MICROBIOLOGICAL SPOILAGE OF COTTAGE CHEESE

By

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A THESIS

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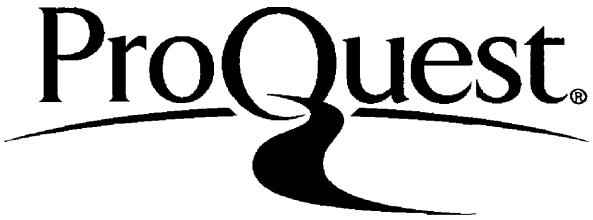

ABSTRACT

Twenty-five cultures were isolated from spoiled cheese and agar exposure plates. The cultures were identified, and the type of spoilage produced by nineteen of the organisms individually or in combination with S. lactis on sterile cottage cheese curd was determined. The effect of incubation temperature, pH, sodium chloride, heat, chlorine, quaternary, iodophor, and sorbic acid on the growth of these organisms was also determined.

Cottage cheese contaminated with the spoilage organisms was washed with waters containing acids and germicides to determine if different washing procedures would increase the shelf-life of the product. Various acids and starter were added to cheese dressings which had been inoculated with the organisms to determine if these additives would increase the shelf-life of cottage cheese.

The isolated organisms belonged to the genera Achromobacter, Alcaligenes, Bacillus, Bacterium, Corynebacterium, Escherichia, Micrococcus, Pseudomonas, Rhodotorula, Torulopsis, Geotrichum, Mucor, and Penicillium.

When inoculated into sterile cottage cheese, the degree of defect produced by species of certain genera could not be correlated

with the presence or absence of S. lactis. However, pH was correlated with degree of defect, since in nine of eleven instances where there were differences in both the degree of defect and the pH, the defects were more pronounced at the higher pH levels regardless of whether these were produced by the pure cultures individually or combined with S. lactis.

After seven days at 3° and 10° C., eight and sixteen of the nineteen organisms, respectively, exhibited visible growth, demonstrating that low temperatures fail to completely control many of the organisms.

Reduced pH levels caused reduced growth of the organisms as two, four, and nine of the sixteen tested showed little growth at pH levels of 5.0, 5.2, and 5.4 or below, respectively.

Seven percent sodium chloride was the critical concentration, since fifteen of the nineteen organisms grew in 5 percent, while only four of the nineteen grew in 7 percent sodium chloride.

Bacillus firmus was the only culture which survived 143° F. for 30 minutes. When heated at 120° F. for 15 minutes in skim milk, only two of the nineteen organisms were destroyed. At this same temperature and holding time in whey, only two of the nineteen organisms survived.

Fifty parts per million of chlorine with 5 minutes of exposure destroyed eighteen of the nineteen, while fifty parts per million of quaternary with 10 minutes of exposure destroyed only eleven of the nineteen organisms. Fifty parts per million of iodophor with 1 minute of exposure destroyed thirteen of the nineteen cultures.

At pH 5.2 and above, the concentrations of sorbic acid used were ineffective, while at pH 5.0 and 4.8 the viability of the cultures was decreased as the sorbic acid content increased.

The addition of acetic, citric, and sorbic acids to the wash water of cottage cheese inoculated with various spoilage organisms usually increased the shelf-life of the product.

Sorbic acid and starter added to cheese dressing containing various spoilage organisms also increased the shelf-life of cottage cheese.

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INTRODUCTION

Cottage cheese is becoming an increasingly popular product with the consuming public. The per capita consumption has increased from 0.9 pounds in 1935 to 3.1 pounds in 1953.[1] This product has been a means of alleviating milk surplus problems in certain areas during the summer months and has proved a profitable item for the dairy plant operator.

However, the cottage cheese industry is faced with a number of problems of increasing importance. The present trend is toward centralization of plants, resulting in longer hauling distances and longer storage before sale. Cottage cheese makers are producing a sweet curd, low acid cheese which is necessary to meet consumer demands. The pH of this cheese is not sufficiently low to retard the development of many spoilage organisms. Inadequate refrigeration in retail outlets and household refrigerators is common. Psychrophilic bacteria, yeasts, and molds can grow and cause undesirable changes in low acid cottage cheese. The defects normally

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[1] Agricultural Statistics (1937, 1955). United States Dept. of Agriculture. U.S. Government Printing Office, Washington, D.C.

encountered are mold mycelia and surface slime, along with stale, moldy, bitter, yeasty, or fruity flavors. More information about the specific organisms causing these defects would be beneficial to dairy plant operators.

The study herein reported was initiated to determine specific organisms responsible for cottage cheese spoilage and methods of controlling their growth. A number of organisms causing spoilage in cottage cheese were isolated and identified. The effect of pH, incubation temperature, heat, salt, sorbic acid, and chlorine, iodophor, and quaternary compounds on the growth of these organisms was determined. Cottage cheese wash waters and creaming mixtures were treated with various compounds in an attempt to determine methods of increasing the shelf-life of cottage cheese inoculated with the spoilage organisms. It was felt that if treatments could be discovered which would add a few days to the shelf-life of cottage cheese the project would be worth while.

REVIEW OF LITERATURE

Psychrophilic bacteria are organisms which are able to grow at refrigerator temperatures. These organisms prefer temperatures of 60° to 70° F., but many which produce undesirable changes in dairy products grow quite well at 40° to 50° F. or lower. Many investigators have reported on the occurrence of these organisms in dairy products, particularly milk.

Conn (1903) stated that milk preserved at 50° F. remains sweet for a long time, but the bacteria which eventually grow are more undesirable than those which grow at higher temperatures. He further stated that old milk, even though perfectly sweet, is unfit for market. Mott and Mazer (1938) reported that milk quality deteriorated, sometimes quite rapidly, when held at 40° F. This deterioration was found to be caused by bacterial growth. Green and Jezeski (1954) showed that a large number of organisms important to the dairy industry are able to grow at refrigerator temperatures. In some cases, they found that cooling from 5° to 0° C. was as effective as cooling from 30° to 5° C. in increasing keeping quality. Chaffee (1952) reported considerable increases in

numbers of organisms in milk held in refrigerated storage, resulting in a marked deterioration of the product.

Burgwald and Josephson (1947) demonstrated that psychrophilic bacteria which develop in refrigerated milk are primarily responsible for the deterioration of the product and apparently are responsible for the development of acid which is produced in milk stored at 40° F. Morris (1942) found that certain samples of raw milk held overnight at 4° C. decolorized methylene blue in less than 8 hours. He isolated Pseudomonas organisms which grew rapidly in milk held at 4° C. Subsequent studies showed farm water supplies to be a possible source of these organisms.

Thomas et al. (1947) isolated and identified as to genera, a number of organisms capable of growing at refrigerator temperatures. The most common genera appeared to be Achromobacter, followed by Flavobacterium, Pseudomonas, and Alcaligenes. Milk with normal taste and odor contained 10,000,000 per ml. or more of the above organisms. Olson et al. (1953a) reported psychrophilic bacteria were always found in raw milk supplies, and extensive proliferation of these bacteria will inevitably occur if such milk is held at low temperatures. Erdman and Thornton (1951a, 1951b) investigated psychrophilic bacteria in sixty-one samples of milk and cream, and found them to be present in all samples

examined. Organisms were isolated and identified as belonging to the genera Aerobacter, Escherichia, Flavobacterium, Lactobacillus, Pseudomonas, and Streptococcus. Mikolajcik and Burgwald (1953) stated that some nonpsychrophilic organisms develop psychrophilic tendencies after storage at low temperatures. Dahlberg (1946) showed that the coliform count of freshly pasteurized milk may increase considerably during 4 days' storage at 45° to 50° F.

Jezeski and Macy (1946) isolated and classified as to genera a number of proteolytic and lipolytic cultures from butter and water which were capable of vigorous growth at 8° C. Genera isolated were Pseudomonas, Flavobacterium, Alcaligenes, and Achromobacter. A nonlactose fermenting yeast was also isolated but not identified. Further studies showed that these organisms were capable of causing undesirable odors in sterile cream incubated at refrigerator temperatures. Lawton and Nelson (1954) reported upon the isolation of a number of psychrophilic bacteria from commercial milk. Eight of these cultures were selected as being representative and were tentatively identified as (1) Pseudomonas ovalis, (2) Pseudomonas fluorescens, (3) Pseudomonas arvilla, (4) Pseudomonas cruciviae, (5) Pseudomonas aquatile, (6) Pseudomonas fluorescens (possibly viscosa), (7) Pseudomonas geniculata, and (8) Pseudomonas species.

Boyd et al. (1953) reported that psychrophilic bacteria are important in the keeping quality of commercially pasteurized and homogenized milk. Olson et al. (1953b) found that psychrophilic bacteria developed more rapidly in recombined milk than in the concentrated milk from which it was prepared.

Morrison and Hammer (1941) showed that psychrophilic Pseudomonas fragi is quite widespread in dairy products and materials coming in contact with dairy products. Nashif and Nelson (1952, 1953a, 1953b, 1953c) found that the lipase of Ps. fragi was capable of causing extensive breakdown of fat in cream and butter. Purko et al. (1952) and Nelson (1952) reported that Geotrichum candidum produced a rapid and extensive hydrolysis of butterfat in cream. Harmon and Nelson (1955a) demonstrated that the presence of Streptococcus lactis was inhibitory to the population development of Ps. fragi, but stimulated the population development of G. candidum in cream. However, they found that the presence of S. lactis and the accompanying lowering of pH reduced the lipase activity and water-insoluble acid production of G. candidum and Ps. fragi and the protease activity of Ps. fragi. Van der Zant and Nelson (1953) demonstrated that certain strains of S. lactis are proteolytic by measuring the increase in soluble nitrogen, tryptophan, and tyrosine in milk inoculated with these strains. Peters and

Nelson (1948) found that under certain conditions Mycotorula lipolytica possessed extremely high lipolytic activity.

Investigations on the microflora of blue cheese slime by Hartley and Jezeski (1954) revealed the presence of species of Micrococcus along with Bacterium linens and Bacterium erythrogenes. Macy and Erekson (1937) studied the slime of several varieties of semisoft cheeses and found that Bact. linens was the organism most commonly isolated. Alford and Frazier (1950) isolated Micrococcus freundenreichii, Micrococcus caseolyticus, and Micrococcus conglomeratus from raw milk cheddar cheese. They believed these organisms were important in the ripening process.

Heat Resistance of Psychrophiles

Thomas et al. (1947) showed that species of Achromobacter, Flavobacterium, Pseudomonas, and Alcaligenes do not survive pasteurization at 143° F. for 30 minutes. Davis and Babel (1954) demonstrated that some species of organisms in the genera Proteus, Pseudomonas, Aerobacter, Alcaligenes, and Achromobacter are quite heat labile. None of the organisms studied in these genera survived pasteurization at 143° F. for 30 minutes. Erdman and Thornton (1951a, 1951b) reported that species of Aerobacter, Escherichia, Flavobacterium, Lactobacillus, Pseudomonas, and

Streptococcus rarely survive pasteurization. Watrous (1953) stated that psychrophilic bacteria do not withstand pasteurization, but are found in commercially pasteurized milk as a result of postpasteurization contamination. Olson et al. (1953a) demonstrated that psychrophilic bacteria found in raw milk are destroyed by proper pasteurization. Atheron et al. (1953) and Watrous et al. (1952) found that bottled milk and cream normally contained psychrophiles, but as a postpasteurization contaminant, since laboratory pasteurization at 143° F. for 30 minutes destroyed these organisms.

Rogick and Burgwald (1952) found that psychrophiles do not survive pasteurization by the vat or the HTST method, but pasteurized milk after bottling and storage invariably contains psychrophiles. They demonstrated that most of the organisms are facultative rather than true psychrophiles. Kennedy and Weiser (1950), in a study of fifteen psychrophilic bacterial cultures, showed that the majority were quite heat labile, but some were not completely destroyed by pasteurization at 145° F. for 30 minutes. The optimum growth temperature of seven of these fifteen organisms was closer to 10° than to 20° C., and none grew at 35° C.

Sherman et al. (1941) stated that pasteurized milk keeps two to three times as long as raw milk of substantially the same quality or bacterial content. They suggested that this was due to the

complete destruction of certain kinds of bacteria. Reinoculation of pasteurized milk with minute amounts of raw milk decreased its keeping quality to a point substantially the same as that of raw milk. They also reported that spoilage of milk held just above the freezing point was due primarily to gram-negative nonsporeforming rods of the Pseudomonas group. Kaufmann and Andrews (1954) demonstrated that cultures of Pseudomonas viscosa and Pseudomonas mephitica were readily destroyed by pasteurization at 143° F. for 30 minutes or by the HTST method when heated in skim milk.

In addition to the above-mentioned studies concerning heat resistance of organisms of dairy origin, Ruyle and Sognefest (1951) studied thermal resistance of a facultative aerobic sporeforming bacterium in evaporated milk. This organism caused the milk to take on a custardlike consistency and was identified as Bacillus subtilis. A minimum of 16.4 minutes at 243° F. was required to destroy the spores of this organism. Myhr and Olson (1952) tested thirty cultures of Micrococci and found only five of these survived heating at 143° F. for 30 minutes.

Defects in Dairy Products Caused by the Growth of Psychrophilic Organisms

Parker and Elliker (1952, 1953) reported that Pseudomonas fragi, Ps. viscosa, and Alcaligenes metalcaligenes have the ability

to reduce the biacetyl content of cottage cheese, thereby destroying the desirable flavor and aroma of the product. According to these workers, destruction of biacetyl and loss of flavor component resulted prior to the appearance of a slimy defect. Bacillus graveolens, Ps. fluorescens, Pseudomonas putrefaciens, Ps. fragi, Escherichia coli, Aerobacter aerogenes, Proteus vulgaris, Alcaligenes fecalis, Streptococcus paracitrovorous, S. lactis, and Streptococcus cremoris were tested by Elliker and Horrall (1943) and Elliker (1945) for their effect on the biacetyl content and flavor of butter. S. lactis was the only organism which showed little or no effect on the biacetyl content of butter. Wales (1956) demonstrated that G. candidum, Penicillium frequentans, Ps. fragi, Ps. fluorescens, Pseudomonas desmolyticum, Pseudomonas tralucida, Rhodotorula flava, Torulopsis candida, Escherichia freundii, Achromobacter eurydice, and Alc. metalcaligenes reduced but did not completely destroy biacetyl in cottage cheese stored at 40° and 50° F. The reduction was more pronounced at 50° F. He showed further that cheese exhibiting heavy slime formation still contained some biacetyl.

Numerous investigators including Tuckey (1951a, 1951b), Young (1953), Baker (1953), Kester (1954), and Elliker (1954) have reported on improved methods of assuring a high quality cottage cheese. They are in general agreement that there is a need for

better plant sanitation in most dairies. Deane et al. (1953) and Rhodes and Harmon (1954) investigated the quality of commercial cottage cheese and found considerable variation among samples examined.

Lyons and Mallmann (1954) found that 64 percent of cottage cheese samples packaged in the plant and 95 percent of bulk samples contained coliform bacteria. They further showed that some pathogenic organisms inoculated into cottage cheese eventually die off, the death rate increasing as the pH decreased. However, they reported that destruction is slow enough to present a possible public health hazard. Geairain and Geairain (1951) revealed that Mycobacterium tuberculosis remained viable for 14 days in cottage cheese made from raw milk.

Davis and Babel (1954) isolated and classified a number of slime-producing organisms from cottage cheese. The organisms belonged to the following genera: Proteus, Pseudomonas, Aerobacter, Alcaligenes, and Achromobacter. Collins (1955a) studied factors involved in the control of gelatinous curd defects in cottage cheese and found that pH and temperature are very important in retarding or controlling this defect. Low temperature and pH levels were shown to retard development of the gelatinous curd defect. These studies also showed that a fruity odor was the first defect produced

by Alc. metalcaligenes and Ps. viscosa. Deane et al. (1953) found that slime eventually developed on cottage cheese having pH levels below 4.7 when received. Parker et al. (1950, 1951) and Elliker et al. (1952) also studied organisms associated with a gelatinous curd type of spoilage. They found that Ps. viscosa produced a yellow or brown slime, while Alc. metalcaligenes and Ps. fragi produced a translucent, yellow, gelatinous film around each curd particle.

Control of Psychrophiles by Sanitizers

Three types of sanitizers are currently considered to be useful in destroying organisms detrimental to dairy products. These are the chlorine, quaternary, and iodophor compounds. Various authors including Johns (1947, 1948a, 1948b), Shere (1948), Dvorkovitz and Crocker (1950), and Parker and Elliker (1951) have compared chlorine and quaternary compounds. Many advantages and disadvantages have been shown for each. Yancy and Faber (1952) showed that alkyl dimethyl benzyl ammonium chloride (quaternary) is destructive to some organisms associated with bovine mastitis. Krog (1942) and Davis (1947) demonstrated that quaternary compounds are effective in sanitizing properly cleaned dairy equipment. DuBois and Dibblee (1946) found that these compounds are effective against the common milk-souring organisms.

Lundstedt (1950) warned against the use of quaternary compounds because they are effective against gram-positive desirable dairy organisms and less effective against the gram-negative, undesirable ones. Parker et al. (1953) observed hypochlorites to have a more rapid germicidal action than quaternary compounds against Ps. viscosa, Ps. fluorescens, Ps. putrefaciens, Ps. fragi, and Alc. metalcaligenes. Many other authors including Harper et al. (1948), Klarmann and Wright (1944, 1946), Rahn and van Eseltine (1947), Elliker (1950), Hucker and van Eseltine (1948), Ridenour and Armbruster (1948), Weber and Black (1948), and Soike et al. (1952) have reported on methods of testing quaternary compounds and factors affecting their germicidal activity.

Collins (1955b) showed that 3 to 5 parts per million of residual chlorine at pH 6.0 was effective in destroying cultures of Ps. fragi, Ps. viscosa, Alc. metalcaligenes, E. coli, and A. aerogenes. The effectiveness of chlorine was reduced as pH was increased and temperature decreased. Most authors generally agree that quaternary compounds are most effective at high pH levels and against gram-positive types of organisms. These compounds are not as greatly affected by organic matter as the hypochlorites. The hypochlorites are most effective at low pH levels and against gram-negative types of organisms. The hypochlorites possess less

organism specificity than the quaternary ammonium compounds, but are more greatly affected by organic matter.

Johns (1954) compared Iobac and Iosan (iodophors) to chlorine and quaternary compounds in destroying Micrococcus pyogenes variety aureus, E. coli, and Ps. aeruginosa. The iodophors compared very favorably with a quick acting hypochlorite, especially in the presence of added skim milk. The other compounds tested were much slower. The iodophors showed greater capacity, destroying many more increments of test organisms than other germicides tested. Certain nonionic constituents of iodophors had a bacteriostatic effect on spores of Bacillus subtilis, indicating exceptionally favorable results against this organism. Mueller (1955) reported that 25 parts per million of available iodine compared favorably with 100 parts per million of available chlorine in killing E. coli, Salmonella typhosa, M. pyogenes variety aureus, and Ps. aeruginosa in the presence of hard water and added organic matter. He also stated that 12.5 parts per million of available iodine was equivalent to 100 parts per million of available chlorine in destroying Ps. aeruginosa and M. pyogenes variety aureus in distilled or hard water, but not in the presence of added organic matter. Gershenfeld (1955) found that iodophors and iodine-iodide compounds are effective as sanitizing agents in concentrations from

25 to 75 parts per million of free iodine. He states that 25 parts per million of free iodine is equivalent to 200 parts per million of available chlorine. Lazarus (1954, 1955) lists numerous advantages in favor of iodine compounds, which recommend them highly for dairy plant use.

