 wks.
76	Control	6.5	6.0	7.0	7.0	3.0	4.0	4.0	3.0
77	47	6.5	6.0	8.5	7.0	4.0	6.5	5.5	5.5
78	57	7.0	4.5	8.0	7.0	5.0	7.5	5.0	5.5
79	100	6.0	6.0	6.0	6.0	7.0	5.5	6.0	7.5
80	Control	7.0	5.0	9.0	5.0	4.5	3.0	4.0	3.5
81	438	7.0	6.0	7.0	4.0	6.0	5.0	5.0	6.0
82	839	5.5	3.5	5.5	8.0	8.0	7.5	7.5	7.5
83	840	7.0	7.0	9.0	5.0	4.5	6.0	5.0	5.5
84	Control	5.5	7.0	7.5	7.5	3.0	4.5	4.0	4.0
85	843	4.0	6.5	7.5	7.5	6.5	7.5	7.0	7.5
86	845	5.0	6.0	7.5	4.5	3.0	5.0	3.5	3.5
87	846	6.0	6.5	7.5	3.5	5.5	7.5	4.5	5.0
88	Control	7.0	7.0	7.5	4.0	4.0	5.0	4.5	4.0
89	M.L.	5.0	6.0	9.0	8.0	5.0	8.0	5.5	5.5
90	848	6.0	4.5	6.5	7.0	5.5	6.0	7.0	6.0
91	848	6.0	5.0	8.0	6.0	5.5	6.5	6.0	6.5
112	Control	5.5	5.0	6.5	5.5	5.5	4.0	4.5	5.5
113	839	3.0	5.5	4.5	7.0	4.0	7.0	5.0	4.5
114	843	4.0	3.0	3.0	6.0	3.0	4.0	4.0	3.5
115	848	5.0	4.0	5.5	7.0	5.5	5.5	5.5	6.0
116	Control	4.0	5.5	3.0	5.0	3.0	5.0	4.0	4.0
117	846	3.5	3.5	5.0	7.5	5.5	6.5	6.0	6.5
118	100	5.0	5.0	4.5	5.0	4.5	3.0	4.0	3.5
119	M.L.	4.0	3.5	4.0	7.5	7.0	8.0	6.0	6.0
124	Control	4.0	5.5	6.0	4.0	3.5	3.0	3.0	4.0
125	M.L.	5.0	7.5	5.0	7.5	7.5	4.5	5.5	7.0
126	843	3.5	3.5	5.0	7.5	4.5	4.5	4.5	4.0
127	848	6.0	8.5	5.0	5.5	5.0	6.5	4.5	7.0

* Defect Comments are for cheese 12 weeks old.

Table 4

a lipolytica to Pasteurized Milk

Flavor Score			Total Volatile Acidity			Defect Comments*
4 wks.	8 wks.	12 wks.	4 wks.	8 wks.	12 wks.	
4.0	3.0	3.5	7.40	11.90	22.00	Nutty, musty
5.5	5.5	5.5	6.50	15.20	35.00	Nutty
5.0	5.5	5.0	5.60	17.00	24.50	Sl. nutty
6.0	7.5	4.5	9.00	12.60	25.70	Sour, nutty
4.0	3.5	3.5	7.30	14.50	27.00	Unclean, cowy, sour
5.0	6.0	5.5	8.60	18.00	27.20	Unclean
7.5	7.5	8.0	19.30	35.00	51.40	Sl. excessive volatile acids
5.0	5.5	6.0	8.30	20.10	33.20	Sl. unclean
4.0	4.0	4.0	6.80	19.00	35.00	Nutty, burned, unnatural
7.0	7.5	8.0	20.50	30.00	50.40	
3.5	3.5	5.5	8.50	14.50	36.80	Musty
4.5	5.0	7.0	9.60	23.60	47.00	
4.5	4.0	4.5	8.00	12.70	21.30	Nutty, lacks pepper
5.5	5.5	8.0	13.90	29.60	43.70	Sl. excessive sharpness
7.0	6.0	7.0	13.90	31.10	39.80	Excessive sharpness, soapy
6.0	6.5	7.5	14.50	31.30	45.00	Excessive sharpness, sl. soapy
4.5	5.5	5.0	7.25	11.90	13.30	Unclean, sl. bitter
5.0	4.5	7.0	8.00	14.70	18.00	
4.0	3.5	4.0	7.90	15.10	14.40	Yeasty, unnatural, sl. sour
5.5	6.0	5.5	7.70	14.60	16.00	Sl. sour, sl. unclean
4.0	4.0	5.0	7.00	8.00	13.00	Sour
6.0	6.5	7.5	16.70	28.40	33.00	Sl. sour, sharp
4.0	3.5	4.5	9.20	12.50	14.70	Unclean, sour, unnatural
6.0	6.0	8.0	13.40	25.00	23.40	
3.0	4.0	4.0	10.00	14.40	20.80	Sour, cheddary, unnatural
5.5	7.0	6.5	13.50	24.40	40.70	Cheddary, soapy
4.5	4.0	5.0	10.30	16.14	19.60	Cheddary, sl. bitter
4.5	7.0	7.0	10.80	19.50	26.60	Cheddary, unnatural

flavor score and the total volatile acidity of the cheese most at 12 weeks in the first trial were exactly the same although the order was slightly different. Those strains were 839, 843, M.L., 848 and 846. Second and third trials were made with some of these organisms, and are reported in the lower part of the table. Strain M.L. increased the flavor score, defect score, and total volatile acidity considerably in all three trials at 12 weeks. Strains 839 and 846 gave the same results in two trials. Strain 848 increased the flavor score and total volatile acidity in three of four trials, and improved the defect score at 12 weeks in all four trials. Strain 843 increased the flavor score and total volatile acidity in one of three trials and improved the defect score in two of three trials. Some of the cheese made with these strains were criticized for being excessively sharp or soapy, indicating that the fat hydrolysis was too great. Probably this condition could be corrected by the use of a smaller amount of culture in the cheese.

In previous studies there was some indication that the moisture content of the cheese might have an effect upon the flavor development and fat breakdown in the cheese. An attempt was made to produce a high moisture and a low moisture cheese for the testing of five different strains of M. lipolytica. Each strain used was inoculated into sterilized homogenized milk, incubated for 48 hours at 30°C.,

and used at the rate of 0.3 percent in the milk for cheese. In each trial the first lot of cheese was dipped 2.0 hours after cutting in an attempt to make it a high moisture cheese and the second lot was dipped 2.5 to 2.75 hours after cutting to make it a low moisture cheese. The whey acidity at dipping was 0.050 to 0.085 percent higher for the lots of cheese held 2.5 to 2.75 hours after cutting than for those held 2.0 hours after cutting. The control of the amount of moisture in the cheese was not successful, as the moisture contents of the cheese in trials 1, 3, and 6 are reversed. The moisture content of the cheese possibly was a function of the activity of the lactic culture after the cheese was dipped rather than of the manufacturing procedure. This entire group of cheese was not paraffined and these cheese developed a red slime which was not typical of the other lots. The scores and total volatile acidities for these cheese are presented in Table 5.

No correlation is shown between the flavor scores and moisture contents of these cheese. The small increases in the total volatile acidities from the eighth to the twelfth week in several of these lots of cheese indicate that in some instances the normal ripening stopped at 8 weeks. This may be a reason for the lack of correlation between the 12 week flavor scores and the total volatile acidities. Some of the cheese in this group as well as in others had a high

Tabl

Effect of the Moisture Content of Blue Cheese, Made With Different Strai

Lot No.*	Strain No.	Moisture %	Mold Score 4 wks.	Mold Score 8 wks.	Mold Score 12 wks.	Defect Score 4 wks.	Defect Score 6 wks.	Defect Score 12 wks.	
128	839	47.60	6.5	5.0	4.0	4.5	3.0	6.5	
130	839	48.70	7.5	7.5	4.5	6.5	4.0	6.5	
129	843	49.10	7.0	8.0	4.0	6.5	4.5	6.5	
131	843	46.95	7.0	7.5	4.5	6.5	5.5	6.5	
132	846	46.85	3.5	5.0	4.5	3.0	4.0	3.5	
134	846	49.05	5.5	4.0	7.0	5.0	5.5	3.0	
133	848	48.25	6.0	5.0	5.5	4.0	4.5	4.5	
135	848	46.50	4.5	4.0	4.0	3.5	5.0	5.0	
136	843	47.20	4.0	2.0	4.5	2.5	3.5	3.5	
138	843	46.85	5.0	5.5	5.0	6.5	3.0	5.0	
137	M.L.	43.45	6.5	4.5	4.0	3.5	4.5	4.5	
139	M.L.	46.90	4.0	4.0	5.5	7.0	5.0	7.0	

* The curd for the first cheese of each pair was held in the whey for

** Defect comments are for cheese 12 weeks old.

e 5

e of Mycotorula lipolytica added, Upon Flavor and Total Volatile Acidity

Flavor Score			Total Volatile Acidity			Defect Comments**
wks.	8 wks.	12 wks.	4 wks.	8 wks.	12 wks.	
3.5	3.5	4.0	9.80	22.00	25.10	Sl. sour
6.0	4.0	5.5	9.50	19.70	21.10	Sl. sour
5.5	5.0	5.0	10.00	23.60	20.70	Sl. sour
5.5	5.5	5.5	12.10	24.70	23.60	Sl. sour
3.0	3.5	4.0	11.00	22.00	31.40	Sour, sl. yeasty
7.0	6.0	4.5	13.50	20.00	36.00	Sour, yeasty
5.0	5.0	5.5	11.00	19.70	22.60	Sour, sl. yeasty
4.0	5.5	5.0	12.80	20.00	24.40	Sour, sl. yeasty
3.0	3.5	3.5	9.30	9.70	17.00	Unclean, ammoniacal
5.0	5.0	6.0	11.00	10.50	17.50	Sl. unclean
3.5	4.5	4.5	12.60	13.50	24.90	Unclean, ammoniacal
7.0	6.5	7.5	16.80	22.40	30.50	

! hours, while that for the second was held 2.5 - 2.75 hours.

flavor score and a relatively low total volatile acidity, indicating the presence of flavor constituents other than volatile acids. The flavor scores at 8 weeks without exception, and at 12 weeks with one exception, were higher on the cheese made from curd held longer in the whey after cutting, despite the comparatively unfavorable showing of the addition of M. lipolytica cultures and the lack of flavor development in certain of the cheese after the eighth week. The ammoniacal flavor defect encountered in two instances was apparent only in the cheese made from curd held in the whey the shorter time after cutting. These trials indicated some beneficial effects of holding the curd in the whey longer before dipping and allowing more acid to develop; however, not enough trials were made to establish definitely the value of such a procedure in the manufacture of blue cheese from pasteurized milk. More study upon this point would seem desirable.

Use of Mold Cultures

Lane and Hammer (28) made blue cheese from unhomogenized raw milk and used eight different strains of mold, six of which were Penicillium roqueforti strains, in an attempt to determine differences in flavor, fat acidity, and total volatile acidity due to the mold strain. Some strains consistently gave cheese with high fat acidities, total

volatile acidities, and good flavor, while others gave cheese with low values and poor flavor. There was a general correlation between flavor, and the fat acidity and total volatile acidity of the cheese. Thibodeau and Macy (37) have found that enzyme production by P. roqueforti may vary widely from strain to strain. These findings were the basis for a study of mold strains suitable for use in pasteurized milk blue cheese.

Eighteen strains of Penicillium were assembled from various sources as follows:

Strain No.	Source of strain
1	French Roquefort.
2	Treasure Cave culture.
3	R_2 isolated by M. B. Michaelian from blue cheese.
4	R_4 isolated by M. B. Michaelian from blue cheese.
5	R_5 isolated by C. E. Parmelee from blue cheese 1944.
6	R_6 isolated by C. E. Parmelee from blue cheese 1945.
7	R_7 isolated by C. E. Parmelee from blue cheese 1945.
8	833-P. roqueforti group - Lane's No. 9.
9	French roquefort from Dr. Babel.
10	923-No. 150 P. gorgonzola - Bisurge.
11	830-P. roqueforti group - Lane's No. 6.
12	832-P. roqueforti group - Lane's No. 8.
13	836a-P. roqueforti III from Danish Roquefort.