Cheese Additives

Elliker (1954) recommended the addition of 5 to 10 parts per million of available chlorine to cottage cheese wash water to destroy organisms present in the water supply. He demonstrated the ineffectiveness of washing cheese curd with chlorinated water to remove organisms from the curd. Marquardt (1953, 1954) reported that the addition of 2.0 percent starter to cottage cheese creaming mixtures partially controlled surface slime in the product. He also stated that the addition of 7.5 parts per million of active available iodine to the final wash water retarded the development of water-borne organisms causing the slimy condition.

Beneke and Fabian (1955) demonstrated that sorbic acid is inhibitory to organisms commonly associated with strawberry and tomato spoilage. Costilow et al. (1955), Sheneman and Costilow (1955), and Phillips and Mundt (1950) worked with various organisms

and phases of the cucumber fermentation industry. They found that sorbic acid was effective in preserving sweet cucumber pickles when used in conjunction with acetic acid in sucrose solutions and was effective at low pH levels against yeasts common to cucumber fermentations.

Smith and Rollin (1954a, 1954b) reported that wrappers treated with sorbic acid were effective in inhibiting molds on cheese. The addition of 0.05 percent sorbic acid to process cheese inhibited molds. According to these workers, this concentration of sorbic acid does not affect the taste, odor, color, or emulsion stability of the cheese. Melnick and Luckmann (1954a, 1954b) showed that sorbic acid migrates rapidly from the wrapper into all varieties of cheese. They proposed a spectrophotometric method for determination of sorbic acid in cheese and cheese wrappers. The method, which is claimed to be precise and specific, consists of distilling the substance from the sample in the presence of magnesium sulfate and calculating the concentration from the absorbency reading. This method may be valuable in enforcing regulations regarding the addition of sorbic acid to cheese and cheese wrappers.

Melnick et al. (1954c, 1954d) found that sorbic acid is oxidized by the same agents and at the same rates as the polysaturated fatty acids found in vegetable oils and butterfat. The

degradation products also are similar. Deuel et al. (1954) demonstrated that rats metabolize sorbic acid, oxidizing it completely to carbon dioxide and water. Deuel and Alfinslater (1954) reported that tests in two independent laboratories showed that sorbic acid is harmless when fed to dogs at the rate of 5 percent of their diet on a dry weight basis. They concluded that sorbic acid is considerably less toxic than sodium benzoate which has been commonly used in cheese wrappers as a fungistatic agent.

METHODS

Isolation and Identification of Spoilage Organisms in Cottage Cheese

Isolation and Purification

Commercial samples of cottage cheese were collected from retail outlets and manufacturing plants. A portion from each sample of cheese was transferred aseptically into sterile petri plates and incubated at 10° and 21° C. until spoilage occurred. The cheese was considered spoiled when slime, mold growth, or a definitely undesirable odor developed.

Material from samples of cheese suspected of being spoiled by bacteria was streaked on tryptone glucose yeast agar plates. The samples suspected of being spoiled by yeasts and molds were streaked on potato dextrose agar acidified to pH of 3.5 with tartaric acid. The predominant colonies appearing on the plates were transferred to tryptone glucose yeast agar slants and acidified potato dextrose agar slants, respectively. The cultures growing on the slants were then purified by plating, streaking, and shake culture techniques. The shake culture technique involved loop transfers from agar slants and subsequent serial dilutions into

three tubes of melted tryptone glucose yeast agar, followed by plating of the tubes of agar. A total of thirty-two cultures were isolated and purified. These purified cultures were used throughout the entire study.

Identification of the Isolated Cultures

The majority of the media and reagents used in this study were obtained from Difco Laboratories, Detroit, Michigan. The trypticase soy broth was obtained from Baltimore Biological Laboratories, Baltimore, Maryland.

The colony characteristics of the bacterial cultures were obtained by growing the organisms on tryptone glucose yeast agar slants and plates and potato dextrose agar plates. Their growth characteristics were observed in nutrient broth and litmus milk. Burke's modification of the Gram stain and Leifson's flagella stain described in The Manual of Methods for Pure Culture Study of Bacteria (1946) were made from 24-hour-old nutrient broth cultures of the organisms.

Biochemical tests performed included reactions in dextrose, maltose, lactose, sucrose, galactose, xylose, and raffinose broth solutions. Motility determinations were performed in a medium consisting of 10.0 g. of tryptose, 5.0 g. of sodium chloride, 5.0 g.

of agar and 1 liter of distilled water. The ability of the organisms to liquefy gelatin, reduce nitrates to nitrites, utilize citrates as a sole source of carbon, produce acid from dextrose (methyl red reaction), form acetyl-methyl-carbinol from dextrose (Voges-Proskauer reaction), hydrolyze starch, and produce urease, hydrogen sulfide, and indole was determined using appropriate media and reagents described in the Difco Manual (1953). The ability of the organisms to use ammonium salts as a sole source of nitrogen was demonstrated using a medium consisting of 15.0 g. of agar, 1.0 g. of ammonium di-hydrogen phosphate, 10.0 g. of glucose, 0.2 g. of potassium chloride, 0.2 g. of magnesium sulfate, 0.01 g. of brom cresol purple, and 1 liter of distilled water.

Inoculations into the appropriate test media were made from 24-hour-old nutrient broth cultures of the organisms being tested. The inoculated media were incubated at 21° C. for the required length of time and the appropriate tests performed.

Bergey's Manual of Determinative Bacteriology by Breed et al. (1948) and Agriculture Monograph No. 16, Aerobic Spore-forming Bacteria by Smith et al. (1952) were used as guides for classification of the isolated cultures.

The appearance of the yeast cultures was noted on tryptone glucose yeast agar slants, in nutrient broth and litmus milk. The

ability of the organisms to assimilate and ferment dextrose, maltose, lactose, galactose, sucrose, mannose, and raffinose was demonstrated as recommended by Lodder and Kreger van Rij (1952). Their ability to reduce nitrates to nitrites was demonstrated as described in the Difco Manual (1953) using sulfanilic acid and a-naphthylamine reagent solutions. Sporulation tests were performed by growing the yeast cultures for 1 month on V-8 vegetable juice agar slants and then examining microscopically for spores. The yeast cultures were also examined for their ability to produce starchlike compounds as recommended by Lodder and Kreger van Rij (1952). The Yeasts: A Taxonomic Study by these authors was used as a guide in classification of the isolated yeast cultures.

Morphological characteristics of the molds were obtained by growing them on Czapek, Sabouraud, and tryptone glucose yeast agar. The cultures grown on Sabouraud agar were stained using lacto-phenol cotton-blue stain as outlined by Alexopoulos and Beneke (1952). Identification of the mold cultures was made using morphological characteristics and the appropriate identification keys found in Gilman (1945) and Raper et al. (1949).

The thirty-two cultures which were isolated and purified were characterized as follows: twenty-one bacteria, eight molds, and three yeasts. Eight bacterial cultures and one yeast were isolated

from tryptone glucose yeast agar plates exposed in the cottage cheese processing rooms for 10 minutes. The elimination of duplicate cultures from the isolates left eighteen bacterial cultures, four molds, and three yeasts which were carried to complete tentative identification.

Type of Spoilage Produced by Pure Cultures of Cottage Cheese Spoilage Organisms

Nineteen representative organisms from the group were selected for use in further studies involving cottage cheese. The cultures were carried in trypticase soy broth since this medium supported the growth of all organisms quite well. Inoculations were made from 24-hour-old broth cultures into the cheese in all instances.

Appearance of Cheese When Spoilage Organisms Are in Pure Culture and in Combination with S. lactis

Cottage cheese was placed in layers approximately 1/8 inch deep in petri plates. The plates were covered with saran and sterilized with an electron beam machine at a dosage of 5.5×10^6 rep. The saran was replaced with sterilized glass covers. The pH of the sterilized cheese was determined and then the plates were inoculated with 1 ml. amounts of 24-hour-old broth cultures of the organisms. One set of plates was inoculated with pure

cultures of the spoilage organisms and a second set was inoculated in the same manner, but received also an inoculation of S. lactis. The inoculated plates were incubated at 21° C. until spoilage occurred or it was apparent that no visible spoilage would occur. The plates containing the pure cultures and the ones containing pure cultures in combination with S. lactis were compared and photographed. The entire contents of each plate was then mixed well, placed in a 50 ml. beaker, and the pH determined using a Beckman Model G pH meter.

Characteristics of Cottage Cheese Spoilage Organisms

The effect of incubation temperature, pH, sodium chloride, heat, chlorine, iodophor, quaternary, and sorbic acid on the growth of nineteen representative organisms from the group was determined. These characteristics were determined in trypticase soy broth or modified broth solutions whenever possible. Inoculations were made from 24-hour-old broth cultures into the appropriate media.

Temperature Limits of Growth

Sterile tubes of broth inoculated with one loopful of the cultures were incubated at 3°, 10°, 20°, 35°, and 50° C., and

examined after 3 and 7 days of incubation. Increased turbidity of the broth was accepted as evidence of growth of the organism.

Effect of pH on Growth Rate

The cultures were tested for growth in broth adjusted to pH levels of 4.0, 4.4, 4.6, 4.8, 5.0, 5.2, 5.4, 6.7, and 7.4 with 85 percent (reagent grade) lactic acid. The required amount of acid was added to the broth prior to autoclaving at 15 pounds pressure for 15 minutes. This autoclaving did not appreciably change the pH of the medium. Inoculations were made and the tubes incubated at 21° C. for 3 days and then examined for growth. The extent of growth was determined by measuring the increase in turbidity with a Klett-Summerson colorimeter equipped with a number 42 filter. The colorimeter was standardized with uninoculated control tubes of broth at each pH level.

Sodium Chloride Tolerance

The cultures were tested for salt tolerance in broth solutions containing 1, 3, 5, 7, and 10 percent sodium chloride added to the medium prior to sterilization. The inoculations were made and tubes incubated at 21° C. for 3 days and then examined for growth. An increase in turbidity of the broth as compared to an

uninoculated control was accepted as evidence of growth of the organism.

Heat Tolerance

Twenty-four-hour-old skim milk cultures of each of the organisms were tested for tolerance to heating at 143° F. for 5, 10, 20, and 30 minutes. The cultures were heated in capillary tubes 1.5 × 90 mm. and then cooled immediately. The capillaries were incubated at 21° C. for 2 days and the contents plated using tryptone glucose yeast agar to determine organism survival. The cultures were also tested for tolerance to 120° F. for 15 minutes, which corresponds to the cooking temperature of cottage cheese. Twenty-four-hour-old skim milk cultures and whey cultures prepared from 24-hour-old agar slants of the organisms were heated for 15 minutes at 120° F. in capillary tubes and then cooled immediately. The capillaries were incubated and plated as described above to determine organism survival.

Resistance to Germicides

The cultures were tested for resistance to solutions of 10, 25, and 50 parts per million of chlorine, quaternary, and iodophor for 1, 5, and 10 minutes' exposure. Fresh stock solutions containing

100 parts per million of chlorine, quaternary, and iodophor were prepared for each determination. The chlorine and iodophor stock solutions were standardized by sodium thiosulfate titration and the quaternary stock solution by the Harper-Elliker method described in the Klenzade Dairy Sanitation Handbook (1954). Stock solutions were stored in 1 gallon capacity, tightly closed, brown glass bottles. One hundred ml. portions containing 10, 25, and 50 parts per million of chlorine, quaternary, and iodophor solutions were prepared from the stock solutions by mixing the appropriate amount of the stock solution with sterile distilled water just prior to testing each culture. One ml. of a 24-hour-old broth culture of the test organism was added to each 100 ml. portion of disinfectant and the mixture well shaken. At intervals of 1, 5, and 10 minutes, loop inoculations were made from the organism-disinfectant mixture into sterile tubes of broth. The inoculated tubes were incubated at 21° C. for 3 days and then examined for growth. An increase in turbidity was accepted as evidence of growth of the organism.

Tolerance to chlorine. The chlorine tolerance of nineteen cottage cheese spoilage organisms was performed as outlined above. The stock solution of chlorine was prepared from a sodium hypochlorite solution containing 8.4 percent chlorine.

Tolerance to quaternary. The ability of nineteen cottage cheese spoilage organisms to tolerate quaternary compounds was demonstrated as outlined above. The stock solution of quaternary was prepared from a 12.8 percent active alkyl dimethyl benzyl ammonium chloride solution obtained from the Klenzade Products Company, Beloit, Wisconsin.

Tolerance to iodophor. The ability of nineteen cottage cheese spoilage organisms to tolerate iodophor compounds was demonstrated as outlined above. The stock solution of iodophor was prepared from a 4.77 percent active Iobac solution obtained from Lazarus Laboratories, Inc., Buffalo, New York.

Resistance to Sorbic Acid

The cultures were tested for resistance to concentrations of 0.05, 0.10, and 0.25 percent sorbic acid at pH levels of 4.8, 5.0, and 5.2 in trypticase soy broth. The broth solutions were made containing the desired sorbic acid concentrations and prior to sterilization the pH was adjusted to the desired level with concentrated (reagent grade) lactic acid. The cultures were also tested at pH levels of 7.0, 6.8, and 6.0 with sorbic acid concentrations of 0.05, 0.10, and 0.25 percent, respectively. These pH levels resulted when

the desired sorbic acid concentrations were added to the broth with no attempt to control pH. Sterilization by autoclaving at 15 pounds pressure for 15 minutes caused no significant change in pH. The tubes of broth containing sorbic acid were inoculated with one loop-ful of a 24-hour-old broth culture of the organism. The tubes were incubated at 21° C. for 48 hours and the increase in turbidity measured with a Klett-Summerson colorimeter equipped with a number 42 filter. The colorimeter was adjusted using uninoculated control broth tubes at each pH level and sorbic acid concentration. In tubes which showed no increase in turbidity upon visual inspection, the viability of organisms was determined by plating with tryptone glucose yeast agar. An increase in turbidity of the broth was accepted as evidence of growth of the organism, while no increase in turbidity but growth on the plates was accepted as evidence of viability but no growth.

Control of Organisms Causing Spoilage in Cottage Cheese

Treatment of Wash Water

The effectiveness of washing cottage cheese curd with waters containing acetic, citric, lactic, phosphoric, and sorbic acids; and chlorine, iodophor and quaternary compounds in controlling organisms

which cause spoilage in cottage cheese was determined. Twenty pounds of fresh dry cottage cheese curd was obtained from the Michigan State University Dairy Plant or a local dairy. One and one-half pounds of the cheese, which served as an uninoculated control, was removed and washed with 1 liter of cold tap water. The remainder of the cheese was thoroughly washed with 2 liters of water inoculated with 2 ml. of a 24-hour-old broth culture of the spoilage organism. Wash waters were prepared containing sufficient acetic, citric, phosphoric, and lactic acids to reduce the pH of the wash waters to 5.0. Wash water was prepared also which contained 0.05 percent sorbic acid; higher concentrations imparted an objectionable flavor to the cheese. Waters containing 10 parts per million of chlorine and quaternary and 7.5 parts per million of iodophor were prepared from stock solutions containing 100 parts per million of these compounds. One and one-half-pound portions of the contaminated cheese were weighed into 1 liter portions of the prepared wash waters. One lot of 1-1/2 pounds of inoculated cheese was also weighed into 1 liter of plain cold tap water; this was the inoculated control. The wash waters were drained from the curd after 30 minutes of exposure and the dry curd was creamed with sterile cream which contained 14 percent butterfat and 4 percent sodium chloride. The salt was added after autoclaving for 15 minutes at 15 pounds pressure. The cream and

cheese were mixed in proportions which gave a product containing 4 percent butterfat and 1.15 percent salt. The creaming mixture was allowed to stand on the cheese for approximately 1 hour before packaging Each lot of cheese was packaged in three 12-ounce cottage cheese cartons and stored at 50° F. The cheese was examined for appearance of visible spoilage, given a flavor score, and the pH determined initially and at 2-day intervals thereafter until spoilage occurred.

Treatment of Dressing Materials

The effectiveness of treating cottage cheese dressing with citric, lactic, and sorbic acids and starter in controlling organisms which cause spoilage in cottage cheese was determined. Fifteen pounds of fresh dry cottage cheese curd was obtained from the Michigan State University Dairy Plant or a local dairy. Six lots of 1-1/2 pounds of the cheese were placed in separate containers. Five pounds of cream containing 14 percent butterfat was sterilized by autoclaving for 15 minutes at 15 pounds pressure, cooled, and 4 percent salt added. A portion of this cream was added to one lot of 1-1/2 pounds of cheese curd, yielding 4 percent butterfat and 1.15 percent salt in the finished product. This lot of cheese served as the uninoculated control. The remainder of the cream

was contaminated with 1 ml. of a 24-hour-old broth culture of the spoilage organism. A portion of this contaminated cream was added to one lot of 1-1/2 pounds of cheese, yielding a standardized product as before. This lot served as the inoculated control. Fifteen hundredths percent citric and lactic acids, 0.10 percent sorbic acid, and 2.0 percent starter were added, respectively, to four portions of the contaminated cream. These inoculated and treated dressings were then added to the cheese and allowed to stand for approximately 1 hour before packaging. Each lot of cheese was packaged in three 12-ounce cottage cheese cartons and stored at 50° F. The cheese was examined for appearance of visible spoilage, given a flavor score, and the pH determined initially and at 2-day intervals thereafter until spoilage occurred.

RESULTS

Identification of the Isolated Cultures

Tables I, II, III, and IV show the various tests which were performed in identifying the bacterial and yeast cultures. Morphological characteristics used in identifying the mold cultures are described later.

The bacterial cultures were tentatively identified as Achromobacter butyri, Achromobacter eurydice, Alcaligenes metalcaligenes, Bacillus firmus, Bacterium erythrogenes, Corynebacterium filamentosum, Escherichia coli, Escherichia freundii, Escherichia intermedium, Micrococcus aurantiacus, Micrococcus candidus, Micrococcus conglomeratus, Micrococcus epidermidis, Micrococcus flavus, Pseudomonas desmolyticum, Pseudomonas fluorescens, Pseudomonas fragi, and Pseudomonas tralucida. The yeast cultures were tentatively identified as Rhodotorula flava, Torulopsis candida, and Torulopsis versatilis, and the mold cultures as Geotrichum candidum, Mucor plumbeus, Mucor racemosus, and Penicillium frequentans.

Alc. metalcaligenes, C. filamentosum, E. freundii, M. aurantiacus, M. conglomeratus, M. epidermidis, M. flavus, and

TABLE I

PHYSIOLOGICAL, MORPHOLOGICAL, AND BIOCHEMICAL CHARACTERISTICS OF EIGHTEEN BACTERIA ISOLATED FROM SPOILED CHEESE AND AGAR PLATES EXPOSED IN CHEESE PLANTS

Organism	Staining and Morphology		
	Gram	Flagella, Motility	Size and Shape
Ach. butyri	-	-	small short rods
Ach. eurydice	-	-	small short rods
Alc. metalcaligenes	-	-	small medium rods
B. firmus	+	-	large medium rods
Bact. erythrogenes	-+[a]	-	small short rods
C. filamentosum	+	-	large medium rods
E. coli	-	+	small short rods
E. freundii	-	-	small short rods
E. intermedium	-	-	small short rods
M. aurantiacus	+	-	medium cocci
M. candidus	+	-	small cocci
M. conglomeratus	+	-	large cocci
M. epidermidis	+	-	medium cocci
M. flavus	+	-	large cocci
Ps. desmolyticum	-	+	medium short rods
Ps. fluorescens	-	+	medium long rods
Ps. fragi	-	+	medium long rods
Ps. tralucida	-	+	medium long rods

Legend: In carbohydrate reactions, - = no reaction, + = acid produced, ++ = acid and gas produced.

TABLE I (Continued)

Carbohydrate Reactions						
Dextrose	Maltose	Lactose	Sucrose	Galactose	Xylose	Raffinose
+	-	-	-	-	-	-
+	-	-	-	-	-	-
+	-	-	-	-	-	-
+	+	-	-	-	-	-
-	-	-	-	-	-	-
-	-	-	-	-	-	-
++	++	++	++	++	+	++
++	++	++	++	++	++	++
++	++	++	++	++	++	++
-	-	-	-	-	-	-
+	-	+[b]	+[b]	+[b]	+[b]	+[b]
+	-	-	-	+	-	-
+	+	+	+	-	-	-
+	+	-	-	+	-	-
++	++	+	+	++	++	-
+	-	-	-	-	-	-
+	-	-	-	+	+	-
++	++	++	-	++	++	-

[a] Variable gram reaction.

[b] Weak carbohydrate reaction.

TABLE II

PHYSIOLOGICAL AND BIOCHEMICAL CHARACTERISTICS OF EIGHTEEN BACTERIA ISOLATED FROM SPOILED CHEESE AND AGAR PLATES EXPOSED IN CHEESE PLANTS

Organism	Appearance of Growth in Different Media	
	Litmus Milk	Nutrient Broth
Ach. butyri	no reaction	turbid, sediment
Ach. eurydice	alkaline	turbid, sediment
Alc. metalcaligenes	slight alkaline	turbid, sediment
B. firmus	acid digestion	turbid, sediment
Bact. erythrogenes	alkaline coagulation	turbid, sediment
C. filamentosum	no reaction	turbid, sediment
E. coli	acid coagulation	turbid, sediment
E. freundii	acid	turbid, sediment
E. intermedium	acid	turbid, sediment
M. aurantiacus	slight alkaline	turbid, sediment
M. candidus	slight acid	turbid, sediment
M. conglomeratus	no reaction	turbid, sediment
M. epidermidis	acid	turbid, sediment
M. flavus	slight acid	turbid, sediment
Ps. desmolyticum	no reaction	turbid, sediment
Ps. fluorescens	alkaline proteolysis	turbid, sediment
Ps. fragi	acid digestion	turbid, sediment
Ps. tralucida	acid	turbid, sediment

TABLE II (Continued)

Appearance of Growth in Different Media	
Tryptone Glucose Yeast Agar	Potato Dextrose Agar
smooth, brown, mucoid	smooth, brown
smooth, gray, mucoid	smooth, brown
smooth, brown, mucoid	smooth, dull
rough, dull, dry	rough, dull, dry
rough, dark, mucoid	rough, dark
rough, yellow	no growth
smooth, white, mucoid	smooth, brown
smooth, dull, mucoid	smooth, brown
smooth, gray, mucoid	smooth, white
smooth, brown, mucoid	smooth, brown
smooth, white, scant	smooth, brown
smooth, white, mucoid	smooth, brown
smooth, white, scant	no growth
smooth, orange, mucoid	smooth, brown
smooth, brown, mucoid	smooth, brown
rough, green	rough, brown
smooth, brown, mucoid	smooth, brown
smooth, brown	smooth, brown

TABLE III

PHYSIOLOGICAL AND BIOCHEMICAL CHARACTERISTICS OF EIGHTEEN BACTERIA ISOLATED FROM SPOILED CHEESE AND AGAR PLATES EXPOSED IN CHEESE PLANTS

Organism	Physiological and Biochemical Reactions		
	Liquefaction of Gelatin	Reduction of Nitrates	Utilization of Citrates
Ach. butyri	+	-	-
Ach. eurydice	-	-	+
Alc. metalcaligenes	-	-	-
B. firmus	+	+	-
Bact. erythrogenes	+[b]	-	+
C. filamentosum	+	-	-
E. coli	+	+	-
E. freundii	-	+	+
E. intermedium	-	+	+
M. aurantiacus	-	-	-
M. candidus	-	+	-
M. conglomeratus	-	+	-
M. epidermidis	+[b]	+	-
M. flavus	-	-	-
Ps. desmolyticum	-	+	-
Ps. fluorescens	+	+	-
Ps. fragi	+	-	+
Ps. tralucida	-	+	-

[a] Weak H_2S production.

[b] Slow liquefaction of gelatin.

TABLE III (Continued)

Physiological and Biochemical Reactions						
Methyl Red	Voges-Proskauer	Production of Indole	Production of H_2S	Hydrolysis of Starch	Utilization of $NH_4H_2PO_4$ as a Sole Source of N	Production of Urease
-	-	-	+[a]	-	+	-
-	-	-	-	-	+	-
-	-	+	-	-	+	-
+	-	-	-	+	-	-
-	-	-	-	-	-	-
-	-	-	-	-	-	-
+	-	-	-	-	+	-
+	-	+	-	-	+	-
-	-	-	+	-	+	-
-	-	-	-	-	-	-
-	-	-	-	-	-	-
-	-	-	-	+	+	-
+	-	-	-	-	-	-
+	-	-	-	-	-	-
-	-	-	+	-	+	+
-	-	-	+	-	+	-
-	-	-	+	-	+	-
-	-	-	+	-	+	-

TABLE IV

PHYSIOLOGICAL AND BIOCHEMICAL CHARACTERISTICS OF YEASTS CAUSING SPOILAGE IN COTTAGE CHEESE

Test Performed	T. candida	T. versatilis	R. flava
Dextrose			
Assimilation	+	+	+
Fermentation	+	+	-
Maltose			
Assimilation	+	+	+
Fermentation	-	+	-
Lactose			
Assimilation	+	+	+
Fermentation	-	-	-
Galactose			
Assimilation	+	+	+
Fermentation	-	+	-
Sucrose			
Assimilation	+	+	+
Fermentation	-	+	-
Mannose			
Assimilation	+	+	+
Fermentation	-	-	-
Raffinose			
Assimilation	+	+	+
Fermentation	-	-	-
Pellicle formation in broth	+		
Reduction of nitrates			
Formation of spores	-	-	
Reaction in litmus milk	alk. prot.		
Production of starchlike compounds	-	-	-

R. flava were isolated from tryptone glucose yeast agar plates exposed in cottage cheese processing rooms.

The characteristics of Ach. eurydice, M. epidermidis, Ps. desmolyticum, and Ps. fluorescens agreed without discrepancy with those listed by Breed et al. (1948). These authors do not describe Ps. desmolyticum in any great detail. Ps. fluorescens was somewhat difficult to identify, because it did not produce the green fluorescent, water-soluble pigment typical of this organism until after numerous transfers on artificial media.

Ach. butyri gave dark mucoid growth on an agar slant rather than white growth as reported by Breed et al. (1948). The characteristics exhibited by Alc. metalcaligenes also differed from those listed by these authors as follows: acid was produced from glucose and a pellicle was not formed in broth.

The organism tentatively identified as B. firmus exhibited rough surface growth on an agar slant. According to Breed et al. (1948), B. firmus should produce smooth growth on an agar slant. The above authors stated that Bact. erythrogenes turns litmus milk a blood red color and exhibits optimum growth at 28° to 35° C. The organism tentatively identified as Bact. erythrogenes grew best at 21° to 25° C. and failed to turn litmus milk a blood red color.

Breed et al. (1948) stated that C. filamentosum should cause an alkaline reaction in carbohydrates and litmus milk, grow at 37° C., and liquefy gelatin slowly. The organism tentatively identified as C. filamentosum liquefied gelatin rapidly and failed to grow at 37° C. or produce an alkaline reaction in litmus milk and carbohydrates. These discrepancies might be sufficient to cause some doubt as to the true identity of the organism. However, it coincided with the characteristics listed for C. filamentosum more closely than those listed for any other organism.

The organisms tentatively identified as E. coli, E. freundii, and E. intermedium showed characteristics that varied somewhat from those described by Breed et al. (1948). E. coli liquefied gelatin and failed to produce indole. E. freundii failed to produce hydrogen sulfide when grown on lead acetate agar, while E. intermedium produced hydrogen sulfide on this medium. E. coli should produce indole, but not liquefy gelatin. E. freundii should produce hydrogen sulfide on proteose, peptone, ferric citrate agar, while E. intermedium should not produce hydrogen sulfide on this medium. The discrepancies in hydrogen sulfide production might have been due to the difference in medium employed.

M. aurantiacus failed to reduce nitrates or produce acid from carbohydrates and litmus milk. Breed et al. (1948) stated that

this organism reduces nitrates weakly and produces slight acid from carbohydrates and litmus milk. Many of the reactions noted in the tentative identification of M. candidus were extremely weak also. Breed et al. (1948) reported that M. conglomeratus should produce acid from lactose but not hydrolyze starch and M. flavus also should produce acid from lactose and liquefy gelatin. Neither of these organisms produced acid from lactose, while M. conglomeratus hydrolyzed starch and M. flavus failed to liquefy gelatin.

Ps. fragi liquefied gelatin more rapidly but was less proteolytic in litmus milk than reported by Breed et al. (1948). Ps. tralucida varied from the tabulated characteristics in that growth on an agar slant was good rather than scant, sucrose was not fermented, and cellulose was not utilized.

The yeast cultures tentatively identified as R. flava, T. candida, and T. versatilis (Table IV) exhibited characteristics that agreed with those described by Lodder and Kreger van Rij (1952) for these organisms.

Mucor plumbeus, Mucor racemosus, Pen. frequentans, and G. candidum were tentatively identified using characteristics listed in A Manual of Soil Fungi by Gilman (1945). A Manual of the Penicillia by Raper et al. (1949) also was used in identifying Pen. frequentans.

Types of Defects Produced by Spoilage Organisms in Cottage Cheese

The types of defects produced by nineteen organisms causing spoilage in cottage cheese and their effect on the pH of sterile cottage cheese curd when in pure culture and combined with S. lactis are shown in Table V.

M. candidus and M. conglomeratus were the only pure cultures that failed to produce a visible defect, except free whey resulting from reduced pH. The above two cultures and Bact. erythrogenes failed to produce a visible defect when combined with S. lactis, except the appearance of free whey as above.

E. freundii, Ps. fragi, R. flava, Mucor plumbeus, and Pen. frequentans produced approximately the same degree and type of spoilage in pure culture as when combined with S. lactis. Ach. butyri, B. firmus, Bact. erythrogenes, E. coli, M. flavus, and Ps. fluorescens produced characteristic types of spoilage more rapidly in pure culture, whereas Ach. eurydice, Alc. metalcaligenes, Ps. desmolyticum, Ps. tralucida, T. candida, and G. candidum produced characteristic spoilage more rapidly when combined with S. lactis.

Pure cultures of Ach. butyri, Ach. eurydice, Alc. metalcaligenes, Bact. erythrogenes, E. freundii, M. flavus, Ps.

TABLE V

TYPE OF DEFECT AND pH OBTAINED WITH ORGANISMS CAUSING SPOILAGE IN COTTAGE CHEESE IN PURE CULTURE AND COMBINED WITH S. LACTIS

Organism	Initial pH
Bacteria	
Ach. butyri	5.10
Ach. eurydice	5.10
Alc. metalcaligenes	5.10
B. firmus	5.10
Bact. erythrogenes	5.10
E. coli	5.10
E. freundii	5.10
M. candidus	5.10
M. conglomeratus	5.10
M. flavus	5.10
Ps. desmolyticum	5.10
Ps. fluorescens	5.10
Ps. fragi	5.10
Ps. tralucida	5.10
Yeasts	
R. flava	5.10
T. candida	5.10
Molds	
G. candidum	5.10
Mucor plumbeus	5.10
Pen. frequentans	5.10

TABLE V (Continued)

Defect and pH Obtained with Pure Culture		Defect and pH Obtained with Pure Cultures Combined with S. lactis	
Defect	pH	Defect	pH
tapioca slime	5.00	slight tapioca slime	5.20
slight tapioca slime	5.00	tapioca slime	5.25
slight tapioca slime	5.25	tapioca slime	5.40
brown slime, digested	6.35	brown slime, slight digestion	4.75
brown slime, liquefied	5.00	no visible defect	4.60
brown slime, digested	6.35	brown slime, slight digestion	6.25
brown slime	5.25	brown slime	5.50
no visible defect	4.60	no visible defect	4.45
no visible defect	4.60	no visible defect	4.55
yellow buttons	5.20	slight yellow buttons	5.10
slight tapioca slime	5.40	tapioca slime	5.40
green slime	5.10	slight green slime	4.60
light brown slime	5.10	light brown slime	5.15
slight brown slime	5.10	brown slime	5.40
pink slime	5.40	pink slime	4.75
yellow slime, liquefied	5.70	yellow slime, liquefied	5.60
slight gray mold	5.15	gray mold	5.25
brown mold	5.60	brown mold	5.30
green mold	5.40	green mold	5.50

fluorescens, Ps. fragi, Ps. tralucida, and G. candidum altered the pH of the cottage cheese curd 0.05 to 0.15 units. The pH increased 0.30 to 1.25 units in cheese inoculated with pure cultures of B. firmus, E. coli, Ps. desmolyticum, R. flava, T. candida, Mucor plumbeus, and Pen. frequentans, whereas the pH decreased approximately 0.50 units when pure cultures of M. candidus and M. conglomeratus were inoculated into the curd.

Ach. butyri, Ach. eurydice, M. flavus, Ps. fragi, G. candidum, and Mucor plumbeus altered the pH of the cottage cheese curd 0.05 to 0.20 units when combined with S. lactis. When S. lactis was combined with Alc. metalcaligenes, E. coli, E. freundii, Ps. desmolyticum, Ps. tralucida, T. candida, and Pen. frequentans, the pH increased 0.30 to 1.15 units. The pH decreased from 0.35 to 0.75 units in samples inoculated with S. lactis combined with each of the following: B. firmus, Bact. erythrogenes, M. candidus, M. conglomeratus, Ps. fluorescens, and R. flava.

In nine of eleven cases where there were differences in both the degree of defect and the pH, the defects exhibited were more pronounced at the higher pH values regardless of whether these were produced by the pure cultures individually or when combined with S. lactis.

Characteristics of Cottage Cheese Spoilage Organisms

Temperature Limits of Growth

Table VI shows the effect of temperature on the growth of nineteen cottage cheese spoilage organisms when grown in trypticase soy broth.

None of the organisms tested exhibited visible growth in 3 days when held at 3° C. However, Ach. butyri, Ach. eurydice, Alc. metalcaligenes, E. coli, M. flavus, Ps. fragi, Ps. fluorescens, and Mucor plumbeus exhibited slight growth after 7 days at this temperature.

After 3 days at 10° C. Ach. butyri, E. coli, E. freundii, Ps. desmolyticum, and Ps. tralucida exhibited a moderate amount of growth, whereas Ach. eurydice, Alc. metalcaligenes, M. candidus, Ps. fragi, Ps. fluorescens, G. candidum, and Mucor plumbeus showed only a slight amount of growth. Bact. erythrogenes, M. conglomeratus, and Pen. frequentans were the only organisms that failed to grow after 7 days at 10° C.

At 20° C. all cultures tested grew, but exhibited varying amounts of growth.

Ach. butyri, Ach. eurydice, Alc. metalcaligenes, Ps. fluorescens, R. flava, T. candida, Mucor plumbeus, and Pen.

TABLE VI

GROWTH OF COTTAGE CHEESE SPOILAGE ORGANISMS IN TRYPTICASE SOY BROTH AT DIFFERENT INCUBATION TEMPERATURES (THREE TRIALS)

Organism	Incubation Temperatures and Days of Incubation									
	3° C.		10° C.		20° C.		35° C.		50° C.	
	3	7	3	7	3	7	3	7	3	7
Bacteria										
Ach. butyri	-	+	++	+++	+++	+++	-	+	-	-
Ach. eurydice	-	+	+	++	+++	+++	-	+	-	-
Alc. metalcaligenes	-	+	+	++	++	++	-	+	-	-
B. firmus	-	-	-	+	+++	+++	+++	+++	-	-
Bact. erythrogenes	-	-	-	-	+	++	+	++	-	-
E. coli	-	+	++	+++	+++	+++	+++	+++	-	-
E. freundii	-	-	++	+++	+++	+++	+++	+++	-	-
M. candidus	-	-	+	++	++	++	+	++	-	-
M. conglomeratus	-	-	-	-	+	++	+	++	-	-
M. flavus	-	+	-	++	+++	+++	+++	+++	-	-
Ps. desmolyticum	-	-	++	+++	+++	+++	+++	+++	-	-
Ps. fragi	-	+	+	+++	+++	+++	+	++	-	-
Ps. fluorescens	-	+	+	++	+++	+++	-	+		
Ps. tralucida	-	-	++	+++	+++	+++	+++	+++		
Yeasts										
R. flava	-	-	-	+	+	++	-	-	-	-
T. candida	-	-	-	++	+++	+++	-	-	-	-
Molds										
G. candidum	-	-	+	++	++	++	+	++	-	-
Mucor plumbeus	-	++	+	++	++	++	-	+	-	-
Pen. frequentans	-	-	-	-	+	++	-	-	-	-

Legend: - = no growth, + = slight growth, ++ = moderate growth, +++ = profuse growth.

frequentans failed to show growth in 3 days at 35° C., but only R. flava, T. candida, and Pen. frequentans failed to grow after 7 days at this temperature.

None of the organisms studied exhibited growth after 3 or 7 days when held at 50° C.

Effect of pH on Growth Rate

The effect of pH on the growth rate of sixteen organisms causing spoilage in cottage cheese is shown in Figures 1 through 16. The data shown are the results of three trials. The pH values used covered the range normally encountered in cottage cheese. The range was lowered to pH 4.0 to extend below the minimum limit produced by S. lactis. The levels of 6.7 and 7.4 were used because they correspond to the approximate pH of fresh milk and normal trypticase soy broth, respectively.

The amount of growth was determined by measuring the increase in turbidity of the broth with a Klett-Summerson colorimeter. Klett-Summerson readings were not used with the mold cultures because molds tend to form a pellicle rather than a turbidity, and the resulting readings are not representative.