14	836a-*P. roqueforti* II from caves of Blue d'Auvergne.
15	926- No. 155 *P. roqueforti* - Thom.
16	924- No. 150 *P. gorgonzola* - Bisurge.
17	Minnesota strain of *P. roqueforti*.
18	Mold from Langlois blue cheese isolated 1-15-45.

Proteolytic activity of mold strains

In an attempt to pick certain of these mold strains which might be the most suitable for use in pasteurized milk cheese, a study of the proteolytic and lipolytic activity of all the strains was made. Proteolytic activity was studied by use of a single inoculation with each strain in the center of each of three petri dishes previously poured with Czapek's agar to which 10 percent of sterile skim milk had been added. The three sets of plates were incubated at 13, 21, and 30°C., and the diameters of the colony and of the clear zone around the colony were measured at various intervals. The data are recorded in Table 6. Only strain 11 had a clear zone around it at 13°C. in 15 and 20 days; three strains had clear zones at 21°C. in 5 days and seven had clear zones at 21°C. in 7 days; all but four strains had clear zones at 30°C. in 5 days, and all but five had clear zones at 30°C. in 7 days. The width of the clear zone around colonies of different strains incubated for

Table 6

Proteolysis by Different Mold Strains as Measured by the Agar Plate Me

Mold Strain No.	Temperature and Time						
	13°C.				21°C.		
	15 days		20 days		5 days		
	Diameter of Colony	Diameter of Clear Zone	Diameter of Colony	Diameter of Clear Zone	Diameter of Colony	Diameter of Clear Zone	Dia Co
	(mm)	(mm)	(mm)	(mm)	(mm)	(mm)	(
1	37	-	66	-	24	-	
2	30	-	57	-	28	-	
3	25	-	66	-	25	-	
4	38	-	62	-	25	-	
5	40	-	73	-	29	-	
6	36	-	65	-	24	-	
7	35	-	68	-	23	23	
8	33	-	62	-	24	-	
9	52	-	51	-	29	30	
10	58	-	70	-	27	-	
11	43	44	65	67	22	26	
12	45	-	49	-	31	-	
13	30	-	51	-	18	-	
14	40	-	67	-	29	-	
15	30	-	60	-	30	-	
16	38	-	70	-	31	-	
17	52	-	66	-	17	-	
18	40	-	62	-	25	-	

Table 6

the Agar Plate Method at Three Temperatures

nperature and Time of Incubation of Plates								
21°C.				30°C.				
5 days		7 days		5 days		7 days		
ter: Diameter of Clear Zone	: Diameter of Colony	: Diameter of Clear Zone		: Diameter of Colony	: Diameter of Clear Zone	: Diameter of Colony	: Diameter of Clear Zone	
) (mm)	(mm)	(mm)		(mm)	(mm)	(mm)	(mm)	
: -	: 33	: 37	:	13	: 18	: 20	: 26	
: -	: 45	: -	:	23	: 25	: 40	: 42	
: -	: 35	: -	:	14	: 18	: 21	: 28	
: -	: 37	: -	:	14	: 15	: 21	: 24	
: -	: 41	: -	:	14	: 18	: 23	: 27	
: -	: 31	: 35	:	15	: 20	: 23	: 31	
: 23	: 33	: 37	:	17	: 22	: 23	: 32	
: -	: 32	: 35	:	16	: 21	: 24	: 29	
: 30	: 35	: 37	:	22	: -	: 27	: -	
: -	: 44	: -	:	9	: -	: 25	: -	
: 26	: 33	: 38	:	21	: 29	: 30	: 41	
: -	: 42	: -	:	22	: 29	: 30	: 42	
: -	: 27	: 28	:	14	: 20	: 17	: 27	
: -	: 43	: -	:	19	: 19	: 33	: -	
: -	: 37	: -	:	20	: -	: 32	: -	
: -	: 47	: -	:	22	: -	: 29	: -	
: -	: 31	: -	:	20	: 27	: 26	: 38	
: -	: 47	: -	:	9	: 19	: 14	: 26	

the same length of time at the same temperature varied greatly, as did the size of colony. In general, the colonies grown at higher temperatures were not so large as those grown at lower temperatures, but were more dense.

Lipolytic activity of mold strains

The lipolytic activity of the strains of mold was studied by a procedure similar to that described for proteolysis except that 0.5 ml. of a 3.0 percent butterfat emulsion in 0.5 percent agar was added to each plate before it was poured with Czapek's agar to which nile-blue sulfate had been added in a concentration of 1 to 15,000. No milk was used in the agar. The three sets of plates were incubated at the same temperatures and for the same lengths of time as in the study of proteolysis. The degree of lipolysis was indicated by plus and minus signs, and the data are presented in Table 7. The mold cultures were lipolytic over a wide temperature range, although the greatest amount of lipolysis was at 21°C. for 7 days. Large variation was shown in the lipolytic activity of the various strains of mold by this method.

The work of Thibodeau and Macy (37) showed that lipase production by P. roqueforti is increased greatly if the sugar in Czapek's agar is replaced by peptone. The lipase production by these 18 strains was measured by a procedure identical to that described in the preceding paragraph

Table 7

Lipolysis by Different Mold Strains as Measured by the Agar Plate Met

Mold Strain No.	Temperature and Time: 13°C. 15 days: Diameter of Colony	13°C. 15 days: Degree of Lipolysis	13°C. 20 days: Diameter of Colony	13°C. 20 days: Degree of Lipolysis	21°C. 5 days: Diameter of Colony	21°C. 5 days: Degree of Lipolysis	Diame of Colo
	(mm)		(mm)		(mm)		(m
1	30	-	35	-	27	-	3
2	21	-	26	-	14	+	1
3	29	-	34	++	23	++	2
4	30	-	33	++	21	-	2
5	38	+	46	+	27	+	3
6	34	-	35	+	28	-	3
7	33	-	43	-	23	+	3
8	30	-	34	+	23	+	2
9	34	++	38	++	27	-	3
10	33	+	38	+	24	-	2
11	38	-	42	-	27	+	3
12	28	+	33	+	26	++	2
13	27	-	32	-	22	-	3
14	45	+	54	++	31	+++	4
15	27	-	31	-	19	-	2
16	38	-	43	++	23	+	2
17	31	+	36	+	20	++	2
18	41	+	44	+	29	+	3

Table 7

Agar Plate Method at Three Temperatures

rature and Time of Incubation of Plates

21°C.				30°C.			
ays	7 days			5 days		7 days	
Degree of Lipolysis	Diameter of Colony (mm)	Degree of Lipolysis		Diameter of Colony (mm)	Degree of Lipolysis	Diameter of Colony (mm)	Degree of Lipolysis
-	37	-		12	-	17	-
+	16	++		12	-	16	-
++	31	++		14	+	18	+
-	29	-		11	+	18	+
+	31	+		12	-	15	+
-	36	-		13	-	20	+
+	30	+		11	-	15	+
+	29	+		12	-	17	+
-	38	+		19	-	22	+
-	29	-		15	+	20	-
+	33	++		8	-	13	-
++	29	+++		16	+	20	++
-	31	-		13	+	17	++
+++	44	+++		21	++	28	++
-	29	+		12	+	19	+
+	26	+		15	-	16	-
++	26	++		7	-	12	-
+	35	++		8	-	12	-

except that the sugar in the medium was replaced by 0.3 percent peptone. The lipolysis by all strains, at all temperatures, for all the lengths of time used was very strong and would have been indicated by three plus signs. It was so strong by all strains that no differentiation between strains could be made.

Test of mold strains in cheese

With the aid of the data of Tables 6 and 7, eight mold strains were selected to give as many combinations of proteolytic and lipolytic activity as possible. Mold powder was prepared with each of these strains by the method of Hussong and Hammer (21). A lot of homogenized raw milk cheese was made with each mold strain by the method of Lane and Hammer (28), and a lot of homogenized pasteurized milk cheese was made with each mold strain by the method described under "Methods". The scores, total volatile acidities and defect comments for these cheese are given in Table 8.

The flavor scores, total volatile acidities, and fat acidities of the cheese made from pasteurized milk are quite consistently lower than those of the cheese made from raw milk. All average values are lower as shown at the bottom of the table. Strains 17, 7, 6, and 3 gave the best results in raw milk cheese, and strains 13, 6, 7, and 17 gave the best results in pasteurized milk cheese. Three of the four strains occur in both lists, although in reverse order.

Table 8

Comparison of Score, Total Volatile Acidity, and Fat Acidity of Blue C
Using Eight Strains of Penicilli

Lot No.	Treatment of Milk	Mold Strain No.	Mold Score 4 wks.	Mold Score 18 wks.	Flavor Score 4 wks.	Flavor Score 18 wks.	Total Volatile Acidity 4 wks.	Total Volatile Acidity 14 wks.
17	Raw	3	5.0	8.0	4.5	6.0	16.00	47.20
13	Past.	3	3.0	5.0	3.0	3.0	9.00	30.40
18	Raw	6	7.0	9.0	6.0	6.5	15.50	51.80
14	Past.	6	6.0	5.0	3.0	5.0	7.40	28.00
19	Raw	7	7.0	5.5	5.0	7.0	16.70	51.00
15	Past.	7	5.0	6.0	4.0	4.5	7.30	42.50
20	Raw	11	7.0	9.0	2.5	5.0	13.20	44.30
16	Past.	11	4.0	8.0	4.0	4.0	6.50	36.60
5	Raw	12	6.5	9.5	3.0	3.5	14.00	44.00
9	Past.	12	7.0	5.5	4.5	3.0	5.80	11.00
6	Raw	13	4.0	4.0	5.0	5.0	17.00	45.00
10	Past.	13	3.0	7.0	2.0	5.5	7.60	24.00
7	Raw	14	4.0	3.0	4.0	4.5	16.00	64.60
11	Past.	14	4.0	8.0	4.0	3.5	7.60	34.20
8	Raw	17	9.0	9.0	4.5	7.5	11.60	43.60
12	Past.	17	7.0	6.0	1.0	4.5	5.80	18.00
Av.	Raw		6.19	7.12	4.31	5.62	15.00	48.94
Av.	Past.		4.87	6.31	3.19	4.12	7.12	28.09

* Defect Comments are for cheese 18 weeks old.

Table 8

ldity, and Fat Acidity of Blue Cheese Made from Raw and Pasteurized Milk Using Eight Strains of Penicillia

Flavor Score		Total Volatile Acidity		Fat Acidity		Defect Comments*
4 wks.	18 wks.	4 wks.	14 wks.	4 wks.	14 wks.	
4.5	6.0	16.00	47.20	22.20	27.30	Evidence of two molds
3.0	3.0	9.00	30.40	2.00	11.50	Nutty, undesirable
6.0	6.5	15.50	51.80	21.25	22.80	
3.0	5.0	7.40	28.00	2.85	14.50	Nutty
5.0	7.0	16.70	51.00	23.10	36.15	
4.0	4.5	7.30	42.50	2.00	8.07	Nutty
2.5	5.0	13.20	44.30	22.10	33.25	Soapy
4.0	4.0	6.50	36.60	2.85	9.70	Soapy
3.0	3.5	14.00	44.00	20.95	22.30	Sweet, unnatural
4.5	3.0	5.80	11.00	11.90	8.84	Nutty
5.0	5.0	17.00	45.00	23.45	29.65	
2.0	5.5	7.60	24.00	2.75	11.72	Nutty
4.0	4.5	16.00	64.60	25.60	32.97	Lacking
4.0	3.5	7.60	34.20	3.05	12.60	Nutty
4.5	7.5	11.60	43.60	22.25	28.87	
1.0	4.5	5.80	18.00	2.35	9.00	Nutty
4.31	5.62	15.00	48.94	22.61	29.16	
3.19	4.12	7.12	28.09	3.72	10.74	

eeks old.