Effect of pH on the growth of organisms causing spoilage in cottage cheese

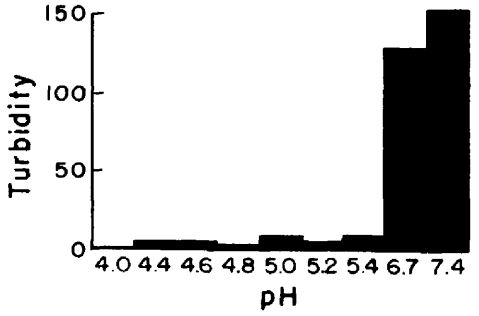

Fig. 1. E. Coli

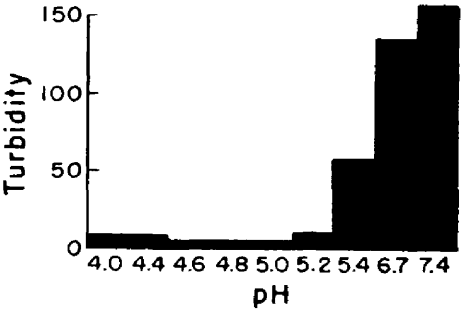

Fig. 2. E. freundii

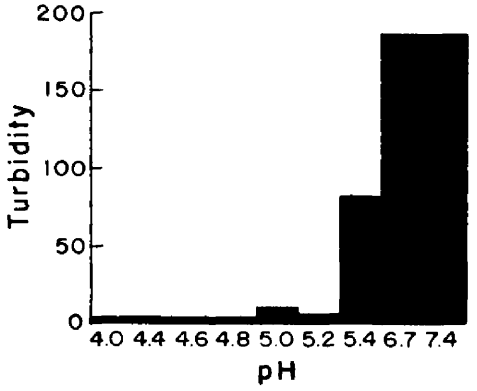

Fig. 3. Ps. desmolyticum

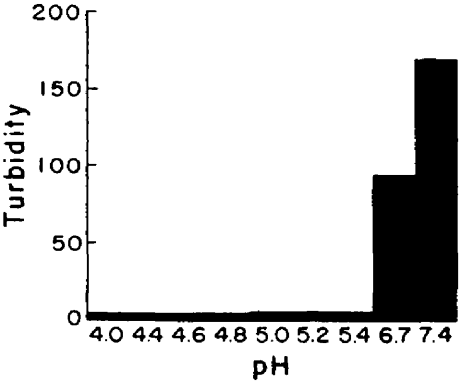

Fig. 4. Ps. fluorescens

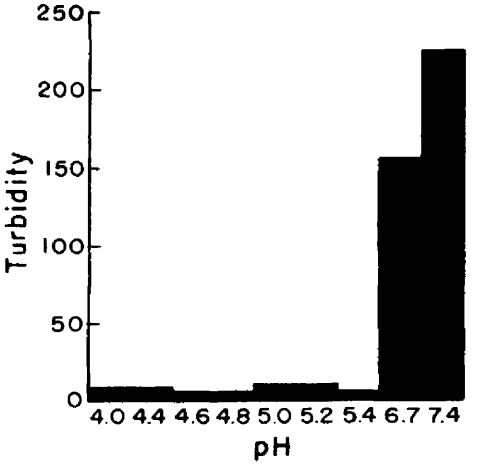

Fig. 5. Ps. fragi

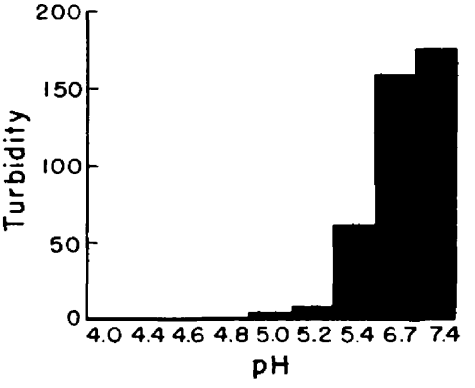

Fig. 6. Ps. tralucida

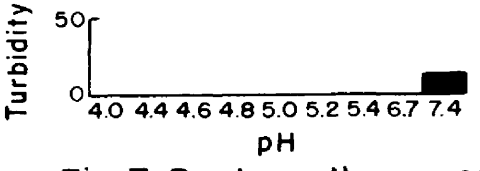

Fig. 7. Bact. erythrogenes

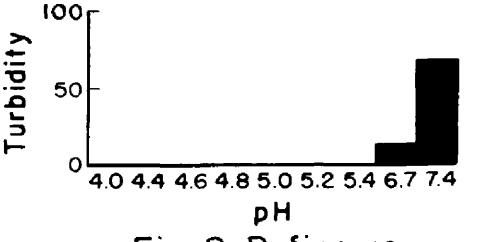

Fig. 8. B. firmus

Effect of pH on the growth of organisms causing spoilage in cottage cheese

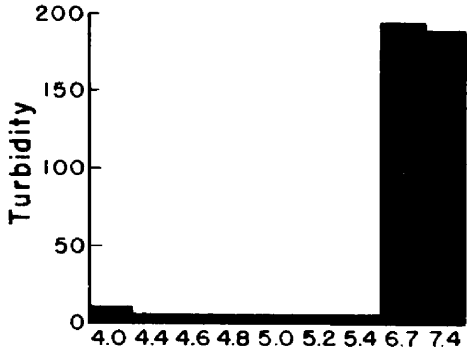

Fig. 9. Ach. butyri

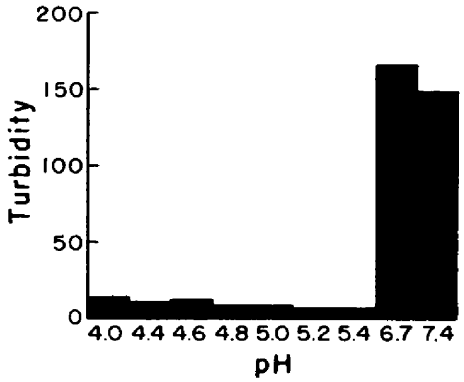

Fig. 10. Ach. eurydice

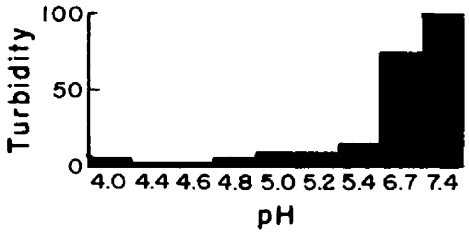

Fig. 11. M. flavus

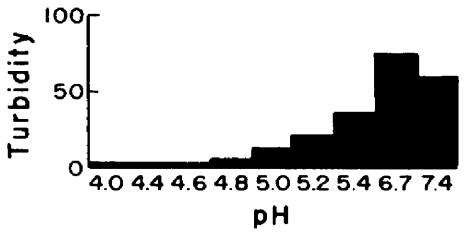

Fig. 12. M. candidus

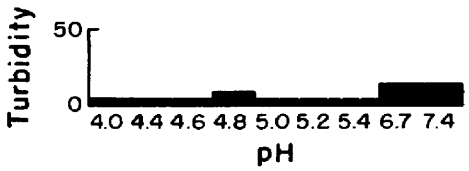

Fig. 13. M. conglomeratus

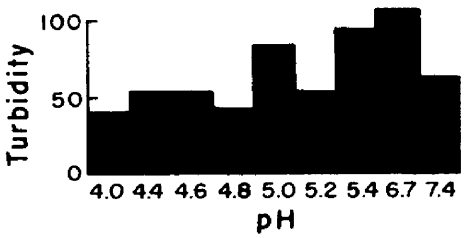

Fig. 14. T. candida

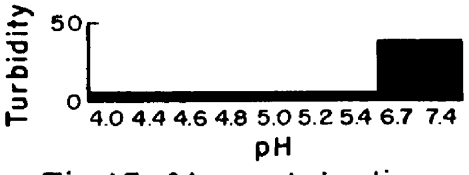

Fig. 15. Alc. metalcaligenes

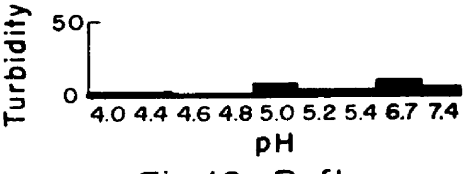

Fig. 16. R. flava

E. coli and E. freundii exhibited little growth at pH 5.2 and below, as shown in Figures 1 and 2. Both cultures grew better at 7.4 than any other pH used.

Ps. desmolyticum and Ps. tralucida (Figures 3 and 6) exhibited little growth at pH 5.2 and below, while Ps. fragi and Ps. fluorescens (Figures 4 and 5) showed little growth at pH 5.4 and below. Ps. desmolyticum seemed to exhibit more growth at pH 6.7 to 7.4, with the optimum probably being at approximately 7.0 Ps. fragi, Ps. fluorescens, and Ps. tralucida exhibited more growth at 7.4 than any other pH employed.

Figures 7 and 8 show that B. firmus and Bact. erythrogenes also exhibited little growth at pH 5.4 and below, and both organisms seemed to have an optimum pH of 7.4 or above.

Ach. butyri, Ach. eurydice, and Alc. metalcaligenes (Figures 9, 10, and 15) exhibited little growth at pH 5.4 and below. Their optimum seems to be approximately pH 6.7 to 7.0. Alc. metalcaligenes exhibited less growth than Ach. butyri or Ach. eurydice.

Figures 11, 12, and 13 show that M. flavus and M. candidus exhibited little growth at pH 5.0 and below, while M. conglomeratus showed little growth at pH 5.4 and below. The optimum pH for M. candidus and M. conglomeratus appeared to be approximately 6.7 to 7.0, while M. flavus grew better at 7.4 than any other pH used.

R. flava (Figure 16) exhibited little growth at any pH used. The growth of T. candida (Figure 17) fluctuated slightly, but grew well throughout the pH range studied. This organism exhibited the greatest amount of growth at pH 6.7.

Sodium Chloride Tolerance

The effect of various concentrations of sodium chloride in trypticase soy broth, on the growth of nineteen organisms causing spoilage in cottage cheese is shown in Table VII.

Bact. erythrogenes failed to tolerate 1 percent sodium chloride. M. candidus tolerated 1 but not 3 percent, while R. flava, and Pen. frequentans tolerated 3 but not 5 percent sodium chloride. Ach. butyri, Ach, eurydice, Alc. metalcaligenes, B. firmus, E. freundii, Ps. desmolyticum, Ps. fragi, Ps. fluorescens, Ps. tralucida, G. candidum, and Mucor plumbeus tolerated 5 but not 7 percent sodium chloride. E. coli was the only organism that tolerated 7 but not 10 percent sodium chloride. M. conglomeratus, M. flavus, and T. candida tolerated 10 percent sodium chloride.

Heat Tolerance

Table VIII shows the resistance of nineteen organisms causing spoilage in cottage cheese to heating at 143° F. in skim milk.

TABLE VII

GROWTH OF COTTAGE CHEESE SPOILAGE ORGANISMS IN TRYPTICASE SOY BROTH CONTAINING DIFFERENT CONCENTRATIONS OF SODIUM CHLORIDE (THREE TRIALS)

Organism	Percent NaCl (w/v)					
	0	1	3	5	7	10
Bacteria						
Ach. butyri	+	+	+	+	-	-
Ach. eurydice	+	+	+	+	-	-
Alc. metalcaligenes	+	+	+	+	-	-
B. firmus	+	+	+	+	-	-
Bact. erythrogenes	+	-	-	-	-	-
E. coli	+	+	+	+	+	-
E. freundii	+	+	+	+	-	-
M. candidus	+	+	-	-	-	-
M. conglomeratus	+	+	+	+	+	+
M. flavus	+	+	+	+	+	+
Ps. desmolyticum	+	+	+	+	-	-
Ps. fragi	+	+	+	+	-	-
Ps. fluorescens	+	+	+	+	-	-
Ps. tralucida	+	+	+	+	-	-
Yeasts						
R. flava	+	+	+	-	-	-
T. candida	+	+	+	+	+	+
Molds						
G. candidum	+	+	+	+	-	-
Mucor plumbeus	+	+	+	+	-	-
Pen. frequentans	+	+	+	-	-	-

TABLE VIII

RESISTANCE OF COTTAGE CHEESE SPOILAGE ORGANISMS TO HEATING AT 143° F. IN SKIM MILK (FIVE TRIALS)

Organism	Minutes Heated at 143° F.				
	0	5	10	20	30
Bacteria					
Ach. butyri	+	+	-	-	-
Ach. eurydice	+	+	-	-	-
Alc. metalcaligenes	+	+	-[a]	-	-
B. firmus	+	+	+	+	+
Bact. erythrogenes	+	-	-	-	-
E. coli	+	-	-	-	-
E. freundii	+	-	-	-	-
M. candidus	+	+[b]	-	-	-
M. conglomeratus	+	-	-	-	-
M. flavus	+	+[b]	-	-	-
Ps. desmolyticum	+	-	-	-	-
Ps. fragi	+	+[b]	-[a]	-	-
Ps. fluorescens	+	-	-	-	-
Ps. tralucida	+	+	+[b]	-	-
Yeasts					
R. flava	+	-	-	-	-
T. candida	+	-	-	-	-
Molds					
G. candidum	+	-	-	-	-
Mucor plumbeus	+	-	-	-	-
Pen. frequentans	+	-	-	-	-

[a]Growth in one of five trials.

[b]Growth in four of five trials.

Bact. erythrogenes, E. coli, E. freundii, M. conglomeratus, Ps. desmolyticum, Ps. fluorescens, R. flava, T. candida, G. candidum, Mucor plumbeus, and Pen. frequentans failed to survive 143° F. for 5 minutes. Ach. butyri, Ach. eurydice, Alc. metalcaligenes, M. candidus, M. flavus, and Ps. fragi survived 10 but not 20 minutes at this temperature. B. firmus was the only organism that survived for 30 minutes at 143° F. Subsequent heat studies showed B. firmus was still viable after 120 minutes at this temperature.

Further studies were made by heating the nineteen cultures in skim milk and whey at 120° F. for 15 minutes. This temperature and time corresponds approximately to the cooking temperature and holding time used in manufacturing cottage cheese. Table IX shows that Bact. erythrogenes and M. conglomeratus were the only organisms destroyed by heating at 120° F. for 15 minutes in skim milk at pH 6.7, while B. firmus and G. candidum were the only cultures which survived this temperature and time in whey at pH 4.55.

Resistance to Germicides

Tolerance to chlorine. The growth of nineteen spoilage organisms after exposure to distilled water solutions of 10, 25, and 50

TABLE IX

RESISTANCE OF COTTAGE CHEESE SPOILAGE ORGANISMS TO HEATING AT 120° F. FOR 15 MINUTES IN SKIM MILK AND WHEY (THREE TRIALS)

Organism	Substrate	
	Skim Milk	Whey
Bacteria		
Ach. butyri	+	-
Ach. eurydice	+	-
Alc. metalcaligenes	+	-
B. firmus	+	+
Bact. erythrogenes	-[a]	-
E. coli	+	-
E. freundii	+	-
M. candidus	+	-
M. conglomeratus	-	-
M. flavus	+	-
Ps. desmolyticum	+	-
Ps. fragi	+	-
Ps. fluorescens	+	-
Ps. tralucida	+	-
Yeasts		
R. flava	+	-
T. candida	+	-
Molds		
G. candidum	+	+[b]
Mucor plumbeus	+	-
Pen. frequentans	+[b]	-

[a] Growth in one of three trials.
[b] Growth in two of three trials.

parts per million of chlorine at pH 7.5 and 25° C. for 1, 5, and 10 minutes is shown in Table X.

R. flava was the most susceptible organism to chlorine, apparently being destroyed in 1 minute in 10 parts per million. Bact. erythrogenes and M. candidus survived 1 but not 5 minutes, while Ach. eurydice survived 5 but not 10 minutes in the above concentration.

Bact. erythrogenes, M. candidus, and R. flava were apparently destroyed in 1 minute in 25 parts per million of chlorine, while Alc. metalcaligenes survived 1 but not 5 minutes in this concentration. Ach. eurydice, E. freundii, Ps. desmolyticum, Ps. fluorescens, Ps. fragi, Ps. tralucida, and G. candidum survived 5 but not 10 minutes in this concentration of chlorine.

B. firmus, M. flavus, T. candida, and Mucor plumbeus were the only organisms that survived 1 minute in 50 parts per million of chlorine, and B. firmus was the only organism that survived 5 and 10 minutes in this concentration.

Tolerance to quaternary. The growth of nineteen spoilage organisms after exposure to distilled water solutions of 10, 25, and 50 parts per million of quaternary at pH 6.5 and 25° C. for 1, 5, and 10 minutes is shown in Table XI.

TABLE X

GROWTH OF COTTAGE CHEESE SPOILAGE ORGANISMS AFTER EXPOSURE TO DIFFERENT CONCENTRATIONS OF CHLORINE SOLUTIONS FOR VARIOUS TIME INTERVALS (THREE TRIALS)

Organism	Parts per Million of Chlorine and Minutes of Exposure								
	10 ppm			25 ppm			50 ppm		
	1	5	10	1	5	10	1	5	10
Bacteria									
Ach. butyri	+	+	+	+	+	+	-[a]	-	-
Ach. eurydice	+	+	-	+	+	-	-	-	-
Alc. metalcaligenes	+	+	+	+	-[a]	-	-	-	-
B. firmus	+	+	+	+	+	+	+	+	+
Bact. erythrogenes	+	-[a]	-	-	-	-	-	-	-
E. coli	+	+	+	+	+	+	-	-	-
E. freundii	+	+	+	+	+	-[a]	-	-	-
M. candidus	+	-[a]	-	-	-	-	-	-	-
M. conglomeratus	+	+	+	+	+	+	-	-	-
M. flavus	+	+	+	+	+	+	+	-	-
Ps. desmolyticum	+	+	+	+	+	-	-	-	-
Ps. fluorescens	+	+	+	+	+	-	-	-	-
Ps. fragi	+	+	+	+	+	-[a]	-	-	-
Ps. tralucida	+	+	+	+	+	-	-	-	-
Yeasts									
R. flava	-	-	-	-	-	-	-	-	-
T. candida	+	+	+	+	+	+	+[b]	-	-
Molds									
G. candidum	+	+	+	+	+	-	-	-	-
Mucor plumbeus	+	+	+	+	+	+	+[b]	-	-
Pen. frequentans	+	+	+	+	+	+	-	-	-

[a] Growth in one of three trials.

[b] Growth in two of three trials.

TABLE XI

GROWTH OF COTTAGE CHEESE SPOILAGE ORGANISMS AFTER EXPOSURE TO DIFFERENT CONCENTRATIONS OF QUATERNARY SOLUTIONS FOR VARIOUS TIME INTERVALS (THREE TRIALS)

Organism	Parts per Million of Quaternary and Minutes of Exposure								
	10 ppm			25 ppm			50 ppm		
	1	5	10	1	5	10	1	5	10
Bacteria									
Ach. butyri	+	+	+	+	+	+	+	+	+
Ach. eurydice	+	+	+	+	+	+	+	+	+
Alc. metalcaligenes	+	+	+	+	+	+	+	+	+
B. firmus	+	+	+	+	+	+	+	+	+
Bact. erythrogenes	-	-	-	-	-	-	-	-	-
E. coli	+	+	+	+	+	+	+	+	-[a]
E. freundii	+	+	+	+	+	+	+	+	-
M. candidus	+	+	+	-	-	-	-	-	-
M. conglomeratus	-	-	-	-	-	-	-	-	-
M. flavus	+[b]	-	-	-	-	-	-	-	-
Ps. desmolyticum	+	+	+	+	+	+	+	+	+
Ps. fluorescens	+	+	+	+	+	+	+	+	+
Ps. fragi	+	+	+	+	+	+	+	+	+
Ps. tralucida	+	+	+	+	+	+	+	+	+
Yeasts									
R. flava	-[a]	-[a]	-	-	-	-	-	-	-
T. candida	+	+	+	+	+	+[a]	-	-	-
Molds									
G. candidum	+	+	+	+	+	+	+[b]	-	-
Mucor plumbeus	+[b]	+	+	+	+	+	+	+	+
Pen. frequentans	+	+	+	+	+	+	+[b]	-	-

[a]Growth in one of three trials.

[b]Growth in two of three trials.