Strain 6 was found to be the best single strain for use in cheese from both raw and pasteurized milk. Strain 11 is of interest because it has the ability to hydrolyze fat, as shown by the high total volatile acidities in cheese from both raw and pasteurized milk, but it lacks the ability to build up fine flavor as shown by the soapy defect in both types of cheese. Strain 14 produced high total volatile acidity in both types of cheese, but did not produce fine flavor. This strain was the most actively lipolytic strain as shown in Table 6.

All lots of cheese from pasteurized milk, with one exception, were criticized for having a nutty flavor. This flavor seems to be somewhat characteristic of blue cheese made from pasteurized milk to which no enzyme preparation has been added. The data indicate that it was not possible to find a mold strain among those tried that was superior for use in making blue cheese from pasteurized milk.

Use of a blue cheese emulsion in milk for cheese

An attempt was made to improve the flavor of blue cheese from pasteurized milk by the addition of various amounts of an emulsion of a well cured, fine flavored blue cheese to the milk in the hope that some of the enzymes might be carried over. This proved to be very unsuccessful, apparently because of the transfer of defect producing

microorganisms, presumably yeasts, in the cheese emulsion. All lots of cheese (21 to 28 inclusive) made by this method were very yeasty and were discarded at the end of 4 weeks.

Use of mold-enzyme preparation in cheese

The work of Thibodeau and Macy (37) and the tests for lipolysis made of the mold strains on Czapek's agar in which the sugar was replaced by peptone indicated the possibility of making an enzyme preparation for addition to the cheese by growing mold on this type of medium. These workers found that Czapek's solution plus 0.1 percent agar plus either skim milk or peptone gave the best mold growth, and that Czapek's solution without sugar, plus 0.3 percent peptone and 0.3 percent butterfat gave the highest lipase activity. A medium which was a combination of these two was made and had this composition:

Sodium nitrate	2.00 grams
Monopotassium phosphate	1.00 grams
Potassium chloride	.50 grams
Magnesium sulfate	.50 grams
Ferrous sulfate	.01 grams
Peptone	3.00 grams
Butterfat	100.00 grams
Agar	5.00 grams
Water	1000.00 ml.

Due to difficulty in sterilization, the butterfat was sterilized separately and added to the semi-solid medium at the time of inoculation. The medium was dispensed in bottles which were only half filled so the butterfat could be emulsified by shaking.

The mold-enzyme preparation was made by inoculating the above medium with mold spores, emulsifying the melted butterfat into the medium and placing the medium in previously sterilized 2800 ml. Fernbach flasks to a depth not to exceed one inch. The mold was allowed to grow for 7 days at room temperature, with shaking at 2 day intervals to break up the surface felt. This mold-enzyme preparation made with 4 different mold strains was added to 4 lots of pasteurized milk at the rate of 0.55 percent and the milk was made into cheese. The scores, total volatile acidities, and fat acidities of this cheese are given in Table 9.

At 12 weeks all four lots of cheese had considerable peppery flavor but were too rancid to score, indicating that too much of the preparation had been added. This is substantiated by the exceedingly high total volatile acidities and fat acidities. In these cheese the defect scores and flavor scores are quite closely correlated but there seems to be no relationship between them and the mold scores. Strains 4 and 17 gave the highest flavor and defect scores at 4 weeks.

The preceding trials indicated some possibilities for

Table 9

Effect of the Addition of 0.55 Percent of Mold-Enzyme Preparation to

Lot No.	Mold Strain No.	Mold Score 4 wks.	Mold Score 12 wks.	Defect Score 4 wks.	Defect Score 12 wks.	Flavor Score 4 wks.	Flavor Score 12 wks.	Total Volatil Acidity 4 wks.	Total Volatil Acidity 12 wks
48	4	4.0	5.0	7.0	- **	6.5	- **	48.10	107.00
49	12	5.0	6.5	4.0	-	5.5	-	25.20	38.90
50	14	4.0	6.0	4.0	-	4.0	-	22.40	64.00
51	17	6.5	4.0	5.5	-	6.0	-	63.00	99.60

* Defect Comments are for the scores at four weeks.

** Hydrolysis of fat too extensive to permit accurate scoring at 12

Table 9

Enzyme Preparation to Pasteurized Milk

r Score	Total Volatile Acidity		Fat Acidity		Defect Comments *
12 wks.	4 wks.	12 wks.	4 wks.	12 wks.	
- **	48.10	107.00	45.13	98.55	Excessive fatty acids
-	25.20	38.90	26.90	56.48	Some ketone, sour, unclean
-	22.40	64.00	20.38	50.83	Yeasty, unclean, lacking
-	63.00	99.60	48.76	112.63	Some ketone, excessive rancidity

weeks.

ccurate scoring at 12 weeks.

the special mold culture if the right mold strain was selected, and if the proper concentration of mold-enzyme preparation were used in the cheese. A number of mold strains were chosen for various reasons to be used in further trials with this method. Strain 4 was chosen because it was one of the best in the preceding trial and because it was the strain being used in the commercial production of mold powder. Strain 12 was the poorest strain in Table 8 and one of the poorest in Table 9. Strain 6 was the best strain for both raw and pasteurized milk cheese in Table 8. Strain 13 gave about equal and average results for both raw and pasteurized milk cheese as indicated in Table 8. Strain M_8 was a new strain isolated from an excellent blue cheese obtained from Maytag Dairy Farms, Newton, Iowa.

Mold-enzyme preparations were made with each of these strains of mold and were added to three vats of milk at the rates of 0.05, 0.10 and 0.25 percent. The scores, total volatile acidities, and defect comments on these lots of cheese are presented in Table 10.

All lots of cheese, with 2 exceptions, made with added mold-enzyme preparation had higher flavor and defect scores at 8 and 12 weeks than the corresponding control cheese made without added mold-enzyme preparation. In general the higher concentrations of mold-enzyme preparation resulted in the higher flavor scores at 12 weeks. In one case the

Effect of the Addition of Varying Amounts of Mold-Enzyme Preparati

Lot No.	Mold Strain No.	Mold-Enzyme Prep. Used %	Mold Score 4 wks.	Mold Score 8 wks.	Mold Score 12 wks.	Defect Score 4 wks.	Defect Score 8 wks.	Defect Score 12 wks.
92:	4	0.00	4.0	4.5	7.0	4.0	3.5	2.0
93:	4	0.05	4.5	6.0	7.5	7.0	5.5	7.0
94:	4	0.10	3.5	3.5	4.5	6.0	5.5	5.0
95:	4	0.25	3.5	3.5	4.5	4.5	6.0	7.0
96:	12	0.00	6.0	6.0	9.0	5.5	3.0	3.5
97:	12	0.05	8.0	7.5	7.5	2.0	5.0	5.0
98:	12	0.10	8.0	6.0	8.0	3.0	4.0	4.0
99:	12	0.25	8.0	7.5	6.0	4.0	6.0	7.0
100:	6	0.00	5.0	5.5	8.0	6.0	4.0	2.0
101:	6	0.05	6.0	3.5	7.5	7.5	7.0	6.5
102:	6	0.10	4.0	4.5	3.5	4.5	7.0	6.5
103:	6	0.25	3.5	3.5	4.5	7.0	5.5	5.0
104:	13	0.00	3.5	6.5	5.0	2.0	3.0	2.0
105:	13	0.05	7.5	6.5	5.0	4.5	4.5	5.5
106:	13	0.10	6.5	3.5	4.0	5.5	4.0	6.0
107:	13	0.25	5.0	4.0	4.0	7.0	5.5	5.0
108:	M6	0.00	4.5	4.5	4.0	7.5	4.5	3.0
109:	M6	0.05	6.0	4.5	6.5	6.5	4.5	5.0
110:	M6	0.10	3.5	4.5	2.5	4.0	3.5	5.0
111:	M6	0.25	5.5	4.5	5.0	7.5	5.0	5.0
140:	6	0.00	5.5	5.0	5.0	4.5	5.0	2.5
141:	6	0.05	6.5	3.5	4.5	7.0	6.5	5.0
142:	6	0.10	4.5	4.5	4.0	7.0	6.5	7.5
143:	6	0.25	4.0	4.0	4.5	5.0	5.5	6.0
144:	M6	0.00	7.5	4.5	5.5	4.0	3.0	3.0
145:	M6	0.05	7.5	4.5	5.0	4.5	4.5	4.0
146:	M6	0.10	4.5	4.0	3.5	6.5	6.5	5.5
147:	M6	0.25	3.0	5.5	4.5	6.5	5.5	6.5

* Defect Comments are for cheese 12 weeks old.

Table 10

on of Five Strains of Mold to Pasteurized Milk

Flavor Score			Total Volatile Acidity			Defect Comments*
4 wks.	8 wks.	12 wks.	4 wks.	8 wks.	12 wks.	
3.5	3.5	3.0	8.60	12.80	28.60	Sour, musty, cheddary
3.0	4.5	5.5	9.30	16.40	22.30	Sl. nutty
4.5	4.0	6.0	9.80	14.70	26.00	Sour, sl. unnatural
5.5	5.5	7.0	9.50	19.00	36.50	Sl. nutty
3.0	4.0	4.0	10.00	11.10	15.20	Sour, fermented
3.5	6.0	6.0	7.50	12.00	15.70	Sl. sour, sl. fermented
4.0	5.5	6.0	7.50	18.00	28.40	Unclean, sour, fermented
5.5	7.0	7.5	12.00	21.40	32.80	Sl. fermented
4.0	3.5	2.5	6.00	10.00	22.50	Musty, unnatural
6.5	6.0	7.0	12.10	21.50	42.50	Unnatural
7.0	7.0	7.0	21.00	35.10	51.05	Unnatural
7.5	5.5	6.5	28.20	61.50	78.80	Excessive sharpness
3.0	3.5	3.0	9.10	14.40	14.50	Musty, sour, unnatural
4.5	5.0	5.0	9.70	14.20	14.50	Unnatural
4.5	5.0	5.0	9.25	12.50	15.60	Sl. unnatural
5.5	5.5	5.5	8.00	12.50	16.40	Unnatural
4.5	4.5	4.0	7.10	13.30	15.40	Musty, yeasty, sour
5.5	4.5	5.0	7.30	14.00	17.50	Unnatural, sl. sour, cheddary
5.0	3.5	5.5	11.30	18.50	17.00	Unnatural, sl. sour, cheddary
6.6	6.0	6.0	15.70	29.00	29.20	Unnatural, sl. sour, cheddary
4.0	4.0	2.5	11.80	9.50	17.80	Yeasty, sour, unclean
7.0	5.0	7.0	17.40	23.00	37.00	Sl. unclean, sl. fermented
7.5	7.0	7.5	29.00	25.20	57.00	Sl. soapy
6.5	6.0	6.5	43.05	37.00	80.50	Soapy, excessively sharp
3.0	4.0	3.5	8.05	11.00	20.50	Fermented, sour, sl. musty
4.5	6.0	4.5	10.60	16.70	29.00	Sour, sl. fermented
5.5	7.0	6.0	12.70	18.40	28.20	Sl. sour
6.5	6.0	7.0	25.00	24.50	50.70	Sl. soapy

flavor scores at 8 and 12 weeks were reduced because of excessive fat hydrolysis.

With all the mold strains except 13 a definite increase occurred in the total volatile acidities at 8 and 12 weeks with increase in the concentration of mold-enzyme preparation used. The 12 week value for total volatile acidity of the control cheese made with mold strain 4 appears to be out of line with the 4 and 8 week values and the other 12 week values in that series. Strains 4, 12 and 6 appeared to cause the most improvement in the flavor of the cheese, while strain 13 appeared to cause the least improvement of the flavor of the cheese. Strain 6 consistently gives the greatest increase in total volatile acidity of any of those used. It gives the best flavor also particularly when the total volatile acidity is in the range in which 30 to 60 ml. of 0.10 N sodium hydroxide are required to titrate the volatile acids in the first 1000 ml. of distillate from 200 g. of cheese.

Relation of Chemical Analyses to Flavor Score

Relation of protein degradation to flavor score

Total nitrogen, amino nitrogen, and nitrogen fractions soluble and insoluble in phosphotungstic acid and in trichloroacetic acid were determined on certain lots of

cheese. The values for these determinations are given in Appendix B.

The amino nitrogen values for lots 29 to 47 and 52 to 63 at 12 weeks plotted against the flavor scores for that same time are shown in Fig. 1. Values for certain other lots upon which data concerning protein degradation were obtained could not be plotted because these lots were not scored at 12 weeks. The line of linear regression as estimated by the method of least squares according to Snedecor (35) is shown. The point A is located by the two mean values. The slope of the line of linear regression is so steep and there are so many points so far from the line, that the amino nitrogen values are shown to have very little relationship to the flavor score of the cheese. Prediction of the flavor score from the amino nitrogen value could not be made with any satisfactory degree of reliability.

The values for the nitrogen fractions soluble in trichloroacetic acid for these same lots of cheese are plotted against the flavor scores in Fig. 2. The line of linear regression and the point A located by the two mean values are shown. The slope of this line is greater than that of the one for the amino nitrogen values, indicating that values for the nitrogen fraction soluble in trichloroacetic acid have even less relationship to the flavor score of the cheese than do the amino nitrogen values.

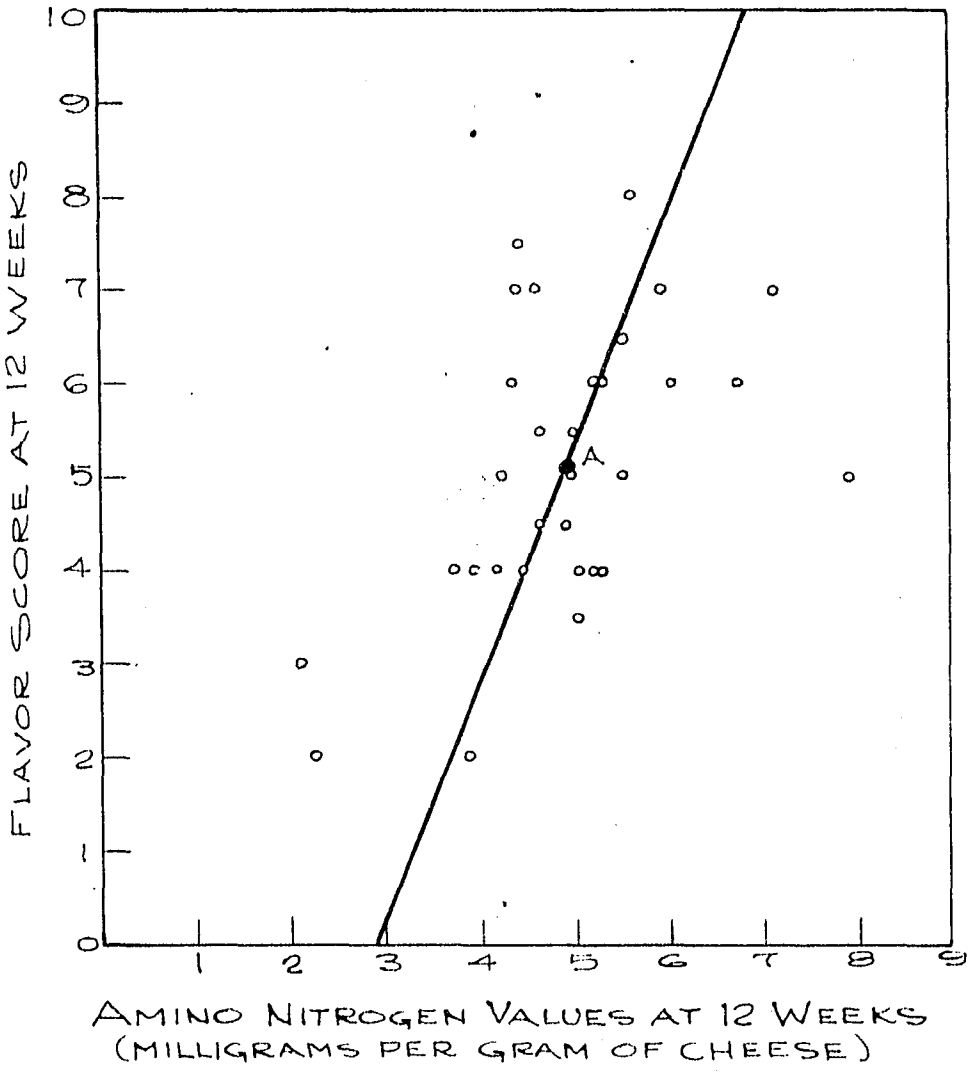
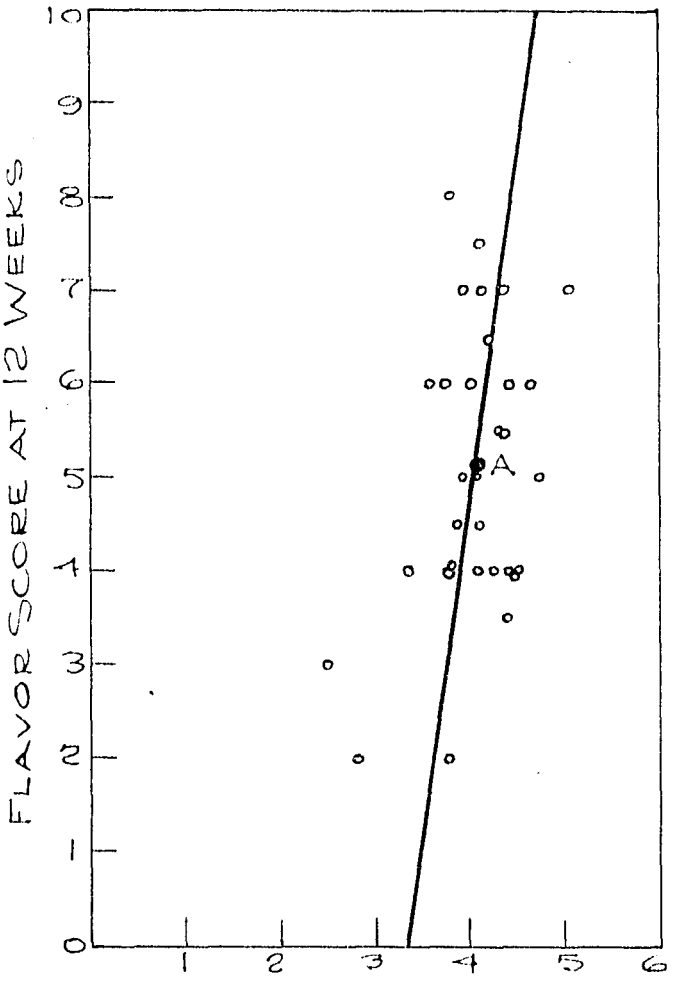


FIG. 1 RELATION OF AMINO NITROGEN VALUES TO FLAVOR SCORE



NITROGEN FRACTION SOLUBLE IN TRICHLOROACETIC ACID AT 12 WEEKS.
(MILLILITERS 0.1N ACID EQUIVALENT TO NITROGEN FROM 0.5g. OF CHEESE.)

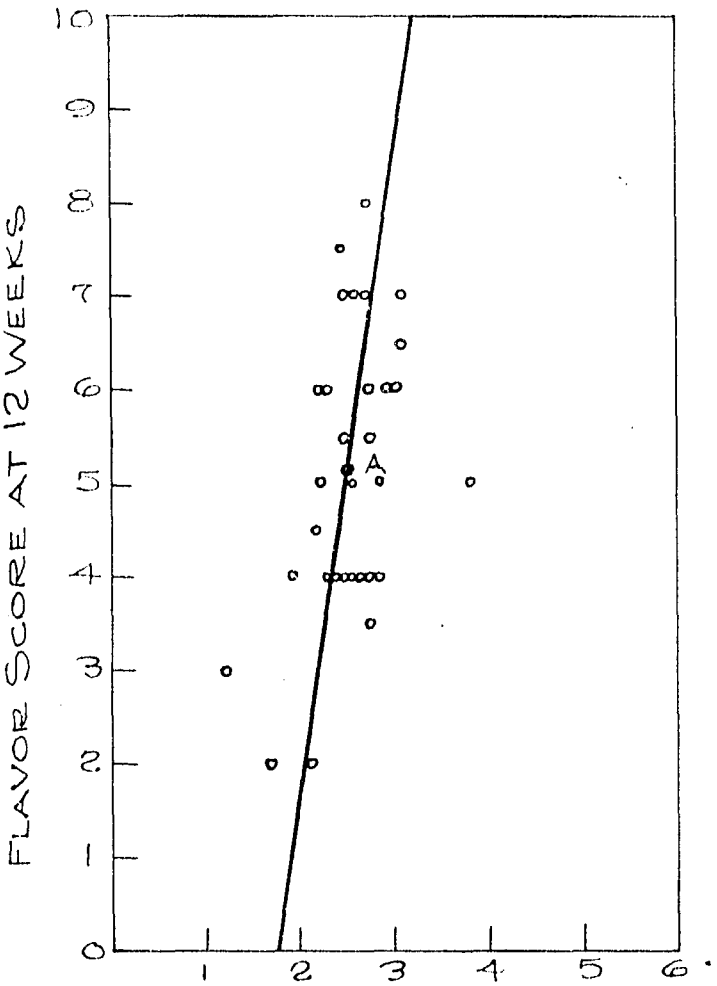
FIG. 2 RELATION OF NITROGEN FRACTION SOLUBLE IN TRICHLOROACETIC ACID TO FLAVOR SCORE.

The values for the same lots of cheese of the nitrogen fraction soluble in phosphotungstic acid are plotted against the flavor scores in Fig. 3. The line of linear regression and the point A located by the two mean values are shown. The slope of this line is about the same as that for the line of the nitrogen fraction soluble in trichloroacetic acid and is more steep than that of the line of the amino nitrogen values. Therefore, values for the nitrogen fraction soluble in phosphotungstic acid would have about the same significance in predicting the flavor scores of blue cheese as the values for the nitrogen fraction soluble in trichloroacetic acid and less significance than the amino nitrogen values.

Because the degree of protein degradation in the cheese had so little apparent relationship to the flavor score, determination of the nitrogen fractions was discontinued with lot 71 at 4 weeks and lot 63 at 12 weeks.