Bact. erythrogenes, M. conglomeratus, and R. flava appeared to be the most susceptible organisms to quaternary, being destroyed in 1 minute in 10 parts per million of this compound. M. flavus survived 1 but not 5 minutes in 10 parts per million of quaternary.

M. candidus and M. flavus were destroyed in 1 minute in 25 parts per million of quaternary.

T. candida, G. candidum, and Pen. frequentans survived 1 but not 5 minutes in 50 parts per million of quaternary, while E. coli and E. freundii survived 5 but not 10 minutes in this concentration. Ach. butyri, Ach. eurydice, Alc. metalcaligenes, B. firmus, Ps. desmolyticum, Ps. fluorescens, Ps. fragi, Ps. tralucida, and Mucor plumbeus survived 10 minutes in 50 parts per million of quaternary.

Tolerance to iodophor. The growth of nineteen spoilage organisms after exposure to distilled water solutions containing 10, 25, and 50 parts per million of iodophor at pH 3.5 and 25° C. for 1, 5, and 10 minutes is shown in Table XII.

Among the organisms tested, Bact. erythrogenes was the most susceptible to the iodophor and was apparently destroyed in 1 minute in 10 parts per million of this compound. Also it was the only organism destroyed in 5 and 10 minutes in 10 parts per million of iodophor.

TABLE XII

GROWTH OF COTTAGE CHEESE SPOILAGE ORGANISMS AFTER EXPOSURE TO DIFFERENT CONCENTRATIONS OF IODOPHOR SOLUTIONS FOR VARIOUS TIME INTERVALS (THREE TRIALS)

Organism	Parts per Million of Iodophor and Minutes of Exposure								
	10 ppm			25 ppm			50 ppm		
	1	5	10	1	5	10	1	5	10
Bacteria									
Ach. butyri	+	+	+	+	+	+	-	-	-
Ach. eurydice	+	+	+	+	+	+	+	+[a]	+
Alc. metalcaligenes	+	+	+	+	+	+	-	-	-
B. firmus	+	+	+	+	+	+	+	+	+
Bact. erythrogenes	-	-	-	-	-	-	-	-	-
E. coli	+	+	+	+	+	+	-	-	-
E. freundii	+	+	+	+	+	+[a]	-	-	-
M. candidus	+	+	+	+	+	+	+	-	-
M. conglomeratus	+	+	+	+	+	+	-[b]	-	-
M. flavus	+	+	+	+	+	+	-	-	-
Ps. desmolyticum	+	+	+	+	+	+	-	-	-[b]
Ps. fluorescens	+	+	+	+	+	+	-	-[b]	-
Ps. fragi	+	+	+	+	+	+	-	-	-
Ps. tralucida	+	+	+	+	+	+	+	+[a]	+
Yeasts									
R. flava	+	+	+	-	-[b]	-	-	-	-
T. candida	+	+	+	+	+	+	-	-	-
Molds									
G. candidum	+	+	+	+	+	+	+	+	+
Mucor plumbeus	+	+	+	+	+	+	+	+	+
Pen. frequentans	+	+	+	+	+	+	+	+	+

[a]Growth in two of three trials.

[b]Growth in one of three trials.

R. flava was destroyed in 1 minute in 25 parts per million and was the only organism in addition to Bact. erythrogenes which was destroyed in 5 and 10 minutes in this concentration of iodophor.

Ach. butyri, Alc. metalcaligenes, E. coli, E. freundii, M. conglomeratus, M. flavus, Ps. desmolyticum, Ps. fluorescens, Ps. fragi, and T. candida were destroyed in 1 minute in 50 parts per million of iodophor, while M. candidus survived 1 but not 5 minutes in this concentration. Ach. eurydice, B. firmus, Ps. tralucida, G. candidum, Mucor plumbeus, and Pen. frequentans survived 10 minutes in 50 parts per million of iodophor.

Resistance to Sorbic Acid

The effect of various concentrations of sorbic acid at different pH levels on the viability of organisms causing spoilage in cottage cheese is shown in Table XIII. The data shown are the results of duplicate trials when the organisms were grown in trypticase soy broth.

Bact. erythrogenes failed to furvive at pH 5.7 with 0.25 percent sorbic acid, while R. flava showed variable results at this pH and sorbic acid concentration, with one of two trials showing survival. All other organisms survived at pH 5.7 and above with sorbic acid concentrations of 0.05, 0.10, and 0.25 percent.

TABLE XIII

GROWTH OF COTTAGE CHEESE SPOILAGE ORGANISMS AFTER EXPOSURE TO DIFFERENT CONCENTRATIONS OF SORBIC ACID AT VARIOUS pH LEVELS (TWO TRIALS)

Organism	pH of Medium and			
	pH 7.3	pH 6.7	pH 6.4	pH 5.7
	0.00	0.05	0.10	0.25
Bacteria				
Ach. butyri	+++	+++	+++	+++
Ach. eurydice	+++	+++	+++	+++
Alc. metalcaligenes	+++	+++	+++	+++
B. firmus	+++	+++	+++	+++
Bact. erythrogenes	+++	+++	+++	-
E. coli	+++	+++	+++	+++
E. freundii	+++	+++	+++	+++
M. candidus	+++	+++	+++	+++
M. conglomeratus	+++	+++	+++	+
M. flavus	+++	+++	+++	+++
Ps. desmolyticum	+++	+++	+++	+++
Ps. fluorescens	+++	+++	+++	+++
Ps. fragi	+++	+++	+++	+++
Ps. tralucida	+++	+++	+++	+++
Yeasts				
R. flava	+++	+++	+	*
T. candida	+++	+++	+++	+++
Molds				
G. candidum	+++	+++	+++	+++
Mucor plumbeus	+++	+++	+++	+++
Pen. frequentans	+++	+++	+++	+++

Legend: - = no growth, + = slight growth, ++ = moderate growth, +++ = profuse growth, * = one positive and one negative growth on duplicate trials.

TABLE XIII (Continued)

Percent Sorbic Acid w/v											
pH 5.2				pH 5.0				pH 4.8			
0.00	0.05	0.10	0.25	0.00	0.05	0.10	0.25	0.00	0.05	0.10	0.25
+++	+++	+++	++	+++	+	*	-	++	-	-	-
+++	+++	+++	*	+	*	-	-	+	-	-	-
+	+	*	*	*	*	*	-	*	-	-	-
++	++	+	+	++	++	+	+	+	++	++	+
*	-	-	-	-	-	-	-	-	-	-	-
+++	+++	+++	*	+++	++	*	-	+++	+	*	-
+++	+++	+++	+++	+++	+++	+	-	+++	+	*	-
+++	+++	+++	+++	+++	+++	+++	+	+++	+++	+++	+
+	+	++	+	+	+	*	-	+	*	-	-
++	++	++	+	++	+	+	+	+++	+	+	+
+++	+++	+++	+++	+++	+++	+	-	+++	++	*	-
*	-	-	-	-	-	-	-	-	-	-	-
++	+	+	+	+	*	*	-	+	-	-	-
+++	+++	+++	+++	+++	++	*	-	+++	+++	*	-
+	-	-	-	+	-	-	-	*	-	-	-
+++	+++	+++	+++	+++	+++	+++	*	+++	+++	+++	-
+++	+++	+++	+	+++	+++	+++	*	+++	+++	*	-
+++	+++	+++	+	+++	+	*	*	+++	*	*	-
+++	+++	+++	+	+++	++	+	*	+++	+++	+	-

Bact. erythrogenes and Ps. fluorescens showed variable results at pH 5.2 even in the absence of sorbic acid; both cultures showed survival in one of two trials. The above-mentioned organisms and R. flava failed to survive at pH 5.2 with 0.05 percent sorbic acid. Alc. metalcaligenes exhibited variable results at pH 5.2 with 0.10 percent sorbic acid, surviving in one of two trials. Ach. eurydice, Alc. metalcaligenes, and E. coli showed variable results at pH 5.2 with 0.25 percent sorbic acid, with one of two trials showing survival.

Bact. erythrogenes and Ps. fluorescens failed to survive at pH 5.0 even in the absence of sorbic acid, while Alc. metalcaligenes gave variable results at this pH in the absence of sorbic acid, with one of two trials showing survival. R. flava failed to survive at pH 5.0 with 0.05 percent sorbic acid. Ach. eurydice, Alc. metalcaligenes, and Ps. fragi exhibited variable results at pH 5.0 with 0.05 percent sorbic acid, with survival in one of two trials. Ach. eurydice failed to survive at pH of 5.0 with 0.10 percent sorbic acid, while Ach. butyri, Alc. metalcaligenes, E. coli, M. conglomeratus, Ps. fragi, Ps. tralucida, and Mucor plumbeus showed variable results at pH 5.0 with 0.10 percent sorbic acid, surviving in one of two trials. Ach. butyri, Alc. metalcaligenes, E. coli, E. freundii, M. candidus, Ps. desmolyticum, Ps. fragi, and Ps. tralucida failed

to survive at pH 5.0 with 0.25 percent sorbic acid; whereas, T. candida, G. candidum, Mucor plumbeus, and Pen. frequentans showed variable results at this pH and sorbic acid concentration, with survival in one of two trials.

At pH 4.8 with no sorbic acid added, Bact. erythrogenes and Ps. fluorescens failed to survive, while Alc. metalcaligenes and R. flava gave variable results, with survival in one of two trials. Ach. butyri, Ach. eurydice, Alc. metalcaligenes, Ps. fragi, and R. flava failed to survive at pH 4.8 with 0.05 percent sorbic acid, while M. conglomeratus and Mucor plumbeus exhibited variable results at this pH and sorbic acid concentration, with one of two trials showing survival. M. conglomeratus failed to survive at pH 4.8 with 0.10 percent sorbic acid, while E. coli, Ps. desmolyticum, Ps. tralucida, G. candidum, and Mucor plumbeus gave variable results at this pH and sorbic acid concentration. B. firmus, M. candidus, and M. flavus were the only organisms tested that survived at pH 4.8 with 0.25 percent sorbic acid.

The extent of growth was determined by measuring turbidity with a Klett-Summerson colorimeter, but the results of these determinations are not shown. Considerable difficulty was encountered in obtaining reliable readings, particularly at the lower pH levels. This might have been due to some precipitation of the protein

constituents in the medium caused by the addition of the sorbic and lactic acids, since colorimetric readings above and below zero were obtained in trials which exhibited viability and nonviability upon plating.

Control of Spoilage in Cottage Cheese

Treatment of Wash Water

The effect of treating cottage cheese wash water with various acids and germicides to control spoilage caused by Ach. butyri, Ach. eurydice, Alc. metalcaligenes, B. firmus, E. coli, E. freundii, M. candidus, M. conglomeratus, Ps. desmolyticum, Ps. fluorescens, Ps. fragi, Ps. tralucida, R. flava, T. candida, Pen. frequentans, and G. candidum is shown in Tables XIV through XXI. Some of the samples failed to develop the type of spoilage characteristic of the organism inoculated into the cheese, and the pH of the samples increased or decreased in some cases without regard as to the type of organism inoculated into the cheese. Probably this was due to the highly variable microflora or contamination which the cheese normally contains.

The sample of cheese inoculated with Ach. butyri (Table XIV) and washed with water containing acetic acid spoiled in 14

TABLE XIV

THE EFFECT OF WASH WATER TREATMENT ON CONTROLLING VARIOUS SPOILAGE ORGANISMS IN COTTAGE CHEESE STORED AT 50° F.

Treatment	Description	Initial pH	Initial Flavor Score	Days Until Spoilage	Type of Spoilage	Final pH
		Spoilage by Ach. butyri				
Controls						
Uninoculated		4.98	39.0	14	mold	4.62
Inoculated		5.10	39.0	6	tapioca	5.27
Acids						
Acetic	pH 5.0	5.00	39.0	14	pink slime	4.81
Citric	pH 5.0	5.13	39.0	6	tapioca	5.20
Lactic	pH 5.0	5.13	39.0	6	tapioca	5.20
Phosphoric	pH 5.0	5.15	39.0	6	tapioca	5.16
Sorbic	pH 5.0	5.05	38.0	6[a]	chemical[a]	5.10
Germicides						
Chlorine	10 ppm	5.17	39.0	6	tapioca	5.18
Iodophor	10 ppm	5.25	39.0	6	tapioca	5.28
Quaternary	10 ppm	5.13	39.0	6	tapioca	5.30
		Spoilage by Ach. eurydice				
Controls						
Uninoculated		5.02	39.5	12	wheyed off	4.30
Inoculated		4.95	39.5	6	tapioca	4.51
Acids						
Acetic	pH 5.0	5.01	39.5	12	wheyed off	4.30
Citric	pH 5.0	4.90	39.5	6	tapioca	4.61
Lactic	pH 5.0	5.05	39.5	6	tapioca	4.50
Phosphoric	pH 5.0	5.05	39.5	6	tapioca	4.49
Sorbic	0.05%	5.10	38.5	6	tapioca	4.72
Germicides						
Chlorine	10 ppm	5.00	39.5	6	tapioca	4.80
Iodophor	10 ppm	4.98	35.0[b]	6	tapioca	4.65
Quaternary	10 ppm	4.98	39.5	6	tapioca	4.50

[a]Discarded at six days with a chemical flavor, but appearance of the cheese was good.

[b]Halogen flavor.

days. However, spoilage was not characteristic of that normally produced by Ach. butyri. Within 6 days a tapioca slime characteristic of Ach. butyri formed on all the remaining samples except the one washed with water containing sufficient sorbic acid to lower the pH of the water to 5.0. This sample exhibited a definite chemical flavor and was discarded.

Wheying-off at a pH of 4.3 was apparent after 12 days in the uninoculated control and the sample of cheese inoculated with Ach. eurydice (Table XIV) and washed with water containing acetic acid. In 6 days the remainder of the samples were spoiled by a tapioca slime typical of Ach. eurydice. A halogen flavor was detectable in cheese washed with water containing 10 parts per million iodophor. It should be noted that the tapioca slime developed on cheese exhibiting the halogen flavor as well as cheese with pH ranging from 4.5 to 4.8.

An atypical spoilage developed in 6 days on the uninoculated control and on the sample inoculated with Alc. metalcaligenes (Table XV) and washed with water containing acetic acid. The sample washed with water containing sorbic acid and all remaining samples developed a tapioca slime characteristic of Alc. metalcaligenes after 6 and 4 days, respectively.

TABLE XV

THE EFFECT OF WASH WATER TREATMENT ON CONTROLLING VARIOUS SPOILAGE ORGANISMS IN COTTAGE CHEESE STORED AT 50° F.

Treatment	Description	Initial pH	Initial Flavor Score	Days Until Spoilage	Type of Spoilage	Final pH
		Spoilage by Alc. metalcaligenes				
Controls						
Uninoculated		4.75	39.0	6	pink slime	4.80
Inoculated		4.88	39.0	4	tapioca	5.00
Acids						
Acetic	pH 5.0	4.85	39.0	6	pink slime	4.90
Citric	pH 5.0	4.83	39.0	4	tapioca	4.91
Lactic	pH 5.0	4.83	39.0	4	tapioca	5.00
Phosphoric	pH 5.0	4.82	39.0	4	tapioca	5.03
Sorbic	0.05%	4.85	39.0	6	tapioca	4.95
Germicides						
Chlorine	10 ppm	4.90	39.0	4	tapioca	5.05
Iodophor	7.5 ppm	4.87	39.0	4	tapioca	5.06
Quaternary	10 ppm	4.88	39.0	4	tapioca	5.08
		Spoilage by B. firmus				
Controls						
Uninoculated		5.00	40.0	10	brown slime	4.98
Inoculated		4.97	40.0	10	brown slime	4.85
Acids						
Acetic	pH 5.0	4.90	40.0	16	wheyed off	4.30
Citric	pH 5.0	5.15	40.0	16	wheyed off	4.50
Lactic	pH 5.0	5.07	40.0	16	brown slime	4.65
Phosphoric	pH 5.0	5.10	40.0	16	brown slime	4.40
Sorbic	0.05%	5.17	40.0	10	brown slime	4.95
Germicides						
Chlorine	10 ppm	5.00	40.0	10	brown slime	4.55
Iodophor	10 ppm	5.17	38.0[a]	10	brown slime	4.80
Quaternary	10 ppm	5.17	40.0	10	brown slime	4.80

[a]Slight halogen flavor.

Wheying-off occurred after 16 days in the samples inoculated with B. firmus (Table XV) and washed with waters containing acetic and citric acids. A brown slime developed on the cheese washed with waters containing lactic and phosphoric acids after 16 days. The remainder of the samples, both uninoculated and inoculated, showed the brown slime in 10 days. Although B. firmus causes a brown slime, this organism might not have been the cause of spoilage in these samples, since the uninoculated control developed the brown slime also.

After 12 days a brown slime characteristic of E. coli appeared on the uninoculated control and on samples inoculated with this organism (Table XVI) and washed with waters containing phosphoric and sorbic acids. The inoculated control and the samples washed with waters containing citric and lactic acids and chlorine and quaternary developed this brown slime in 10 days. The samples washed with waters containing acetic acid and iodophor spoiled in 10 days, but spoilage characteristics were not typical of E. coli.

An atypical fruity flavor developed in the samples inoculated with E. freundii (Table XVI) and washed with waters containing chlorine and sorbic acid in 12 and 14 days, respectively. After 12 days the samples washed with waters containing acetic and phosphoric acids and the iodophor developed defects not characteristic of

TABLE XVI

THE EFFECT OF WASH WATER TREATMENT ON CONTROLLING VARIOUS SPOILAGE ORGANISMS IN COTTAGE CHEESE STORED AT 50° F.

Treatment	De-scrip-tion	Ini-tial pH	Initial Flavor Score	Days Until Spoil-age	Type of Spoilage	Fi-nal pH
		Spoilage by E. coli				
Controls						
Uninoculated		4.82	38.5	12	brown slime	4.96
Inoculated		4.90	38.5	10	brown slime	4.87
Acids						
Acetic	pH 5.0	4.80	38.5	10	mold	4.82
Citric	pH 5.0	4.79	38.5	10	brown slime	4.80
Lactic	pH 5.0	4.76	38.5	10	brown slime	4.72
Phosphoric	pH 5.0	4.81	38.5	12	brown slime	4.85
Sorbic	0.05%	4.77	38.5	12	brown slime	4.85
Germicides						
Chlorine	10 ppm	4.81	38.5	10	brown slime	4.88
Iodophor	7.5 ppm	4.82	38.5	10	pink slime	4.90
Quaternary	10 ppm	4.62	38.5	10	brown slime	4.85
		Spoilage by E. freundii				
Controls						
Uninoculated		4.95	39.0	10	brown slime	4.80
Inoculated		4.90	39.0	10	brown slime	4.78
Acids						
Acetic	pH 5.0	4.90	39.0	12	mold	4.75
Citric	pH 5.0	5.00	39.0	12	brown slime	4.90
Lactic	pH 5.0	5.05	39.0	12	pink slime	4.85
Phosphoric	pH 5.0	4.82	39.0	12	mold	4.85
Sorbic	0.05%	4.91	39.0	14	fruity	4.85
Germicides						
Chlorine	10 ppm	4.93	39.0	12	fruity	4.80
Iodophor	7.5 ppm	4.93	39.0	12	mold	4.80
Quaternary	10 ppm	4.89	39.0	10	brown slime	4.83

E. freundii. The sample washed with water containing citric acid was spoiled with a brown slime in 12 days, while the two controls and the sample washed with water containing quaternary developed brown slime characteristic of E. freundii in 10 days.

The sample inoculated with M. candidus (Table XVII) and the uninoculated control showed free whey after 6 days. No other defect was apparent. Chemical and halogen flavors were noted in the samples washed with waters containing 0.05 percent sorbic acid and 10 parts per million of iodophor, respectively.

Spoilage occurred in all the samples inoculated with M. conglomeratus (Table XVII) and the uninoculated control after 8 to 14 days. This spoilage was due to organisms other than M. conglomeratus, since this organism causes no visible defect other than free whey resulting from acid production. Sorbic acid seemed the most effective compound against the dominant spoilage organism, as 14 days were required for spoilage to occur in the sample washed with water containing this compound.

Among the samples inoculated with Ps. desmolyticum (Table XVIII), the control and the sample washed with water containing lactic acid were fruity in 6 days, while the sample washed with water containing citric acid was bitter in 12 days. After 24 days the sample washed with water containing sufficient sorbic acid to

TABLE XVII

THE EFFECT OF WASH WATER TREATMENT ON CONTROLLING VARIOUS SPOILAGE ORGANISMS IN COTTAGE CHEESE STORED AT 50° F.

Treatment	Description	Initial pH	Initial Flavor Score	Days Until Spoilage	Type of Spoilage	Final pH
		Spoilage by M. candidus				
Controls						
Uninoculated		5.20	39.0	6	wheyed off	4.70
Inoculated		5.10	39.0	6	wheyed off	4.70
Acids						
Acetic	pH 5.0	5.05	39.0	6	wheyed off	4.70
Citric	pH 5.0	5.12	39.0	6	wheyed off	4.85
Lactic	pH 5.0	5.12	39.0	6	wheyed off	4.70
Phosphoric	pH 5.0	5.09	39.0	6	wheyed off	4.80
Sorbic	0.05%	5.10	37.0[a]	6	wheyed off	4.70
Germicides						
Chlorine	10 ppm	5.10	39.0	6	wheyed off	4.70
Iodophor	10 ppm	5.10	38.0[b]	6	wheyed off	4.80
Quaternary	10 ppm	5.12	39.0	6	wheyed off	4.80
		Spoilage by M. conglomeratus				
Controls						
Uninoculated		4.85	39.0	8	pink slime	4.80
Inoculated		4.85	39.0	10	pink slime	4.90
Acids						
Acetic	pH 5.0	4.83	39.0	10	mold	4.88
Citric	pH 5.0	4.85	39.0	10	pink slime	4.80
Lactic	pH 5.0	4.81	39.0	10	pink slime	4.71
Phosphoric	pH 5.0	4.82	39.0	10	pink slime	4.85
Sorbic	0.05%	4.82	39.0	14	pink slime	4.87
Germicides						
Chlorine	10 ppm	4.88	39.0	10	pink slime	4.83
Iodophor	7.5 ppm	4.85	39.0	10	pink slime	4.89
Quaternary	10 ppm	4.88	39.0	10	pink slime	4.94

[a] Chemical flavor

[b] Slight halogen flavor.

TABLE XVIII

THE EFFECT OF WASH WATER TREATMENT ON CONTROLLING VARIOUS SPOILAGE ORGANISMS IN COTTAGE CHEESE STORED AT 50° F.

Treatment	De-scrip-tion	Ini-tial pH	Initial Flavor Score	Days Until Spoil-age	Type of Spoilage	Fi-nal pH
		Spoilage by Ps. desmolyticum				
Controls						
Uninoculated		4.70	40.0	20	wheyed off	4.40
Inoculated		4.88	40.0	6	fruity	5.00
Acids						
Acetic	pH 5.0	4.80	40.0	20	wheyed off	4.50
Citric	pH 5.0	4.75	40.0	12	bitter	4.60
Lactic	pH 5.0	4.85	40.0	6	fruity	5.00
Phosphoric	pH 5.0	4.85	40.0	20	wheyed off	4.75
Sorbic	pH 6.0	4.85	39.0[a]	24[b]	(b)	4.50
Germicides						
Chlorine	10 ppm	4.93	40.0	20	wheyed off	4.81
Iodophor	10 ppm	4.92	40.0	20	wheyed off	4.50
Quaternary	10 ppm	4.92	40.0	20	wheyed off	4.55
		Spoilage by Ps. fluorescens				
Controls						
Uninoculated		4.95	38.5	14	bitter	4.60
Inoculated		5.15	39.5	8	green slime	5.02
Acids						
Acetic	pH 5.0	5.00	40.0	8	green slime	5.05
Citric	pH 5.0	5.15	40.0	14	bitter	4.40
Lactic	pH 5.0	5.12	40.0	6	green slime	4.40
Phosphoric	pH 5.0	5.15	39.5	8	green slime	4.90
Sorbic	pH 5.0	5.10	36.0[c]	14	chemical[c]	4.90
Germicides						
Chlorine	10 ppm	5.17	40.0	6	green slime	4.65
Iodophor	10 ppm	5.20	38.5[d]	6	green slime	4.41
Quaternary	10 ppm	5.21	40.0	6	green slime	4.35

[a] Slight chemical flavor.

[b] Still marketable after twenty-four days.

[c] Discarded due to chemical flavor.

[d] Halogen flavor detected in fresh cheese only, disappeared after two days.

lower the pH of the water to 6.0 was still marketable. The remainder of the samples including the uninoculated control were sour and wheyed-off after 20 days.

The development of a green slime was apparent in 6 days on the samples inoculated with Ps. fluorescens (Table XVIII) and washed with waters containing lactic acid, and chlorine, iodophor, and quaternary compounds. This green slime appeared in 8 days on the inoculated control and the samples washed with waters containing acetic and phosphoric acids. After 14 days the uninoculated control and the sample washed with water containing citric acid were bitter. The sample washed with water containing sufficient sorbic acid to lower the pH of the water to 5.0 was belatedly discarded after 14 days because of a definite chemical flavor. A halogen flavor was noted in the sample washed with water containing 10 parts per million of iodophor when the cheese was fresh, but the flavor disappeared after 2 days of storage.

Among the samples inoculated with Ps. fragi (Table XIX), the control and the samples washed with waters containing acetic, citric, lactic, and phosphoric acids, iodophor, and quaternary developed a bitter flavor not characteristic of Ps. fragi in 6 days. Within 8 days the samples washed with waters containing sorbic acid and chlorine and the uninoculated control also developed this bitter flavor.

TABLE XIX

THE EFFECT OF WASH WATER TREATMENT ON CONTROLLING VARIOUS SPOILAGE ORGANISMS IN COTTAGE CHEESE STORED AT 50° F.

Treatment	De-scrip-tion	Ini tial pH	Initial Flavor Score	Days Until Spoil-age	Type of Spoilage	Fi-nal pH
		Spoilage by Ps. fragi				
Controls						
Uninoculated		4.98	39.0	8	bitter	4.80
Inoculated		4.99	39.0	6	bitter	4.75
Acids						
Acetic	pH 5.0	4.93	39.0	6	bitter	4.82
Citric	pH 5.0	5.02	39.0	6	bitter	4.70
Lactic	pH 5.0	5.05	39.0	6	bitter	4.75
Phosphoric	pH 5.0	5.00	39.0	6	bitter	4.73
Sorbic	0.05%	5.03	39.0	8	bitter	4.70
Germicides						
Chlorine	10 ppm	5.04	39.0	8	bitter	4.70
Iodophor	7.5 ppm	5.00	39.0	6	bitter	4.75
Quaternary	10 ppm	5.03	39.0	6	bitter	4.68
		Spoilage by Ps. tralucida				
Controls						
Uninoculated		5.00	40.0	16	bitter	4.45
Inoculated		4.90	40.0	16	bitter	4.55
Acids						
Acetic	pH 5.0	4.90	40.0	16	bitter	4.38
Citric	pH 5.0	4.92	40.0	16	bitter	4.30
Lactic	pH 5.0	4.90	40.0	16	bitter	4.40
Phosphoric	pH 5.0	4.90	40.0	16	bitter	4.40
Sorbic	0.05%	4.95	40.0	16	bitter	4.50
Germicides						
Chlorine	10 ppm	4.90	40.0	16	bitter	4.45
Iodophor	10 ppm	4.80	40.0	16	bitter	4.43
Quaternary	10 ppm	4.95	40.0	16	bitter	4.60

After 16 days the complete set of samples inoculated with Ps. tralucida (Table XIX) and the uninoculated control were discarded with a bitter flavor not characteristic of Ps. tralucida.

Table XX shows that the samples inoculated with R. flava and washed with waters containing acetic acid, chlorine, iodophor, and quaternary as well as the control, developed pink slime characteristic of R. flava in 4 days, while the samples washed with waters containing citric, lactic, and phosphoric acids showed pink slime in 6 days. After 18 days the sample washed with water containing sorbic acid was discarded because of staleness, but had not developed the pink slime defect.

When inoculated into the cheese, T. candida (Table XX) caused the control and the sample washed with water containing iodophor to develop a yeasty flavor in six days. Within six days the samples washed with waters containing acetic, lactic, and phosphoric acids, and quaternary developed a yellow slime characteristic of T. candida, while the samples washed with waters containing citric and sorbic acids and chlorine required 8 days to develop this slime.

The sample inoculated with Pen. frequentans (Table XXI) and washed with water containing sorbic acid developed typical blue-green

TABLE XX

THE EFFECT OF WASH WATER TREATMENT ON CONTROLLING VARIOUS SPOILAGE ORGANISMS IN COTTAGE CHEESE STORED AT 50° F.

Treatment	Description	Initial pH	Initial Flavor Score	Days Until Spoilage	Type of Spoilage	Final pH
		Spoilage by R. flava				
Controls						
Uninoculated		4.90	40.0	6	mold	4.80
Inoculated		4.94	40.0	4	pink slime	4.90
Acids						
Acetic	pH 5.0	4.87	40.0	4	pink slime	4.85
Citric	pH 5.0	4.81	40.0	6	pink slime	4.90
Lactic	pH 5.0	4.89	40.0	6	pink slime	4.80
Phosphoric	pH 5.0	4.90	40.0	6	pink slime	4.85
Sorbic	0.05%	4.90	40.0	18	stale	4.55
Germicides						
Chlorine	10 ppm	4.92	40.0	4	pink slime	4.92
Iodophor	7.5 ppm	4.93	40.0	4	pink slime	4.92
Quaternary	10 ppm	4.93	40.0	4	pink slime	4.92
		Spoilage by T. candida				
Controls						
Uninoculated		4.95	39.0	8	pink slime	4.82
Inoculated		4.97	39.0	6	yeasty	4.70
Acids						
Acetic	pH 5.0	4.98	39.0	6	yellow slime	4.85
Citric	pH 5.0	4.95	39.0	8	yellow slime	4.72
Lactic	pH 5.0	4.95	39.0	6	yellow slime	4.65
Phosphoric	pH 5.0	5.00	39.0	6	yellow slime	4.85
Sorbic	0.05%	5.00	39.0	8	yellow slime	4.85
Germicides						
Chlorine	10 ppm	4.95	39.0	8	yellow slime	4.81
Iodophor	7.5 ppm	4.95	38.0[a]	6	yeasty	4.70
Quaternary	10 ppm	4.90	39.0	6	yellow slime	4.83

[a]Slight halogen flavor.

TABLE XXI

THE EFFECT OF WASH WATER TREATMENT ON CONTROLLING VARIOUS SPOILAGE ORGANISMS IN COTTAGE CHEESE STORED AT 50° F.

Treatment	Description	Initial pH	Initial Flavor Score	Days Until Spoilage	Type of Spoilage	Final pH
		Spoilage by Pen. frequentans				
Controls						
Uninoculated		4.92	39.0	10	mold	5.00
Inoculated		4.90	39.0	10	mold	4.83
Acids						
Acetic	pH 5.0	4.95	39.0	10	mold	5.00
Citric	pH 5.0	4.98	39.0	10	mold	5.00
Lactic	pH 5.0	5.00	39.0	10	mold	4.95
Phosphoric	pH 5.0	5.01	39.0	10	mold	5.15
Sorbic	0.05%	4.97	39.0	14	mold	4.92
Germicides						
Chlorine	10 ppm	4.95	39.0	10	mold	5.08
Iodophor	7.5 ppm	4.90	39.0	10	mold	5.02
Quaternary	10 ppm	4.93	39.0	10	mold	4.98
		Spoilage by G. candidum				
Controls						
Uninoculated		4.92	39.0	8	yellow slime	4.92
Inoculated		4.90	38.0	6	mold	4.98
Acids						
Acetic	pH 5.0	4.90	38.0	6	mold	5.05
Citric	pH 5.0	4.86	38.5	6	mold	4.99
Lactic	pH 5.0	4.89	39.0	6	mold	5.05
Phosphoric	pH 5.0	4.90	39.0	6	mold	4.98
Sorbic	0.05%	4.87	39.0	8	mold	4.96
Germicides						
Chlorine	10 ppm	4.93	39.0	6	mold	5.04
Iodophor	7.5 ppm	4.91	39.0	6	mold	4.84
Quaternary	10 ppm	4.90	39.0	6	mold	5.04

mold in 14 days, while the remainder of the samples developed this defect in 10 days.

After 8 days mold growth characteristic of G. candidum developed on the sample inoculated with this organism (Table XXI) and washed with water containing sorbic acid, while the remaining samples developed this mold in 6 days.

Table XXII shows the number of days of increase in shelf-life produced by various wash water treatments when cottage cheese was inoculated with different spoilage organisms.

The shelf-life was increased in six of sixteen trials when acetic and citric acids were added to the wash water. Lactic and phosphoric acids increased the shelf-life in three and five of sixteen trials, respectively, while sorbic acid increased the shelf-life in eleven of sixteen trials. The addition of quaternary, iodophor, and chlorine to the wash water increased the shelf-life in one, two, and four of sixteen instances, respectively.

When spoilage was produced by Ps. fluorescens, the development of green slime was hastened approximately 2 days by the addition of lactic acid, chlorine, iodophor, and quaternary to the wash water.

TABLE XXII

EFFECT OF WASH WATER TREATMENT ON THE SHELF-LIFE OF COTTAGE CHEESE INOCULATED WITH VARIOUS SPOILAGE ORGANISMS

Organism	Treatment of Wash Water and Number of Days Added to Shelf-Life when Compared to an Inoculated Control							
	Acetic Acid	Citric Acid	Lactic Acid	Phosphoric Acid	Sorbic Acid	Chlorine	Iodophor	Quaternary
Ach. butyri	8	0	0	0	0	0	0	0
Ach. eurydice	6	0	0	0	0	0	0	0
Alc. metalcaligenes	2	0	0	0	2	0	0	0
B. firmus	6	6	6	6	0	0	0	0
E. coli	0	0	0	2	2	0	0	0
E. freundii	2	2	2	2	4	2	2	0
M. candidus	0	0	0	0	0	0	0	0
M. conglomeratus	0	0	0	0	4	0	0	0
Ps. desmolyticum	14	6	0	14	18	14	14	14
Ps. fluorescens	0	6	-2	0	6	-2	-2	-2
Ps. fragi	0	0	0	0	2	2	0	0
Ps. tralucida	0	0	0	0	0	0	0	0
R. flava	0	2	2	2	14	0	0	0
T. candida	0	2	0	0	2	2	0	0
Pen. frequentans	0	0	0	0	4	0	0	0
G. candidum	0	0	0	0	2	0	0	0

Treatment of Dressing Materials

The effect of treating cottage cheese dressing material with various acids and starter in controlling spoilage organisms in cottage cheese is shown in Tables XXIII through XXXI.

When samples were inoculated with Ach. butyri (Table XXIII), a typical tapioca slime developed in 4 days on the control and the samples containing lactic acid and starter, while the samples with citric and sorbic acids developed this tapioca slime in 6 days.

Among the samples inoculated with Ach. eurydice (Table XXIII), the control and the sample containing citric acid, as well as the uninoculated control, developed a characteristic tapioca slime in 6 days. Spoilage not representative of Ach. eurydice occurred in the samples containing lactic acid and starter in 6 and 8 days, respectively. After 12 days free whey was evident in the sample containing sorbic acid.

The control sample inoculated with Alc. metalcaligenes (Table XXIV) developed characteristic tapioca slime in 6 days, whereas the defect was delayed until 8 days in samples containing citric and lactic acids. The sample with added starter developed a low pH and tapioca slime in 8 days. The tapioca slime characteristic of Alc. metalcaligenes developed after 10 days in the sample which contained sorbic acid.

TABLE XXIII

THE EFFECT OF DRESSING TREATMENT IN CONTROLLING VARIOUS SPOILAGE ORGANISMS IN COTTAGE CHEESE STORED AT 50° F.

Treatment	Description	Initial pH	Initial Flavor Score	Days Until Spoilage	Type of Spoilage	Final pH
			Spoilage by Ach. butyri			
Controls						
Uninoculated		4.82	39.0	8	mold	4.65
Inoculated		4.90	39.0	4	tapioca	5.03
Acids						
Citric	0.15%	4.85	39.0	6	tapioca	5.00
Lactic	0.15%	4.81	39.0	4	tapioca	5.08
Sorbic	0.10%	4.86	39.0	6	tapioca	5.25
Starter	2.0%	4.86	39.0	4	tapioca	5.15
			Spoilage by Ach. eurydice			
Controls						
Uninoculated		4.67	38.0[a]	6	tapioca	4.82
Inoculated		4.59	38.0[a]	6	tapioca	4.75
Acids						
Citric	0.15%	4.64	38.0[a]	6	tapioca	4.85
Lactic	0.15%	4.60	38.0[a]	6	pink slime	4.78
Sorbic	0.10%	4.60	38.0[a]	12	wheyed off	4.75
Starter	2.0%	4.59	38.0[a]	8	pink slime	4.62

[a]Sour.

TABLE XXIV

THE EFFECT OF DRESSING TREATMENT IN CONTROLLING VARIOUS SPOILAGE ORGANISMS IN COTTAGE CHEESE STORED AT 50° F.

Treatment	Description	Initial pH	Initial Flavor Score	Days Until Spoilage	Type of Spoilage	Final pH
		Spoilage by Alc. metalcaligenes				
Controls						
Uninoculated		5.20	40.0	8	pink slime	5.02
Inoculated		5.20	40.0	6	tapioca	5.15
Acids						
Citric	0.15%	5.21	40.0	8	tapioca	5.05
Lactic	0.15%	5.15	40.0	8	tapioca	5.24
Sorbic	0.10%	5.18	40.0	10	tapioca	5.08
Starter	2.0%	5.20	40.0	8	tapioca[a]	4.53
		Spoilage by B. firmus				
Controls						
Uninoculated		4.62	39.5	8	mold	4.62
Inoculated		4.63	39.5	6	mold	4.69
Acids						
Citric	0.15%	4.58	39.5	6	mold	4.72
Lactic	0.15%	4.57	39.0	8	mold	4.64
Sorbic	0.10%	4.61	39.5	10	mold	4.70
Starter	2.0%	4.61	39.5	6	mold	4.70

[a]Pink slime.

After 6 days spoilage appeared in the control sample inoculated with B. firmus (Table XXIV) as well as those inoculated samples containing citric acid and starter, but spoilage was not typical of B. firmus. The same spoilage occurred in the sample containing lactic acid, the sample containing sorbic acid, and the uninoculated control in 8, 10, and 8 days, respectively.