Relation of fat acidity and total volatile acidity to flavor score

In order to determine whether a relationship between flavor score and fat acidity could be established, the fat acidities at 12 weeks on lots 29 to 47 and 52 to 55, as shown in Tables 1 and 2, are plotted against flavor score at 12 weeks in Fig. 4. The line of linear regression as calculated by the method of least squares according to



NITROGEN FRACTION SOLUBLE IN PHOSPHOTUNGSTIC ACID AT 12 WEEKS
(MILLILITERS 0.1N ACID EQUIVALENT TO NITROGEN FROM 0.5 G. OF CHEESE)

FIG. 3 RELATION OF NITROGEN FRACTION SOLUBLE IN PHOSPHOTUNGSTIC ACID TO FLAVOR SCORE

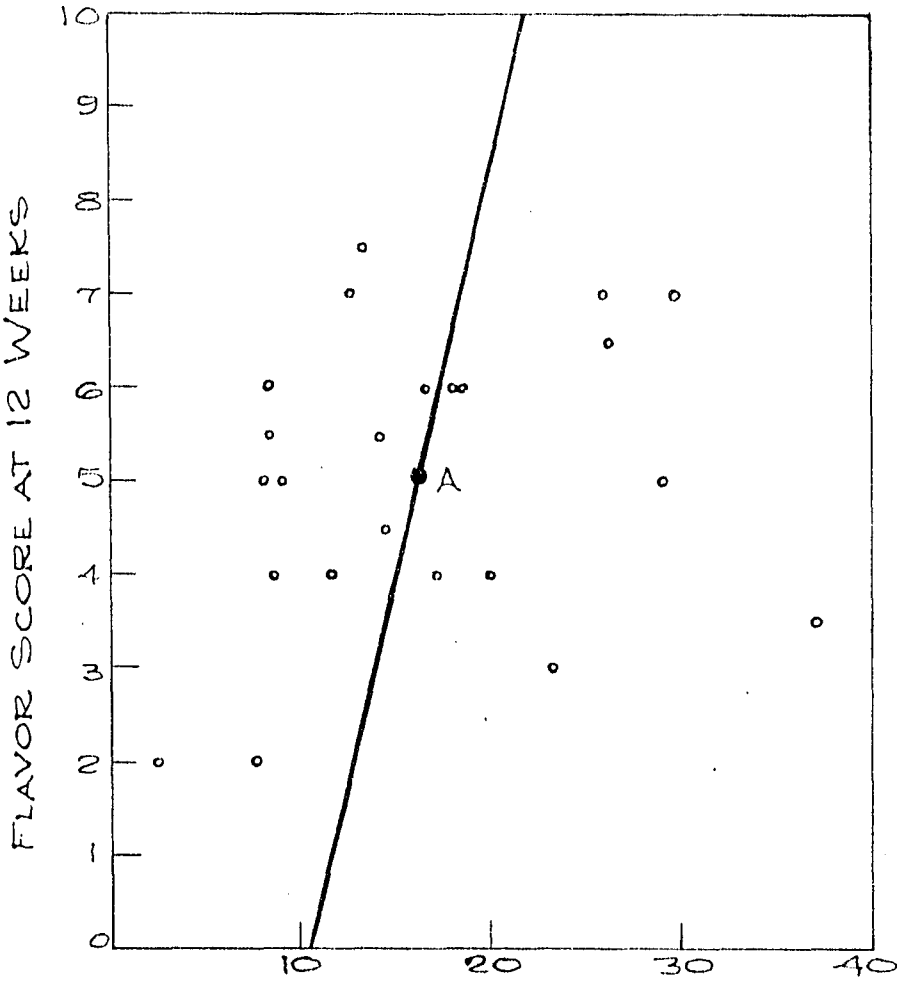


FIG. 4 RELATION OF FAT ACIDITY TO FLAVOR SCORE

Snedecor (35) and the point A located by the mean values are shown. The data reveal very little correlation between the fat acidities and the flavor scores of these lots of blue cheese. Prediction of the probable flavor score from the fat acidity or vice versa would not be possible.

The total volatile acidities were determined on all lots of cheese from 29 to 147 at 12 weeks. The values for the lots to which M. lipolytica cultures were added are shown in Tables 2, 3, 4, and 5. The values for these lots, which are numbers 32 to 35, 56 to 59, 72 to 91, 112 to 119, and 124 to 139, are plotted against the flavor scores in Fig. 5. The line of linear regression and the point A located by the mean values are shown. A fairly close correlation between the total volatile acidity and the flavor score of these lots of cheese is evident.

The total volatile acidity values for the lots of cheese to which the mold-enzyme preparations were added are given in Table 10. The values for lots 92 to 111 and 140 to 147 are plotted against the flavor scores in Fig. 6. Approximately the same correlation is shown in Fig. 6 as in Fig. 5. The two values to the extreme right in Fig. 6 were for cheese that was scored down because of excessive rancidity.

From Fig. 5 and 6, it is apparent that the total volatile acidity is correlated with the flavor score of the cheese more closely than is any of the other chemical

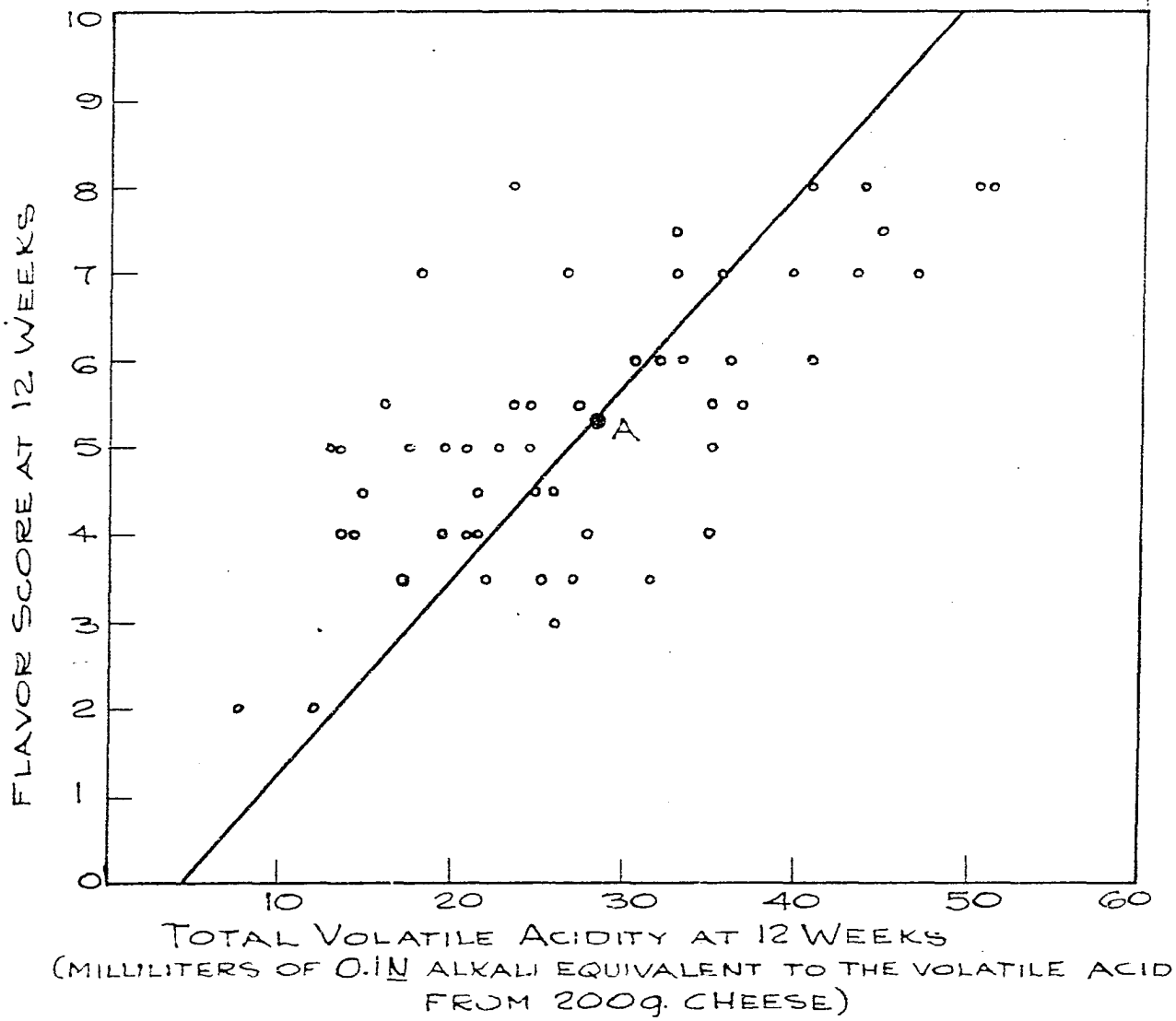


FIG 5 RELATION OF THE TOTAL VOLATILE ACIDITY TO THE FLAVOR SCORE OF CHEESE TO WHICH MYCOTORULA LIPOLYTICA CULTURES HAD BEEN ADDED

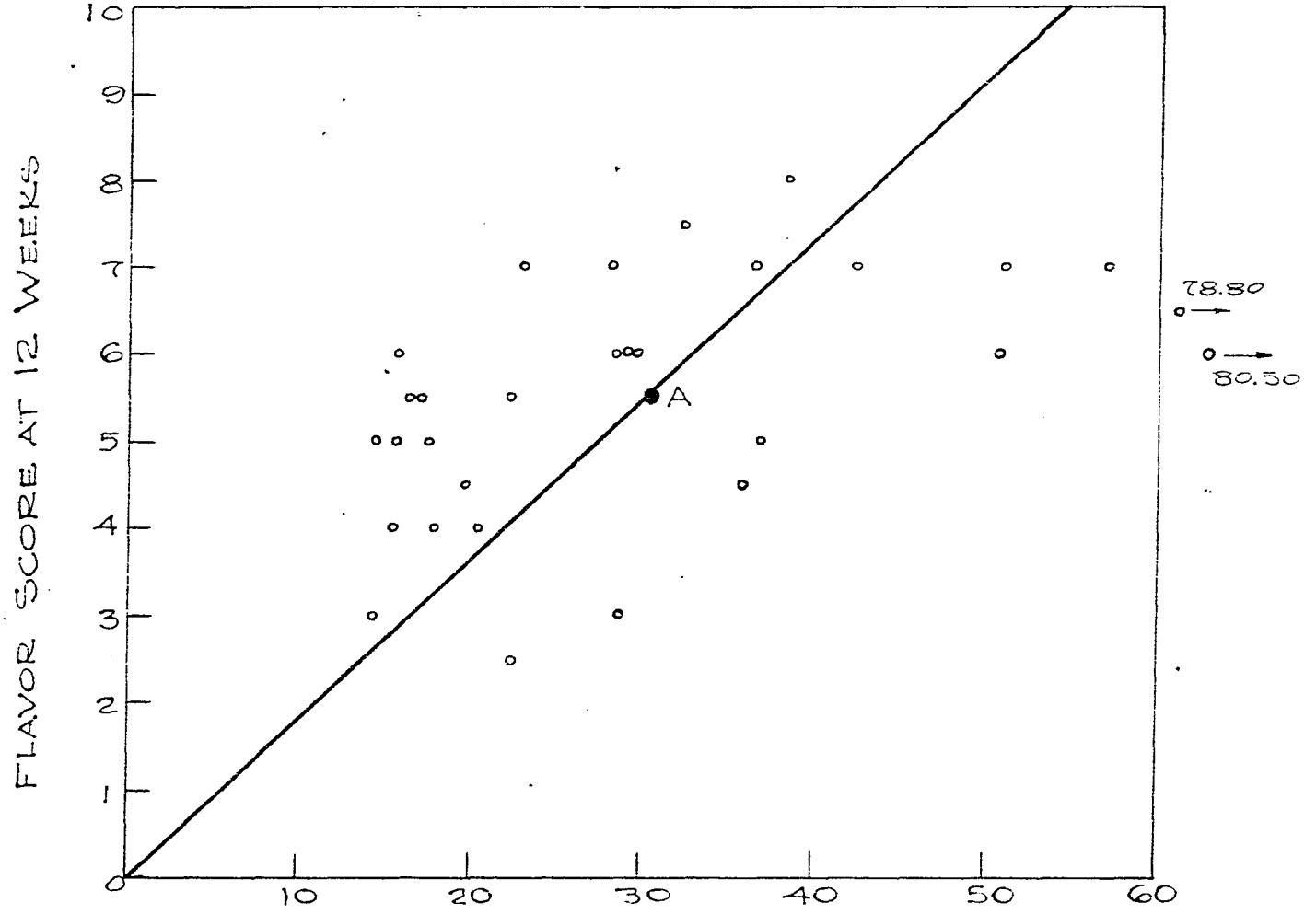


FIG. 6 RELATION OF THE TOTAL VOLATILE ACIDITY TO THE FLAVOR SCORE OF CHEESE TO WHICH MOLD-ENZYME PREPARATION HAD BEEN ADDED.

analyses used. The flavor score could be predicted with some degree of accuracy if the total volatile acidity of the cheese were known. In general, the cheese with highest flavor scores had total volatile acidities in the range in which 30 to 55 ml. of 0.1 N alkali were necessary to titrate the volatile acids in the first 1000 ml. of steam distillate from 200 g. of cheese.