After 6 days characteristic brown slime occurred in the control sample inoculated with E. coli (Table XXV), while the samples containing citric and lactic acids developed this defect in 8 days. The sample containing starter developed a fruity flavor in 8 days, and the sample containing sorbic acid developed a bitter flavor after 10 days. The two latter defects are not characteristic of those produced by E. coli.

Among the samples inoculated with E. freundii (Table XXV), atypical spoilage occurred after 6 days in the uninoculated control, the control, and the sample containing starter. The brown slime defect typical of E. freundii developed on the inoculated control and the samples containing citric and lactic acids in 6 days, while the sample containing sorbic acid required 8 days to develop this defect.

M. candidus produces no visible defect other than free whey resulting from acid development, but after 6 days a pink slime

TABLE XXV

THE EFFECT OF DRESSING TREATMENT IN CONTROLLING VARIOUS SPOILAGE ORGANISMS IN COTTAGE CHEESE STORED AT 50° F.

Treatment	Description	Initial pH	Initial Flavor Score	Days Until Spoilage	Type of Spoilage	Final pH
		Spoilage by E. coli				
Controls						
Uninoculated		4.84	39.0	8	mold	4.78
Inoculated		4.86	39.0	6	brown slime	5.20
Acids						
Citric	0.15%	4.86	39.0	8	brown slime	5.05
Lactic	0.15%	4.90	39.0	8	brown slime	5.05
Sorbic	0.10%	4.90	39.0	10	bitter	4.88
Starter	2.0%	4.82	39.0	8	fruity	4.97
		Spoilage by E. freundii				
Controls						
Uninoculated		5.12	40.0	6	mold	5.18
Inoculated		5.12	40.0	6	brown slime[a]	5.00
Acids						
Citric	0.15%	5.08	40.0	6	brown slime	4.78
Lactic	0.15%	5.10	40.0	6	brown slime	4.72
Sorbic	0.10%	5.12	40.0	8	brown slime	4.93
Starter	2.0%	5.10	40.0	6	mold	4.45

[a] Mold.

developed in most of the samples in the series inoculated with M. candidus (Table XXVI). However, no spoilage occurred in the sample containing sorbic acid.

The samples inoculated with M. conglomeratus (Table XXVI) spoiled in 6 to 10 days with different types of atypical defects. M. conglomeratus produces no visible defect other than free whey resulting from acid development.

All of the samples inoculated with Ps. desmolyticum (Table XXVII) developed mold in 8 days, except the sample containing sorbic acid, which did not show mold until the fourteenth day.

The sample containing lactic acid and inoculated with Ps. fluorescens (Table XXVII) developed a green slime in 6 days. The remainder of the samples developed atypical types of spoilage in 10 days.

All samples inoculated with Ps. fragi (Table XXVIII), as well as the uninoculated control, developed mold in 10 days.

Mold growth occurred in 6 to 8 days in all samples inoculated with Ps. tralucida (Table XXVIII), except the sample containing sorbic acid in which mold was deferred until the sixteenth day.

The characteristic pink slime appeared after 6 to 8 days on all samples inoculated with R. flava (Table XXIX), except the sample

TABLE XXVI

THE EFFECT OF DRESSING TREATMENT IN CONTROLLING VARIOUS SPOILAGE ORGANISMS IN COTTAGE CHEESE STORED AT 50° F.

Treatment	Description	Initial pH	Initial Flavor Score	Days Until Spoilage	Type of Spoilage	Final pH
		Spoilage by M. candidus				
Controls						
Uninoculated		4.60	38.0	6	pink slime	4.72
Inoculated		4.62	38.0	6	pink slime	4.59
Acids						
Citric	0.15%	4.57	38.0	6	pink slime	4.53
Lactic	0.15%	4.58	38.0	6	pink slime	4.53
Sorbic	0.10%	4.62	39.0	12	wheyed off	4.65
Starter	2.0%	4.63	38.0	6	pink slime	4.46
		Spoilage by M. conglomeratus				
Controls						
Uninoculated		4.85	39.0	10	bitter	4.58
Inoculated		4.85	39.0	8	mold	4.75
Acids						
Citric	0.15%	4.84	39.0	10	bitter	4.77
Lactic	0.15%	4.87	39.0	6	bitter	4.95
Sorbic	0.10%	4.83	39.0	10	bitter	4.84
Starter	2.0%	4.88	39.0	10	mold	4.52

TABLE XXVII

THE EFFECT OF DRESSING TREATMENT IN CONTROLLING VARIOUS SPOILAGE ORGANISMS IN COTTAGE CHEESE STORED AT 50° F.

Treatment	Description	Initial pH	Initial Flavor Score	Days Until Spoilage	Type of Spoilage	Final pH
Spoilage by Ps. desmolyticum						
Controls						
Uninoculated		4.65	39.5	8	mold	4.67
Inoculated		4.66	39.5	8	mold	4.71
Acids						
Citric	0.15%	4.60	39.5	8	mold	4.64
Lactic	0.15%	4.62	39.5	8	mold	4.66
Sorbic	0.10%	4.64	39.5	14	mold	4.72
Starter	2.0%	4.68	39.5	8	mold	4.45
Spoilage by Ps. fluorescens						
Controls						
Uninoculated		4.75	37.0[a]	10	mold[b]	4.75
Inoculated		4.72	37.0[a]	10	mold[b]	4.78
Acids						
Citric	0.15%	4.75	37.0[a]	10	mold[b]	4.69
Lactic	0.15%	4.74	37.0[a]	6	green slime	4.61
Sorbic	0.10%	4.71	37.0[a]	10	mold[b]	4.66
Starter	2.0%	4.75	37.0[a]	10	stale	4.68

[a] Sour.

[b] Pink slime.

TABLE XXVIII

THE EFFECT OF DRESSING TREATMENT IN CONTROLLING VARIOUS SPOILAGE ORGANISMS IN COTTAGE CHEESE STORED AT 50° F.

Treatment	Description	Initial pH	Initial Flavor Score	Days Until Spoilage	Type of Spoilage	Final pH
		Spoilage by Ps. fragi				
Controls						
Uninoculated		4.75	37.0[a]	10	mold	4.68
Inoculated		4.73	37.0[a]	10	mold	4.78
Acids						
Citric	0.15%	4.71	37.0[a]	10	mold	4.65
Lactic	0.15%	4.75	37.0[a]	10	mold	4.65
Sorbic	0.10%	4.75	37.0[a]	10	mold	4.73
Starter	2.0%	4.75	37.0[a]	10	mold	4.65
		Spoilage by Ps. tralucida				
Controls						
Uninoculated		4.62	39.5	6	mold[b]	4.72
Inoculated		4.65	39.5	6	mold	4.95
Acids						
Citric	0.15%	4.58	39.5	6	mold	4.78
Lactic	0.15%	4.62	39.5	6	mold	4.86
Sorbic	0.10%	4.58	39.5	16	wheyed off	4.62
Starter	2.0%	4.60	39.5	8	mold	4.47

[a] Sour.

[b] Pink slime.

TABLE XXIX

THE EFFECT OF DRESSING TREATMENT IN CONTROLLING VARIOUS SPOILAGE ORGANISMS IN COTTAGE CHEESE STORED AT 50° F.

Treatment	Description	Initial pH	Initial Flavor Score	Days Until Spoilage	Type of Spoilage	Final pH
		Spoilage by R. flava				
Controls						
Uninoculated		4.90	39.0	10	mold	4.75
Inoculated		4.90	39.0	6	pink slime	4.81
Acids						
Citric	0.15%	4.88	39.0	6	pink slim	4.80
Lactic	0.15%	4.89	39.0	6	pink slime	4.83
Sorbic	0.10%	4.90	39.0	16	wheyed off	4.71
Starter	2.0%	4.89	39.0	8	pink slime	4.70
		Spoilage by T. candida				
Controls						
Uninoculated		5.15	40.0	6	mold	5.12
Inoculated		5.12	40.0	4	yellow slime	5.32
Acids						
Citric	0.15%	5.10	40.0	4	yellow slime	4.98
Lactic	0.15%	5.12	40.0	4	yellow slime	5.02
Sorbic	0.10%	5.15	40.0	6	yellow slime	5.12
Starter	2.0%	5.12	40.0	6	yellow slime	4.51

containing sorbic acid. On the sixteenth day the latter sample showed free whey but no pink slime.

All samples inoculated with T. candida (Table XXIX) developed yellow slime after 4 to 6 days. The samples containing sorbic acid or starter required longer for development of this defect.

Mucor plumbeus grew in 6 days in all the samples inoculated with this organism (Table XXX), except the sample containing sorbic acid which showed growth of Mucor after 14 days.

All samples inoculated with Pen. frequentans (Table XXX) showed mold in 8 to 10 days, with the exception of the sample containing sorbic acid, which delayed mold development until the fourteenth day.

Among the samples inoculated with G. candidum (Table XXI), the control and the samples with citric and lactic acids and starter added developed this mold in 8 days, while the sample with sorbic acid showed mold growth in 12 days.

Table XXXII shows the number of days increase in shelf-life produced by various dressing treatments when cottage cheese was inoculated with spoilage organisms.

The addition of lactic and citric acids to cottage cheese dressing increased the shelf-life in four and five of seventeen trials,

TABLE XXX

THE EFFECT OF DRESSING TREATMENT IN CONTROLLING VARIOUS SPOILAGE ORGANISMS IN COTTAGE CHEESE STORED AT 50° F.

Treatment	De-scrip-tion	Ini-tial pH	Initial Flavor Score	Days Until Spoil-age	Type of Spoilage	Fi-nal pH
		Spoilage by Mucor plumbeus				
Controls						
Uninoculated		4.88	39.0	12	mold	4.71
Inoculated		4.89	39.0	6	mold	4.72
Acids						
Citric	0.15%	4.87	39.0	6	mold	4.78
Lactic	0.15%	4.88	39.0	6	mold	4.80
Sorbic	0.10%	4.87	39.0	14	mold	4.65
Starter	2.0%	4.89	39.0	6	mold	4.80
		Spoilage by Pen. frequentans				
Controls						
Uninoculated		5.10	40.0	12	brown slime	5.15
Inoculated		5.12	40.0	8	mold	5.02
Acids						
Citric	0.15%	5.07	40.0	10	mold	5.00
Lactic	0.15%	5.10	40.0	10	mold	5.05
Sorbic	0.10%	5.11	40.0	14	mold	5.01
Starter	2.0%	5.10	40.0	10	mold	5.08

TABLE XXXI

THE EFFECT OF DRESSING TREATMENT IN CONTROLLING VARIOUS SPOILAGE ORGANISMS IN COTTAGE CHEESE STORED AT 50° F.

Treatment	Description	Initial pH	Initial Flavor Score	Days Until Spoilage	Type of Spoilage	Final pH
		Spoilage by G. candidum				
Controls						
Uninoculated		5.12	40.0	14	brown slime	5.10
Inoculated		5.13	40.0	8	mold	4.98
Acids						
Citric	0.15%	5.10	40.0	8	mold	5.02
Lactic	0.15%	5.11	40.0	8	mold	4.95
Sorbic	0.10%	5.10	40.0	12	mold	4.98
Starter	2.0%	5.12	40.0	8	mold	4.97

TABLE XXXII

EFFECT OF DRESSING TREATMENT ON THE SHELF-LIFE OF COTTAGE CHEESE INOCULATED WITH VARIOUS SPOILAGE ORGANISMS

Organism	Treatment of Dressing and Number of Days Added to Shelf-Life when Compared to an Inoculated Control			
	Citric Acid	Lactic Acid	Sorbic Acid	Starter
Ach. butyri	2	0	2	0
Ach. eurydice	0	0	6	2
Alc. metalcaligenes	2	2	4	2
B. firmus	0	2	4	0
E. coli	2	2	4	2
E. freundii	0	0	2	0
M. candidus	0	0	6	0
M. conglomeratus	2	-2	2	2
Ps. desmolyticum	0	0	6	0
Ps. fluorescens	0	-4	0	0
Ps. fragi	0	0	0	0
Ps. tralucida	0	0	10	2
R. flava	0	0	10	2
T. candida	0	0	2	2
Mucor plumbeus	0	0	8	0
Pen. frequentans	2	2	4	2
G. candidum	0	0	4	0

respectively. Starter and sorbic acid added to the dressing increased the shelf-life in eight and fifteen of seventeen trials, respectively. The shelf-life was decreased by 2 and 4 days, respectively, when lactic acid was added to the dressing of cheese inoculated with M. conglomeratus and Ps. fluorescens.

DISCUSSION

Isolation and Identification

Only four of eighteen bacterial cultures isolated showed characteristics which agreed without discrepancy with those listed by Breed et al. (1948) for these organisms. These four were Ach. eurydice, M. epidermidis, Ps. desmolyticum, and Ps. fluorescens; and of these, Ps. desmolyticum was not described in any detail by the above authors. Ps. fluorescens failed to produce a green pigment when first isolated; however, the organism developed this characteristic after numerous transfers on artificial media. The isolated culture might have been a mutant strain of Ps. fluorescens.

Species are frequently encountered which do not conform to all of the characteristics described by Breed et al. (1948). The variations are attributed to mutant strains, changes in artificial media, and perhaps human error.

The yeast and mold cultures were identified with less difficulty because their characteristics conformed to the keys used in identification. The characteristics used in identification of the mold cultures are not shown because of the detail involved in tabulating

their characteristics. A description of the mold cultures can be found in A Manual of Soil Fungi by Gilman (1945).

Types of Defects Produced by Spoilage Organisms in Cottage Cheese

Cottage cheese is by no means a sterile product, since it is subjected to temperatures no higher than 143° F. The product normally contains a highly variable microflora, which makes it difficult to reproduce the type of spoilage caused by a pure culture of any particular organism. Cottage cheese curd cannot be sterilized by heating, since this process alters the appearance and physical nature of the product. In one portion of this study, the curd was sterilized by an electron beam with a dosage of 5.5×10^6 rep.

Two of the pure cultures and three cultures, when combined with S. lactis, failed to produce a visible defect other than free whey on sterile cottage cheese curd. Five of the nineteen cultures produced approximately the same degree and type of spoilage in pure culture as when combined with S. lactis. Six of the nineteen cultures produced more pronounced defects in pure culture, while six others produced more pronounced defects when combined with S. lactis. These differences in degree of spoilage may be due to higher pH in cultures showing more pronounced spoilage in pure

culture and to symbiotic relationships in instances showing more pronounced defects when cultures were combined with S. lactis. No attempt was made to determine symbiotic relationships in this study.

The degree of defect produced by species of the same genera of the organisms tested could not be correlated with the presence or absence of S. lactis. For example, Ach. butyri and Ps. fluorescens produced more pronounced defects in pure culture, while Ach. eurydice, Ps. desmolyticum, and Ps. tralucida produced more pronounced defects when combined with S. lactis. E. freundii produced approximately the same degree of spoilage in pure culture as when combined with S. lactis, while E. coli produced more pronounced defects when in pure culture. However, there was a correlation between pH and degree of defect. In nine of eleven instances where there were differences in both the degree of defect and the pH, the defects exhibited were more pronounced at the higher pH values regardless of whether these were produced by the pure cultures individually or when combined with S. lactis. These data indicate the importance of low pH in retarding the development of many of the organisms causing spoilage in cottage cheese. This fact is substantiated by Elliker (1952) and Collins (1955a), who reported on

the effectiveness of low pH in retarding the development of gelatinous curd defects.

Characteristics of Organisms Causing Spoilage in Cottage Cheese

Temperature Limits of Growth

The data indicate that many organisms are able to grow--some quite rapidly--at temperatures normally maintained with commercial refrigeration, and that low temperatures alone cannot be expected to prevent microbiological spoilage of cottage cheese.

Eight of the nineteen organisms tested exhibited visible growth after 7 days at 3° C. (37.4° F.). This temperature is lower than normally found in commercial refrigeration of cottage cheese. Harmon et al. (1955b), in a study of forty-eight samples of cheese from twenty-four retail outlets showed that the temperature of commercial display cabinets averaged approximately 45° to 50° F. It would be expected that some organisms would eventually grow in properly refrigerated cottage cheese.

Twelve of the nineteen organisms exhibited varying amounts of growth in 3 days at 10° C. (50° F.). At this temperature, which approximates average commercial conditions, several spoilage organisms multiply quite rapidly and would be expected to cause

spoilage in cheese. This work is supported by Jezeski and Macy (1946) and Thomas et al. (1947), who showed that species of Pseudomonas, Flavobacterium, Alcaligenes, and Achromobacter are able to grow vigorously at 8° C.

Only three of the nineteen cultures failed to grow in 7 days at 10° C. All of the cultures grew at 20° C. in 3 days, and this temperature appeared to be the optimum for most of the organisms. Many of the cultures also grew at 35° C. Kennedy and Weiser (1950) stated that the optimum temperature for seven of fifteen psychrophilic bacteria was closer to 10° than to 20° C. and none grew at 35° C.; however, they did not report the identity of the cultures studied.

Effect of pH on Growth Rate

Eight, four, and two of the sixteen organisms studied exhibited little growth at pH levels of 5.4, 5.2, and 5.0 or below, respectively. Only one organism, R. flava, exhibited little growth within the pH range used, while T. candida grew quite well throughout the range. Fifteen of the sixteen organisms tested exhibited little growth at pH values below 5.0 when growth was measured in trypticase soy broth by determining the increase in turbidity with a Klett-Summerson colorimeter. Small amounts of growth might have been undetected by

the colorimeter. Techniques such as plating to determine growth may be more accurate and desirable than the colorimetric method.

Since little growth occurred at pH of 5.0 and below with fifteen of the sixteen organisms, the data indicate that growth of many spoilage organisms should be retarded in cottage cheese with pH of 5.0 or below. Collins (1955a), Parker et al. (1950), and Elliker et al. (1951) reported that low pH tends to retard development of the gelatinous curd type of spoilage in cottage cheese. However, according to Deane et al. (1953), organisms causing gelatinous curd are not completely inhibited by low pH, and they showed that slime eventually developed on cottage cheese having pH below 4.7. The data herein reported indicate that pH below 5.0 retards the growth of many organisms causing spoilage in cottage cheese.

Sodium Chloride Tolerance

Bact. erythrogenes and M. candidus were the only organisms inhibited by 3 percent sodium chloride and only Bact. erythrogenes was inhibited by 1 percent. Cottage cheese normally contains 1.0 to 1.5 percent sodium chloride. Concentrations above 1.5 percent are not acceptable to the average consumer. The critical concentration of sodium chloride for the organisms tested appeared to be approximately 7 percent, since fifteen of the nineteen organisms

tested grew in a 5 percent, while only four of the nineteen grew in a 7 percent solution. Since 5 to 7 percent salt cannot be used in cottage cheese, the data show that sodium chloride offers little protection against spoilage.

Heat Tolerance

Eleven of the nineteen organisms tested were destroyed in 5 minutes at 143° F., and six others were destroyed in 10 minutes at this temperature. One organism was destroyed in 20 minutes, and only B. firmus survived for 30 minutes at 143° F. B. firmus does not appear as often as some of the other organisms that cause spoilage in cottage cheese. Watrous et al. (1952) and Atherton et al. (1953) reported that psychrophilic bacteria do not withstand laboratory pasteurization at 143° F. for 30 minutes, but they did not specify the organisms studied. The results obtained were supported by Kennedy and Weiser (1950), who showed that the majority of psychrophilic bacterial cultures were quite heat labile, but some were not completely destroyed at 145° in 30 minutes. Thomas et al. (1947) and Davis and Babel (1954) showed that species of Achromobacter, Flavobacterium, Pseudomonas, Alcaligenes, Proteus, and Aerobacter were destroyed at 143° F. in 30 minutes. Erdman and Thornton (1951a, 1951b) reported that species of

Aerobacter, Escherichia, Flavobacterium, Lactobacillus, Pseudomonas, and Streptococcus rarely survive pasteurization. Sherman et al. (1941), Rogick and Burgwald (1952), and Kaufmann and Andrews (1954) demonstrated that species of Pseudomonas and other psychrophiles did not survive pasteurization at 143° F. for 30 minutes.