DISCUSSION

The major difference in blue cheese made from pasteurized milk and that made from raw milk is in the amount of flavor that is developed, as has been pointed out earlier. The addition of M. lipolytica cultures or mold-enzyme preparations to the milk increased the total volatile acidities of the cheese, but did not always result in fine-flavored cheese. This is particularly true of cheese which does not have much mold growth. Despite the few exceptions, a quite close relationship between volatile acidity and flavor score was demonstrated as shown in Fig. 5 and 6. Either of the two procedures studied apparently could be used to give any desirable level of fat hydrolysis, and hence each was a method of substituting other lipase for the normal milk lipase. The data obtained did not show as close correlation between the fat acidities and the total volatile acidities as did those of Lane and Hammer (28). This may be due to the fact that they were working primarily with cheese made from raw milk with no special cultures or enzymes added, while the data in the present study were obtained on cheese made from pasteurized milk to which special lipolytic cultures had been added in many instances.

As was indicated in the "Review of Literature" and in

the discussion of Table 5, the volatile acids probably are not the only flavor constituents of blue cheese. Some lots of cheese had relatively high total volatile acidities but were criticized for having a green, mold-like flavor, indicating that some flavor constituents were lacking and the flavor of the cheese was not well-rounded. This was true particularly of the cheese with extensive mold growth after 4 weeks of curing. Other lots of cheese were relatively low in total volatile acidities at 12 weeks but were scored quite high in flavor, because the flavor constituents gave a well-rounded, blue cheese flavor. As has been indicated in the literature, these flavor constituents which are necessary for rounding out the flavor of blue cheese probably are ketones which are formed by the oxidation of the fatty acids by the mold. They most likely do not appear in the cheese at 4 weeks because the concentration of fatty acids has not been built up enough to cause the mold to carry on the abnormal oxidation of the fatty acids which results in the production of the ketones.

The marked differences in the suitability of different strains of Mycotorula or mold would be an important factor to consider in the application of these procedures to commercial practice. Not only would selection of proper original strains be necessary; constant checking also would be needed to insure that the cultures maintained their

original characteristics.

The use of M. lipolytica cultures in cheese on a commercial basis would be a relatively simple procedure. The culture could be prepared by inoculation of sterile homogenized whole milk and incubation at 30°C. for 48 hours. This culture could be added to the milk for cheese at the desired rate which would depend upon the activity of the particular culture and the amount of flavor desired. The use of the mold-enzyme preparation on a commercial basis would be somewhat more complicated, but the reliability of the method and the results obtained might well be worth the added inconvenience. The preparation could be made by inoculation of the modified Czapek's medium with the proper mold strain and incubation in Fernbach flasks at 20 to 25°C. for a week. This preparation should be homogenized into a small amount of milk and added to the milk for cheese at the rate of 0.05 to 0.25 percent, depending upon the amount of flavor desired in the cheese. The attitude of regulatory officials and the definitions for cheese which are being established would be factors to consider in the use of either method.

Some of the results obtained during the course of this work have indicated that several things should be investigated before these methods will be reliable for commercial cheese manufacture. A method should be devised

for rapidly determining the lipolytic activity of the mold-enzyme preparation at the time it is to be used. Further studies should be made on the population of M. lipolytica organisms in the culture at the end of 48 hours incubation. The results obtained indicated there may have been quite a variation in that population. Factors affecting these variations in numbers of organisms should be determined.

The minor difference in blue cheese made from pasteurized milk and that made from raw milk is in the character of the curd that is obtained. The curd from raw milk is tough, pliable, and elastic, while that from pasteurized milk is soft and very brittle. This difference in the character of the curd necessitated the changes in the manufacturing procedure which have been indicated, in order to obtain a cheese that was sufficiently open-bodied to allow mold growth, which in turn would cause fat breakdown and flavor development.

Some improvement in the elasticity of the curd and the openness of the cheese was accomplished by the use of more rennet and by the addition of calcium chloride to the milk before it was set with rennet. However, the character of the curd and the body of the cheese still were not as satisfactory as were those of the cheese made from raw milk. Closely associated with the character of the curd, was the control of the moisture content of the cheese. As

can be seen from the data on composition presented in Appendix A, the cheese made from pasteurized milk usually was high in moisture. The control of the moisture may have been more closely related to the activity of the lactic culture than to the manufacturing procedure, as pointed out in the discussion of Table 5. Cheese which had a moisture content lower than 45 or 46 percent was inclined to be very dry and crumbly. The body of all the cheese made from pasteurized milk was so firm that one was misled as to the moisture content of this cheese. Certainly the procedures used in the manufacture of blue cheese from raw milk cannot be used for the manufacture of blue cheese from pasteurized milk without modification. Further study of modifications which may reduce the moisture content of the cheese and still give a body and texture more open and less fragile would seem desirable, now that means have been found for bringing about the fat degradation which normally is lacking in blue cheese made from pasteurized milk.

SUMMARY

A study was made of some possible means by which blue cheese could be made from pasteurized milk and still develop the flavor characteristic of the product made from raw milk. Previous studies have indicated the effect of pasteurization arises, to a large degree, from the inactivation of the normal milk lipase in cheese made from pasteurized milk.

A total of 143 lots of cheese was made. Seven lots were made with the addition of varying amounts of the lower fatty acids. The addition of the acids improved the flavor of the cheese but did not give the flavor typical of good blue cheese.

The addition of cultures of three lipolytic bacteria and one lipolytic yeast to milk for cheese making was tried. Cultures of the organisms grown in cream containing 18 percent fat were tried in 16 lots of cheese, and cultures of the same organisms grown in homogenized whole milk were tried in 16 lots of cheese. The results in both cases were very similar. M. lipolytica improved the flavor of the cheese and in some cases caused excessive fat breakdown. A. lipolyticus gave the cheese unnatural flavors and did not cause much fat degradation. The cheese made with A. lipolyticum and P. fragi were musty and unclean and showed

no evidence of fat hydrolysis by these organisms.

Eleven M. lipolytica strains collected from different sources were tried in 28 lots of cheese to see if there was any variation in the effect of different strains of the organism in cheese. Most strains improved the flavor of the cheese but some strains appeared to improve the flavor more than others. Of the strains used, 839, 843, M.L., 848, and 846 gave the most improvement in flavor.

A collection of 18 strains of mold of the Penicillium roqueforti type was assembled from various sources. These were tested for lipolytic ability by the nile-blue sulfate technique and for proteolytic ability by Freudenreich's technique (15) on Czapek's agar. Wide variations in lipolytic and proteolytic ability were shown by the different cultures on regular Czapek's agar. The lipolytic ability of the cultures was much greater and about equal for all cultures when the sugar in Czapek's agar was replaced by peptone.

From the results of the above tests, 8 strains were chosen, and a lot of cheese from homogenized raw milk and one from homogenized pasteurized milk were made with each. No particular strain was found to be especially suited to the manufacture of blue cheese from pasteurized milk. Strains 6 and 7 gave the best results in both types of cheese, and strain 17 gave the best result in raw milk cheese.

The addition of an emulsion of a well-cured, fine-flavored cheese to the milk from which blue cheese was made, proved very unsuccessful, apparently due to the transfer of contaminating microorganisms, presumably yeasts, to the milk and subsequent development of a yeasty flavor defect in the cheese.

A mold-enzyme preparation, made by the growth of P. roqueforti on a semi-solid medium low in carbohydrate and containing butterfat, was added to the milk at the rate of 0.55 percent and gave cheese with a very high flavor and excessive rancidity. The same type of preparation was made with 5 strains of penicillia and added to milk in amounts varying from 0.05 to 0.25 percent. All strains brought about an improvement in flavor, although some proved better than others. The optimum amount of mold-enzyme preparation to use was between 0.10 and 0.25 percent, depending upon the sharpness of the cheese desired. Strains 4, 12, and 6 gave the greatest improvement in flavor, while strain 13 gave the least improvement in flavor.

Protein degradation studies were made in an attempt to correlate protein breakdown and flavor development. There is little or no correlation of values for amino nitrogen, nitrogen fraction soluble in trichloroacetic acid, and nitrogen fraction soluble in phosphotungstic acid with the flavor scores of the cheese.

Fat acidity and total volatile acidity were determined

in an attempt to correlate fat degradation and flavor development. There is very little correlation of fat acidity with flavor score of the blue cheese. The values for total volatile acidity correlate fairly well with the flavor scores. The total volatile acidity was the only chemical method employed which could be used to predict the flavor score with any degree of accuracy. Cheese with total volatile acidities in the range in which 30 to 55 ml. of 0.1 N alkali were necessary to titrate the volatile acids in the first 1000 ml. of steam distillate from 200 g. of cheese, generally had the highest flavor scores.

CONCLUSIONS

1. The addition of varying amounts of the fatty acids butyric, caproic, caprylic, capric, and lauric to melted butterfat which was homogenized into cheese milk, improved the flavor of the cheese made from pasteurized milk but did not give flavor entirely typical of good blue cheese.

2. The addition of cultures of Alcaligenes lipolyticus, Achromobacter lipolyticum, and Pseudomonas fragi to milk for cheese making did not improve the flavor of the cheese and did not result in any appreciable amount of additional fat hydrolysis. Cultures of Achromobacter lipolyticum and Pseudomonas fragi appeared to give the cheese musty and unclean flavors.

3. The addition of a culture of Mycotorula lipolytica to milk for cheese making improved the flavor score and increased the total volatile acidity of the cheese.

4. Eleven strains of Mycotorula lipolytica isolated from various products differed markedly in their ability to break down the fat and improve the flavor of blue cheese.

5. Mycotorula cultures prepared in homogenized milk were as effective as those prepared in cream containing 18 percent butterfat in the improvement of the flavor of blue cheese.

6. The 18 strains of mold of the Penicillium roqueforti type tested showed some variation in proteolytic ability as measured on Czapek's agar with 10 percent milk added. They varied widely in lipolytic abilities as measured by the nile-blue sulfate technique with regular Czapek's agar as the basal medium. The same strains were much more strongly lipolytic and showed no appreciable variation in lipolytic ability when measured by the nile-blue sulfate technique with Czapek's agar in which the sugar had been replaced by peptone as the basal medium.

7. The eight strains of Penicillium used in cheese made from raw and pasteurized milk varied greatly in their flavor production and total volatile acid formation. Strains 6 and 7 gave the best results in both raw and pasteurized milk cheese, while strain 17 gave the best result in raw milk cheese.

8. The addition of an emulsion of a well-cured, fine-flavored blue cheese to the milk from which blue cheese was made proved very unsuccessful due to the transfer of contaminating microorganisms, presumably yeasts, to the milk and resulting production of yeasty flavor in the cheese.

9. A mold-enzyme preparation used in lots 48, 49, 50, and 51 at the rate of 0.55 percent in the milk gave cheese with the highest fat acidities and total volatile acidities of any of the cheese made. This same mold-enzyme preparation

used at the rate of 0.10 to 0.25 percent in the milk gave cheese with very good flavor.

10. The mold-enzyme preparations made with 5 strains of Penicillium improved the flavor of cheese in all cases. Strains 4, 12, and 6 gave the greatest improvement in flavor, while strain 13 gave the least improvement in flavor.

11. There was little or no correlation of values for amino nitrogen, nitrogen fraction soluble in trichloroacetic acid, and nitrogen fraction soluble in phosphotungstic acid with flavor scores of the cheese.

12. Total volatile acidities correlated fairly well with the flavor scores, but fat acidities did not. The total volatile acidity was the best index of flavor score of any of the chemical analyses used.

13. Cheese with total volatile acidities in the range in which 30 to 55 ml. of 0.1 N alkali were necessary to titrate the volatile acids in the first 1000 ml. of steam distillate from 200 g. of cheese generally had the highest flavor scores.

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APPENDIX

Appendix A.

Manufacture Data and Analyses of Blue Cheese

Lot No.	Date of Make	Milk Acidity (%)	Whey Acidity* (%)	Fat in Milk (%)	Fat** in Cheese (%)	Moisture** in Cheese (%)	Salt** in Cheese (%)	$CaCl_2$ added to milk (%)
	1945							
5+	6-18	0.200	0.155	3.8	27.86	42.33	3.98	-
6+	6-18	0.200	0.160	3.8	27.49	44.85	4.72	-
7+	6-18	0.200	0.160	3.8	26.41	46.02	4.66	-
8+	6-18	0.200	0.165	3.8	27.51	43.84	4.12	-
9	6-20	0.170	0.160	2.8	28.87	42.29	4.70	-
10	6-20	0.170	0.155	2.8	28.78	41.82	4.27	-
11	6-20	0.170	0.180	2.8	29.27	41.91	4.54	-
12	6-20	0.170	0.155	2.8	29.09	42.84	3.93	-
13	6-22	0.170	0.175	3.5	27.89	44.56	3.54	-
14	6-22	0.170	0.160	3.5	28.62	43.96	4.05	-
15	6-22	0.170	0.165	3.5	27.52	46.32	3.90	-
16	6-22	0.170	0.165	3.5	29.43	44.09	3.52	-
17+	6-27	0.200	0.160	3.6	29.00	44.64	3.69	-
18+	6-27	0.200	0.160	3.6	29.63	43.34	3.81	-
19+	6-27	0.200	0.160	3.6	28.30	45.89	4.18	-
20+	6-27	0.200	0.165	3.6	30.34	43.01	3.94	-
21	9-27	0.170	0.135	4.0	27.45	45.99	3.38	-
22	9-27	0.170	0.135	4.0	27.08	47.03	3.29	-
23	9-27	0.170	0.150	4.0	27.68	45.81	4.65	-
24	9-27	0.170	0.155	4.0	28.83	43.61	4.38	-
25	10-16	0.170	0.150	3.9	25.64	50.35	4.34	-
26	10-16	0.170	0.160	3.9	26.63	47.26	3.96	-
27	10-16	0.170	0.170	3.9	28.54	46.34	3.45	-
28	10-16	0.170	0.170	3.9	27.51	46.20	3.72	-
	1946							
29	1-17	0.175	0.170	3.9	28.92	45.93	3.49	-
30	1-17	0.175	0.165	3.9	30.38	43.90	3.67	-
31	1-17	0.175	0.175	3.9	30.35	43.30	4.02	-
32	1-24	0.190	0.190	4.0	31.58	42.20	4.49	-
33	1-24	0.190	0.190	4.0	29.26	45.40	3.77	-
34	1-24	0.190	0.185	4.0	29.22	45.30	4.16	-
35	1-24	0.190	0.185	4.0	29.08	43.35	4.38	-
36	1-29	0.170	0.165	3.8	29.50	44.95	3.65	-
37	1-29	0.170	0.180	3.8	29.35	45.30	3.52	-
38	1-29	0.170	0.175	3.8	29.10	46.90	3.90	-
39	1-29	0.170	0.175	3.8	29.64	44.45	3.73	-
40	2- 5	0.190	0.160	4.2	28.84	46.35	3.28	-
41	2- 5	0.190	0.170	4.2	33.21	40.00	3.46	-
42	2- 5	0.190	0.180	4.2	32.97	40.80	3.79	-
43	2- 5	0.190	0.175	4.2	30.53	44.50	4.14	-
44	2-12	0.160	0.160	3.6	32.30	42.80	3.39	-

Appendix A. Continued

Manufacture Data and Analyses of Blue Cheese

Lot No.	Date of Make	Milk Acidity (%)	Whey Acidity* (%)	Fat in Milk (%)	Fat** in Cheese (%)	Moisture** in Cheese (%)	Salt** in Cheese (%)	$CaCl_2$ added to milk (%)
45	2-12	0,160	0,165	3,6	30,16	44,80	3,96	-
46	2-12	0,160	0,170	3,6	29,46	46,55	3,69	-
47	2-12	0,160	0,170	3,6	29,93	46,05	3,78	-
48	2-25	0,175	0,180	4,0	27,82	47,05	4,03	-
49	2-25	0,175	0,175	4,0	30,42	43,10	3,74	-
50	2-25	0,175	0,175	4,0	31,58	42,75	3,74	-
51	2-25	0,175	0,175	4,0	27,84	45,40	4,04	-
52	3- 5	0,160	0,170	3,6	30,43	43,95	3,35	-
53	3- 5	0,160	0,165	3,6	30,79	43,10	3,76	-
54	3- 5	0,160	0,170	3,6	28,87	46,00	3,51	-
55	3- 5	0,160	0,170	3,6	29,54	45,30	3,55	-
56	4-26	0,145	0,180	3,7	29,57	45,00	4,14	-
57	4-26	0,145	0,170	3,7	28,49	45,90	4,03	-
58	4-26	0,145	0,195	3,7	28,14	45,00	5,24	-
59	4-26	0,145	0,190	3,7	28,08	45,35	4,16	-
60	5- 3	0,160	0,185	4,1	31,54	42,60	3,49	0,015
61	5- 3	0,160	0,180	4,1	30,97	43,80	3,41	0,015
62	5- 3	0,160	0,175	4,1	30,73	43,80	3,73	0,015
63	5- 3	0,160	0,190	4,1	31,13	43,20	3,64	0,015
64	5-12	0,135	0,150	3,5	31,57	40,90	3,71	0,015
65	5-12	0,135	0,150	3,5	29,89	42,80	3,78	0,015
66	5-12	0,135	0,155	3,5	29,64	43,70	3,94	0,015
67	5-12	0,135	0,150	3,5	30,72	42,40	3,62	0,015
68	5-17	0,150	0,170	3,2	25,62	46,60	3,34	0,015
69	5-17	0,150	0,170	3,2	29,58	44,10	4,28	0,015
70	5-17	0,150	0,170	3,2	26,08	45,55	5,14	0,015
71	5-17	0,150	0,175	3,2	26,63	45,40	4,62	0,015
72	10-11	0,170	0,165	3,4	25,17	47,45	3,48	-
73	10-11	0,170	0,165	3,4	24,58	50,25	3,76	-
74	10-11	0,170	0,165	3,4	25,17	49,60	3,45	-
75	10-11	0,170	0,160	3,4	25,66	48,45	2,96	-
76	10-14	0,160	0,150	3,4	26,54	45,60	3,89	-
77	10-14	0,160	0,145	3,4	27,37	47,40	3,69	-
78	10-14	0,160	0,150	3,4	27,30	43,65	3,88	-
79	10-14	0,160	0,155	3,4	24,72	49,00	4,43	-
80	10-17	0,165	0,160	3,6	28,60	45,50	3,69	-
81	10-17	0,165	0,160	3,6	27,39	47,40	3,59	-
82	10-17	0,165	0,150	3,6	26,27	49,20	3,44	-
83	10-17	0,165	0,155	3,6	26,72	47,60	4,00	-
84	10-22	0,150	0,150	3,4	27,20	47,60	3,11	-
85	10-22	0,150	0,160	3,4	26,31	48,55	3,83	-
86	10-22	0,150	0,150	3,4	25,40	50,25	3,45	-
87	10-22	0,150	0,155	3,4	26,38	48,80	2,84	-
88	10-24	0,170	0,175	3,4	27,97	45,55	3,53	-

Appendix A. Continued

Manufacture Data and Analyses of Blue Cheese

Lot No.	Date of Make	Milk Acidity (%)	Whey Acidity* (%)	Fat in Milk (%)	Fat** in Cheese (%)	Moisture** in Cheese (%)	Salt** in Cheese (%)	$CaCl_2$ added to milk (%)
89	10-24	0.170	0.170	3.4	26.62	46.20	3.74	-
90	10-24	0.170	0.165	3.4	28.12	44.65	4.02	-
91	10-24	0.170	0.175	3.4	27.99	44.65	4.38	-
92	10-28	0.155	0.155	3.2	26.05	47.45	3.40	-
93	10-28	0.155	0.140	3.2	25.92	47.35	3.46	-
94	10-28	0.155	0.140	3.2	25.52	47.95	4.14	-
95	10-28	0.155	0.145	3.2	25.19	47.95	4.21	-
96	11- 5	0.160	0.155	3.6	26.99	46.80	3.79	-
97	11- 5	0.160	0.160	3.6	26.73	46.75	3.93	-
98	11- 5	0.160	0.155	3.6	27.72	45.95	3.51	-
99	11- 5	0.160	0.150	3.6	26.81	47.05	3.73	-
100	11-12	0.175	0.155	3.7	29.17	44.30	3.45	-
101	11-12	0.175	0.150	3.7	27.78	46.20	3.84	-
102	11-12	0.175	0.140	3.7	25.85	50.15	3.55	-
103	11-12	0.175	0.150	3.7	26.57	48.50	3.77	-
104	11-19	0.170	0.185	3.8	26.89	46.95	3.30	-
105	11-19	0.170	0.180	3.8	27.03	47.96	3.45	-
106	11-19	0.170	0.180	3.8	26.87	48.20	3.84	-
107	11-19	0.170	0.185	3.8	26.50	47.90	3.17	-
108	11-26	0.170	0.165	3.7	27.36	47.55	3.70	0.015
109	11-26	0.170	0.165	3.7	29.24	46.40	4.41	0.015
110	11-26	0.170	0.165	3.7	28.12	48.30	3.15	0.015
111	11-26	0.170	0.165	3.7	27.55	48.55	3.36	0.015
112	12- 3	0.170	0.190	3.5	28.05	45.25	3.59	0.015
113	12- 3	0.170	0.210	3.5	26.42	46.40	4.71	0.015
114	12- 3	0.170	0.190	3.5	26.42	47.10	3.81	0.015
115	12- 3	0.170	0.180	3.5	26.93	45.60	4.70	0.015
116	12-10	0.175	0.210	3.3	27.54	45.40	4.38	0.015
117	12-10	0.175	0.190	3.3	25.76	48.35	3.99	0.015
118	12-10	0.175	0.210	3.3	28.36	44.25	3.82	0.015
119	12-10	0.175	0.205	3.3	27.39	46.40	3.62	0.015
120	12-17	0.170	0.190	3.2	28.26	45.90	4.90	0.015
121	12-17	0.170	0.190	3.2	28.70	46.45	4.94	0.015
122	12-17	0.170	0.190	3.2	28.20	46.20	4.57	0.015
123	12-17	0.170	0.185	3.2	26.58	48.00	4.52	0.015
124	12-24	0.190	0.185	3.0	26.76	44.55	4.39	0.015
125	12-24	0.190	0.180	3.0	25.31	47.10	4.51	0.015
126	12-24	0.190	0.180	3.0	25.78	44.90	4.34	0.015
127	12-24	0.190	0.180	3.0	24.96	47.45	4.94	0.015

Appendix A. Continued
Manufacture Data and Analyses of Blue Cheese

Lot No.	Date of Make	Milk Acidity (%)	Whey Acidity* (%)	Fat in Milk (%)	Fat** in Cheese (%)	Moisture** in Cheese (%)	Salt** in Cheese (%)	$CaCl_2$ added to milk (%)
	1947							
128	1-23	0.180	0.180	3.4	25.13	47.60	4.49	0.015
129	1-23	0.180	0.170	3.4	24.72	49.10	4.19	0.015
130	1-23	0.180	0.240	3.4	24.83	48.70	4.47	0.015
131	1-23	0.180	0.220	3.4	25.43	46.95	4.89	0.015
132	1-29	0.185	0.200	3.4	25.34	46.85	4.11	0.015
133	1-29	0.185	0.200	3.4	26.16	48.25	4.07	0.015
134	1-29	0.185	0.260	3.4	24.64	49.05	4.72	0.015
135	1-29	0.185	0.260	3.4	26.80	46.50	4.54	0.015
136	2- 5	0.175	0.200	3.5	27.79	47.20	3.77	0.015
137	2- 5	0.175	0.190	3.5	30.04	43.45	3.68	0.015
138	2- 5	0.175	0.275	3.5	27.91	46.85	4.12	0.015
139	2- 5	0.175	0.275	3.5	27.84	46.90	5.03	0.015
140	2-11	0.160	0.170	3.7	28.80	46.40	3.74	0.015
141	2-11	0.160	0.170	3.7	28.16	47.35	3.69	0.015
142	2-11	0.160	0.170	3.7	28.98	45.85	3.35	0.015
143	2-11	0.160	0.180	3.7	28.62	48.30	4.69	0.015
144	2-18	0.190	0.210	3.0	26.90	48.00	3.91	0.015
145	2-18	0.190	0.200	3.0	27.54	47.20	3.79	0.015
146	2-18	0.190	0.200	3.0	27.68	45.70	4.01	0.015
147	2-18	0.190	0.200	3.0	27.88	46.30	3.91	0.015

* Whey acidity at time curd was dipped
** Analyses were made on 4 week old cheese
\+ Raw milk cheese

Appendix B.
Nitrogen Analyses of Blue Cheese

Lot No.	Age Wks.	Total N.	Amino N.	Precipitating Agent: Trichloroacetic Acid Sol.*	Trichloroacetic Acid Insol.*	Trichloroacetic Acid Total*	Phosphotungstic Acid Sol.*	Phosphotungstic Acid Insol.*	Phosphotungstic Acid Total*
5+	4	10.23	4.33	2.61	7.44	10.05	0.71	9.04	9.75
6+	4	9.92	2.48	1.35	8.09	9.44	0.20	9.31	9.51
7+	4	9.74	2.34	1.56	8.68	10.24	0.56	8.89	9.45
8+	4	10.71	2.75	2.08	7.91	9.99	1.45	9.44	10.89
9	5	10.39	3.17	2.22	7.94	10.16	1.52	8.88	10.40
10	5	10.58	1.96	1.62	9.51	11.13	0.54	11.03	11.57
11	5	10.80	2.17	1.84	8.83	10.67	1.08	9.56	10.64
12	5	10.79	2.68	2.77	7.82	10.59	1.17	10.17	11.34
13	5	11.18	3.06	2.71	8.68	11.39	1.24	10.29	11.53
14	5	11.35	3.39	2.87	8.45	11.32	1.40		
15	5	11.34	4.96	4.52	7.95	12.47	2.18	9.91	12.09
16	5	10.90	6.38	5.47	6.07	11.54	2.71		
17+	5	10.52	2.63	2.95	9.00	11.95	1.51	10.45	11.96
18+	5	11.25	4.03	4.07	8.09	12.16	1.84	9.88	11.72
19+	5	10.87		4.01	7.10	11.11	2.20	8.43	10.63
20+	5	11.87		5.67	6.32	12.19	3.14	8.29	11.43
21	4	10.41	3.75	3.28	7.12	10.40	1.73	9.19	10.92
22	4	10.36	3.20	2.81	7.94	10.75	0.95	9.21	10.16
23	4	10.84	2.68	2.65	9.39	12.04	1.22	10.36	11.58
24	4	10.53	2.86	2.63	8.40	11.03	1.66	9.20	10.86
25	4	9.47	4.06	3.38	6.41	9.79	1.67	8.29	9.96
26	4	9.47	3.71	3.24	7.12	10.36	1.40	8.66	10.06
27	4	10.45	2.91	3.26	8.00	11.26	1.27	9.37	10.64
28	4	10.16	3.04	2.49	7.80	10.29	0.80	9.17	9.97
29	4	9.68		1.79	7.61	9.40	0.28	9.79	10.07
30	4	10.39		1.51	8.81	10.32	0.45	10.21	10.66
31	4	10.75		1.71	9.15	10.86	0.44	9.55	9.99
32	4	10.94		0.76	9.27	10.03	0.26	9.93	10.19
33	4	10.53		1.13	8.78	9.91	0.30	9.41	9.71
34	4	9.95		1.01	8.43	9.44	0.26	9.21	9.47
35	4	10.78		1.29	9.06	10.35	0.40	9.92	10.32
36	4	10.65		1.75	8.64	10.39	0.54	9.85	10.39
37	4	11.16		1.55	8.81	10.36	0.46	9.95	10.41
38	4	10.33		2.23	8.18	10.41	0.53	9.37	9.90
39	4	10.68		2.30	8.26	10.56	0.64	9.46	10.10
40	4	10.48		1.74	8.62	10.36	0.55	9.85	10.40
41	4	11.16		1.76	9.70	11.46	0.40	10.65	11.05
42	4	10.99		1.53	9.02	10.55	0.38	10.40	10.78
43	4	10.57		1.82	8.42	10.24	0.36	9.22	9.58
44	4	10.54		2.25	8.64	10.89	0.77	10.00	10.77
45	4	10.54		1.95	8.71	10.66	0.56	9.74	10.30

Appendix B. Continued
Nitrogen Analyses of Blue Cheese

Lot No.	Age Wks.	Total N. *	Amino N **	Precipitating Agent: Trichloroacetic Acid Sol.*	Trichloroacetic Acid Insol.*	Trichloroacetic Acid Total*	Phosphotungstic Acid Sol.*	Phosphotungstic Acid Insol.*	Phosphotungstic Acid Total*
46	4	10.00		1.89	8.34	10.23	0.90	9.25	10.15
47	4	9.90		2.01	8.41	10.42	0.71	9.25	9.96
48	4	10.46		1.75	8.46	10.21	0.66	9.75	10.41
49	4	11.01		2.97	9.25	12.22	1.06	9.76	10.82
50	4	10.99		1.76	8.65	10.41	0.58	9.88	10.46
51	4	10.71		2.53	8.19	10.72	0.99	9.72	10.71
52	4	10.97		1.82	9.20	11.02	0.66	10.30	10.96
53	4	11.02		2.20	8.90	11.10	0.81	10.05	10.86
54	4	10.52		2.17	8.57	10.74	0.92	9.66	10.58
55	4	10.58		2.29	8.62	10.91	0.90	9.87	10.77
56	4	10.74	2.16	1.89	8.97	10.86	0.46	10.15	10.61
57	4	10.44	2.06	2.04	8.24	10.28	0.54	9.92	10.46
58	4	10.69	1.74	1.28	8.75	10.03	0.25	9.90	10.15
59	4	11.08	1.06	1.79	8.60	10.39	0.35	9.94	10.29
60	4	11.19	1.24	2.30	8.92	11.22	0.50	10.58	11.08
61	4	11.14	.96	2.44	8.88	11.32	0.40	10.42	10.82
62	4	11.05	.62	1.84	8.89	10.73	0.45	9.74	10.19
63	4	10.70	1.24	2.04	8.69	10.73	0.46	9.46	9.92
64	4	11.73	2.24	1.92	9.94	11.86	0.15	11.17	11.32
65	4	12.09	1.95	1.87	9.32	11.19	0.15	10.72	10.87
66	4	10.97	1.78	1.31	8.95	10.26	1.22	10.30	11.52
67	4	11.75	1.59	1.32	9.17	10.49	1.22	10.62	11.84
68	4	12.05	2.16	2.50	9.20	11.70	1.05	10.77	11.82
69	4	11.00	1.55	2.00	8.65	10.65	0.78	9.67	10.45
70	4	12.00	1.70	2.35	9.25	11.60	1.40	10.55	11.95
71	4	11.75	1.94	2.50	9.00	11.50	1.50	10.42	11.92
5	12	11.59	13.96	7.46	3.79	11.25	4.26	6.79	11.05
6	12	10.39	11.01	6.51	3.81	10.32	2.83	7.00	9.83
7	12	10.94	8.93	4.77	5.84	10.61	2.72	8.18	10.90
8	12	11.22	10.76	6.36	4.83	11.19	3.37	7.83	11.20
9	12	11.43	8.58	5.20	6.44	11.64	2.82	8.49	11.31
10	12	11.47	8.62	4.72	6.82	11.54	2.88	8.59	11.47
11	12	11.62	11.25	5.30	5.54	10.84	3.19	7.75	10.94
12	12	11.64	9.73	5.03	6.57	11.60	2.51	8.66	11.17
13	12	10.77	7.73	7.27	4.33	11.60	2.95	8.13	11.08
14	12	11.73	7.21	5.85	4.84	10.69	2.44	9.01	11.45
15	12	10.98	10.92	6.14	4.79	10.93	3.40	7.30	10.70
16	12	10.22	12.96	6.95	3.19	10.14	4.42	5.38	10.80
17	12	10.88	10.48	5.29	5.36	10.65	4.31	6.36	10.67
18	12	11.44	13.62	7.65	3.67	11.32	4.98	6.89	11.87

Appendix B. Continued
Nitrogen Analyses of Blue Cheese

Lot No.	Age Wks.	Total N. *	Amino N. **	Precipitating Agent: Trichloroacetic Acid Sol.*	Trichloroacetic Acid Insol.*	Trichloroacetic Acid Total*	Phosphotungstic Acid Sol.*	Phosphotungstic Acid Insol.*	Phosphotungstic Acid Total*
19	12	10.81	13.20	7.66	3.37	11.03	4.12	6.47	10.59
20	12	11.42	15.08	7.51	4.01	11.52	4.64	6.35	10.99
29	12	10.98	5.22	4.10	6.98	11.08	2.70	8.34	11.04
30	12	10.45	4.36	3.59	6.98	10.57	2.24	7.94	10.18
31	12	10.76	4.91	3.90	7.22	11.12	2.20	8.64	10.84
32	12	11.15	2.22	2.83	8.52	11.35	1.70	9.70	11.40
33	12	10.52	3.89	3.82	6.84	10.66	2.11	8.79	10.90
34	12	10.45	3.92	3.80	6.78	10.58	2.39	8.07	10.46
35	12	10.75	2.12	2.50	8.61	11.11	1.23	9.28	10.51
36	12	10.35	4.99	4.07	6.15	10.22	2.60	7.80	10.40
37	12	10.56	4.40	4.40	6.41	10.81	2.50	8.20	10.70
38	12	10.49	4.73	4.12	5.85	9.97	2.47	7.56	10.03
39	12	10.29	6.75	4.67	5.89	10.56	3.01	7.18	10.19
40	12	10.65	4.26	3.95	6.35	10.30	2.26	8.42	10.68
41	12	12.00	4.49	4.50	7.89	12.39	2.38	9.20	11.58
42	12	10.64	3.74	3.38	7.29	10.67	1.95	8.61	10.56
43	12	10.19	4.67	4.31	5.95	10.26	2.50	7.27	9.77
44	12	10.70	5.00	4.33	6.50	10.83	2.77	8.10	10.87
45	12	10.55	5.24	4.47	6.29	10.76	2.90	7.48	10.38
46	12	10.00	5.56	4.21	6.24	10.45	3.11	7.07	10.18
47	12	10.50	4.60	3.95	6.28	10.23	2.74	7.37	10.11
48	12	10.55	4.06	3.30	6.99	10.29	2.12	8.29	10.41
49	12	11.40	7.06	5.62	5.59	11.21	3.55	7.19	10.74
50	12	11.29	4.00	3.52	7.40	10.92	1.99	8.79	10.78
51	12	11.30	4.00	4.25	7.66	11.91	2.25	8.56	10.81
52	12	11.35	5.01	4.42	6.50	10.92	2.77	8.09	10.86
53	12	11.55	5.52	4.77	6.15	10.92	2.86	7.66	10.52
54	12	11.20	7.16	5.08	5.40	10.48	3.10	7.85	10.95
55	12	11.10	6.02	4.03	6.71	10.74	2.29	8.01	10.30
56	12	11.37	4.18	3.79	7.32	11.11	2.50	9.05	11.55
57	12	11.40	5.24	3.77	7.25	11.02	2.75	8.77	11.52
58	12	11.21	5.93	4.13	7.12	11.25	2.58	8.65	11.23
59	12	11.05	5.63	3.80	7.42	11.22	2.75	8.35	11.10
60	12	11.74	4.63	4.10	7.70	11.80	2.51	8.35	10.86
61	12	10.70	7.91	5.73	5.10	10.83	3.81	6.92	10.73
62	12	11.15	5.06	4.28	6.72	11.00	2.71	8.32	11.03
63	12	11.77	5.26	4.40	7.35	11.75	2.66	8.89	11.55

* Ml. of N/10 HCl equivalent to nitrogen of 0.5 g. of cheese
** Mg. per g. of cheese
\+ Raw milk cheese