The data herein reported and the supporting literature indicate that heating at 143° F. for 30 minutes destroys the majority but not all of the psychrophilic bacteria encountered in dairy products. Generally, the psychrophilic contamination represents post-pasteurization contamination as pointed out by Watrous (1953), Watrous et al. (1952) and Atherton et al. (1953).

Further heat studies showed that Bact. erythrogenes and M. conglomeratus were the only organisms of the nineteen tested that were destroyed at 120° F. in 15 minutes in skim milk. However, B. firmus and G. candidum were the only cultures which survived this temperature and holding time in whey.

Resistance to Germicides

Tolerance to chlorine. Only four of the nineteen organisms tested were destroyed by 10 parts per million chlorine at pH 7.5 and 25° C., and this required 10 minutes of exposure to obtain

positive destruction in three trials. Twenty-five parts per million chlorine was not effective in 1 and 5 minutes, destroying only three and four species, respectively. However, eleven of the nineteen cultures were destroyed in 10 minutes with 25 parts per million chlorine. The 50 parts per million chlorine was considerably more effective as only four cultures survived 1 minute and only one culture, B. firmus, survived 5 and 10 minutes in this concentration.

These data indicate that 50 parts per million of chlorine with 5 minutes of exposure is necessary to destroy most of the organisms under consideration. However, in this study, 1 ml. of a broth culture of the organism was added to 100 ml. of the germicide. This constitutes a considerable amount of organic matter and a much higher organism population than would ever be encountered in a commercial water supply. No attempt was made to determine the residual chlorine or to control the pH. These results show the ineffectiveness of low concentrations of chlorine in the presence of organic matter against organisms causing spoilage in cottage cheese. Investigations should be made using washed cell concentrates and controlled numbers of these nineteen cultures to determine their resistance to chlorine in the absence of excess organic matter. Collins (1955b) showed that 3 to 5 parts per million of residual chlorine at pH 6.0 was effective in destroying Ps. fragi,

Ps. viscosa, Alc. metalcaligenes, E. coli, and A. aerogenes, and Elliker (1954) demonstrated that the addition of 5 to 10 parts per million of chlorine was effective in destroying organisms in the wash water supply.

Tolerance to quaternary. Ten and 25 parts per million of quaternary at pH 6.5 and 25° C. was not effective against the organisms tested, with only five and six cultures, respectively, being destroyed in 10 minutes. Only eleven species were destroyed by 50 parts per million quaternary in 10 minutes. The Micrococci were among the first cultures destroyed as M. conglomeratus and M. flavus failed to survive 1 and 5 minutes, respectively, in 10 parts per million of quaternary. Twenty-five parts per million of quaternary destroyed M. candidus in 1 minute.

Mucor plumbeus was the only mold that survived 5 and 10 minutes in 50 parts per million of quaternary. E. coli and E. freundii required 10 minutes exposure in this concentration of quaternary before destruction was complete, and these were the only gram-negative organisms in this study which were destroyed. The species of Achromobacter and Pseudomonas, along with Alcaligenes metalcaligenes and B. firmus were not destroyed in 10 minutes in 50 parts per million of quaternary These are the

types of organisms usually responsible for the slimy curd or tapioca defects commonly encountered in cottage cheese spoilage. It appears that quaternary is ineffective, even in high concentrations in destroying these types of organisms.

According to the data, quaternary is effective against the gram-positive organisms, with the exception of B. firmus, but ineffective against the gram-negative organisms. These data are supported by the work of various authors including DuBois and Dibblee (1946), Johns (1947, 1948a, 1948b), Shere (1948), Dvorkovitz and Crocker (1950), Lundstedt (1950), and Parker and Elliker (1951), who reported that quaternary ammonium compounds are effective against gram-positive organisms but less effective against gram-negative ones.

Tolerance to iodophor. Ten and 25 parts per million iodophor at pH 3.5 and 25° C. were not effective against the organisms tested. Bact. erythrogenes was the only organism destroyed in 1, 5, and 10 minutes in 10 parts per million. In 25 parts per million Bact. erythrogenes and R. flava were the only cultures destroyed in 1, 5, and 10 minutes. Fifty parts per million of iodophor was considerably more effective as twelve, thirteen, and thirteen of the cultures were destroyed in 1, 5, and 10 minutes, respectively. The

increased exposure times of 5 and 10 minutes did not appear to increase the effectiveness of 50 parts per million of the solution, which indicates that the compound is rapid acting.

Different species of the same genera did not react similarly when exposed to iodophor. Ach. eurydice survived 1, 5, and 10 minutes in 50 parts per million, while Ach. butyri was destroyed in 1, 5, and 10 minutes in this concentration. Ps. tralucida was the only species of Pseudomonas surviving 1, 5, and 10 minutes in 50 parts per million of iodophor. The reasons for different species of the same genera reacting differently to the iodophor is not known.

The ineffectiveness of iodophor against molds should not be overlooked as the mold cultures grew at all concentrations and exposure times used.

In part the above data are supported by Johns (1954), who reported that concentrations of iodophor compared favorably with the same concentrations of chlorine in destroying E. coli. However, the data herein reported do not agree with data by Mueller (1955), who found that 25 parts per million of iodine compared favorably with 100 parts per million of chlorine in killing E. coli, and Gershenfeld (1955), who stated that 25 parts per million of iodophor was equivalent to 200 parts per million of chlorine. Johns (1954) also found that certain nonionic constituents of iodophors had a bacteriostatic

effect on spores of B. subtilis, indicating exceptionally favorable results against this organism, but in this study the iodophor was not effective against B. firmus. These two organisms would be expected to react similarly to iodophor.

When used in the same concentrations with the same exposure times, iodophor appeared to be more effective than quaternary but not as effective as chlorine against the nineteen cultures tested.

Resistance to Sorbic Acid

At pH values of 5.7, 6.4, 6.7, and 7.3, sorbic acid in trypticase soy broth was not effective even in high concentrations against organisms causing spoilage in cottage cheese. Bact. erythrogenes was destroyed, M. conglomeratus was retarded, and R. flava showed variable results at pH of 5.7 with 0.25 percent sorbic acid. The sixteen remaining cultures were able to grow quite well at this pH and sorbic acid concentration. Only a small number of the organisms tested were destroyed by 0.05, 0.10, and 0.25 percent sorbic acid at pH 5.2, but at pH 5.0 and 4.8, the viability of the cultures was decreased considerably as the sorbic acid content increased from 0.05 to 0.25 percent.

The difference in viability among species of the same genera is an interesting observation, but the reasons for these differences

are unknown. Usually the viability of organisms decreased as the sorbic acid content increased and the pH decreased.

These data may not represent the actual value of sorbic acid as an inhibitor of the organisms studied, since the results shown are the viabilities exhibited by the organisms when plated on tryptone glucose yeast agar, rather than a measure of inhibition. Sorbic acid may be bacteriostatic to many of the organisms but not germicidal, and when the acid was diluted during plating, the organisms were able to grow. Loop inoculations were made into the broth solutions containing sorbic acid at the various pH levels, but the actual number of cells or spores added may have varied considerably.

The concentrations of sorbic acid required to destroy the organisms under consideration (Table XIII) were substantially higher than those reported by Sheneman and Costilow (1955) and Beneke and Fabian (1955) as necessary for inhibition. However, the above authors were determining inhibition rather than destruction and were working at pH levels of 4.4 and below, while in the study herein reported, 4.8 was the lowest pH employed.

Although the pH of cottage cheese may not be sufficiently low to obtain the maximum benefit of sorbic acid, the data indicate that at pH values of 4.8 and 5.0, there would be some inhibition

and destruction of bacteria, yeasts, and molds causing spoilage. The addition of sorbic acid to cottage cheese could conceivably add a few days to the shelf-life of the product.

Control of Spoilage in Cottage Cheese

Several instances of spoilage resulting from the development of mold, pink slime, bitter flavors, wheying-off or other atypical types of spoilage in samples inoculated with various spoilage organisms shows the difficulty encountered in controlling or producing a specific type of spoilage in a product such as cottage cheese.

Treatment of Wash Water

The sample washed with water containing acetic acid and inoculated with Ach. butyri was the only one with pH below 5.10 at the time of spoilage, and the low pH rather than the acetic acid might have inhibited the tapioca slime characteristic of Ach. butyri.

Acetic acid added to the wash water appeared to inhibit tapioca slime formation on cottage cheese inoculated with Ach. eurydice. This sample had a pH of 4.3, which may have delayed the formation of slime. However, slime developed in 6 days on the remainder of the samples, some of which had pH values as low as 4.5. This indicates that low pH levels fail to completely inhibit

slime formation. This observation is supported by Deane et al. (1953), who reported that slime eventually developed on cottage cheese having pH below 4.7.

Both species of Achromobacter employed in this study failed to develop tapioca slime on samples washed with water containing acetic acid. The reason is unknown.

Sorbic acid in the wash water inhibited Ach. butyri but failed to inhibit Ach. eurydice. However, the concentrations of sorbic acid used were different, and the amount employed with Ach. butyri imparted an objectionable flavor to the cheese.

Marquardt (1953, 1954) reported that the addition of 7.5 parts per million of iodine to the final wash water retarded the development of water-borne organisms causing the slimy condition. However, in this study the organisms were present on the cheese curd in large numbers rather than in the wash water in small numbers. Previous data illustrate that the iodophor is fast acting, and this compound might be effective against small numbers of organisms in the water supply, especially in the absence of excess organic matter.

It is noteworthy that the sample inoculated with B. firmus and washed with water containing 10 parts per million of iodophor possessed a halogen flavor, but developed the brown slime in 10 days at a pH of 4.80.

E. coli might not have been the cause of spoilage in the samples inoculated with this organism, since the uninoculated control developed the brown slime typical of E. coli also.

Various types of spoilage occurred in 10 to 14 days on all samples inoculated with E. freundii. Sorbic, acetic, citric, lactic, and phosphoric acids, chlorine, and iodophor added to the wash water seemed to increase the shelf-life of cottage cheese somewhat, with sorbic acid being the most effective. Although E. freundii causes a brown slime on cottage cheese, it might not have been the organism causing the slime in this instance, since the uninoculated control developed this defect also.

Table XVII is of little significance, since neither M. candidus nor M. conglomeratus exhibited visible defects other than free whey in sterile cottage cheese curd in a previous experiment. However, it does point out that sorbic acid and iodophor are sometimes detectable upon organoleptic examination when cheese is washed with water containing 0.05 percent and 10 parts per million, respectively, of these compounds.

The sample inoculated with Ps. desmolyticum and washed with water containing sufficient sorbic acid to reduce the pH of the water to 6.0 was still marketable after 24 days. The pH of this latter sample had decreased to 4.50, and this low pH might have

increased the shelf-life of the product more than the added sorbic acid. Since Ps. desmolyticum causes spoilage by the formation of a tapioca slime, this organism probably was not the cause of spoilage in any samples, except the inoculated control and the sample washed with water containing lactic acid, both of which developed fruity flavors. This seems likely, since Collins (1955a) and Elliker et al. (1952) reported a fruity odor precedes the development of the gelatinous curd defect.

Wash waters containing sorbic and citric acids retarded development of green slime in samples inoculated with Ps. fluorescens. The samples washed with waters containing lactic acid, chlorine, iodophor, and quaternary exhibited more rapid slime formation than the inoculated control. This agrees with the findings of Davis and Babel (1954), who showed that washing cottage cheese curd with water containing 100 parts per million of chlorine for 10 minutes enhanced slime formation caused by species in the genera Achromobacter, Aerobacter, Alcaligenes, Proteus, and Pseudomonas. The sample washed with water containing iodophor exhibited a halogen flavor when fresh, but developed green slime in 6 days, which indicates the ineffectiveness of this compound against Ps. fluorescens.

Ps. fragi and Ps. tralucida form a light brown slime on cottage cheese, although the results fail to demonstrate this fact.

In 6 to 16 days, all samples became bitter and the pH decreased. Probably low pH rather than the wash waters retarded the development of the brown slime.

The addition of sorbic acid to the wash water appeared to increase the shelf-life of cottage cheese inoculated with G. candidum and Pen. frequentans by approximately 2 to 4 days, respectively. This was the only compound showing beneficial effects.

Treatment of Dressing Materials

The desired concentrations of the various acids added to the dressing material were determined by preliminary studies. Approximately 0.15 percent citric and lactic acids were found to reduce the pH of the creaming mixture to 5.2, which allowed sufficient safety factor before reaching the iso-electric point of the protein in the creaming mixture. A concentration of 0.10 percent sorbic acid was added to the cream without any detrimental effect on the flavor of the creamed cheese. Two percent starter was used as recommended by Marquardt (1953, 1954), and only slightly affected the pH of the creaming mixture.

The addition of citric and sorbic acids to the creaming mixtures of samples inoculated with Ach. butyri increased the shelf-life approximately 2 days over the remainder of the treated samples.

Sorbic acid added to the creaming mixture of cheese inoculated with Ach. eurydice increased the shelf-life approximately 4 to 6 days longer than the other samples.

Favorable results are shown for sorbic acid in cheese inoculated with Ach. eurydice, while the acid showed no beneficial effect in the samples inoculated with Ach. butyri.

Table XXII indicates that the use of sorbic acid adds approximately 4 days, while incorporation of citric and lactic acids and starter add approximately 2 days to the shelf-life of cottage cheese inoculated with Alc. metalcaligenes.

The entire series of samples inoculated with B. firmus and the uninoculated control developed mold in 6 to 10 days. Sorbic acid appeared to give better results than any other compound used against this natural contaminant. The low pH of the cheese probably retarded the brown slime development of B. firmus, but allowed the mold contaminant to grow readily.

Samples inoculated with E. coli exhibited a somewhat longer shelf-life with all of the additives. Sorbic acid was the most effective, giving an increase of 4 days, while the others gave an increase of 2 days when compared to the inoculated control.

Sorbic acid added to the creaming mixture also appeared to increase the shelf-life of cottage cheese inoculated with E. freundii by approximately 2 days.

The data shown in Table XXVI are insignificant from the standpoint of M. candidus and M. conglomeratus, since neither of these organisms produce visible spoilage in cottage cheese other than free whey.

The results shown in Table XXVII demonstrate the effectiveness of sorbic acid in inhibiting the particular mold which contaminated the samples inoculated with Ps. desmolyticum. The shelf-life was increased approximately 6 days by the addition of this compound. The low pH of the cheese at the time of inoculation probably prevented development of the tapioca slime characteristic of Ps. desmolyticum. This observation is substantiated by Elliker (1952) and Collins (1955a).

Lactic acid in the dressing encouraged the development of green slime in samples inoculated with Ps. fluorescens. After 6 days, green slime developed in the sample containing lactic acid.

The low pH of the cottage cheese curd at the time of inoculation with Ps. fragi probably prevented production of the typical brown slime, and mold growth occurred instead.

The samples inoculated with Ps. tralucida and containing starter and sorbic acid had a shelf-life of 2 and 10 days, respectively, longer than the inoculated control. The spoilage again was due to mold growth, with the exception of the sample containing sorbic acid which wheyed-off. Sorbic acid effectively inhibited the mold contaminant in this group of samples. The low pH of the original cheese probably prevented the development of the brown slime produced by Ps. tralucida.

Sorbic acid was effective in retarding the development of the pink slime of R. flava as the incorporation of this compound increased the shelf-life approximately 10 days.

The addition of sorbic acid to the creaming mixture increased the shelf-life of cottage cheese inoculated with Mucor plumbeus by approximately 8 days when compared to the inoculated control.

Citric and lactic acids and starter in the creaming mixture increased the shelf-life of cottage cheese inoculated with Pen. frequentans by approximately 2 days, while the addition of sorbic acid to the dressing increased the shelf-life by 6 days.

Sorbic acid added to the creaming mixture was effective in controlling spoilage of cottage cheese due to G. candidum, as the shelf-life was increased 4 days by the addition of this compound.

SUMMARY AND CONCLUSIONS

The microorganisms isolated from spoiled cottage cheese and agar exposure plates were identified as follows: Achromobacter butyri, Achromobacter eurydice, Alcaligenes metalcaligenes, Bacillus firmus, Bacterium erythrogenes, Corynebacterium filamentosum, Escherichia coli, Escherichia freundii, Escherichia intermedium, Micrococcus aurantiacus, Micrococcus candidus, Micrococcus conglomeratus, Micrococcus epidermidis, Micrococcus flavus, Pseudomonas desmolyticum, Pseudomonas fluorescens, Pseudomonas fragi, Pseudomonas tralucida, Rhodotorula flava, Torulopsis candida, Torulopsis versatilis, Geotrichum candidum, Mucor plumbeus, Mucor racemosus, and Penicillium frequentans.

Nineteen representative organisms were selected for further study from the twenty-five listed above. When inoculated into sterile cottage cheese curd, five of the nineteen cultures produced approximately the same degree and type of defect in pure culture as when combined with S. lactis.

Six cultures produced more pronounced defects in pure culture, while six others produced more pronounced defects when combined with S. lactis. The degree of defect produced by the

nineteen organisms tested could not be correlated with the presence or absence of S. lactis. However, reduced pH was effective in retarding the development of the defects. In nine of eleven instances where there were differences in both the degree of defect and the pH, the defects were more pronounced at the higher pH levels regardless of whether these were produced by the pure cultures individually or when combined with S. lactis.

The data presented indicate that under normal commercial refrigeration, many of the organisms studied could multiply in cottage cheese and cause spoilage.

Reduced pH levels caused reductions in the growth of the organisms in broth. From the data, it may be concluded that pH levels of 5.0 and below retard the growth of the majority of the organisms studied.

A concentration of 7 percent sodium chloride was required to inhibit the majority of the organisms tested. Since approximately 1.5 percent salt is the maximum concentration acceptable in cottage cheese and approximately 4.0 percent is normal in cottage cheese dressing, it can be concluded that the added salt has little, if any, effect on the shelf-life of cottage cheese.

Heating at 143° F. for 30 minutes destroyed all of the nineteen organisms tested, except Bacillus firmus. Subsequent heat

studies showed that only two organisms were destroyed by heating at 120° F. for 15 minutes in skim milk, but only two cultures survived when heated at this temperature and holding time in whey. This suggests that the cooking process used in manufacturing cottage cheese destroys the majority of spoilage organisms.

All of the organisms except Bacillus firmus were destroyed by 50 parts per million of chlorine in 5 minutes. Only eleven of the nineteen cultures were destroyed in 25 parts per million of chlorine with 10 minutes of exposure. Fifty parts per million of quaternary with 10 minutes of exposure also destroyed only eleven of the nineteen cultures. The quaternary was effective against gram-positive organisms but not effective against the gram-negative types. Fifty parts per million of iodophor with 1 minute of exposure was effective in destroying thirteen of the nineteen cultures, and increased exposures were not significantly more effective. The iodophor was ineffective against the mold cultures tested. Under the conditions of this study, chlorine was most effective, followed by the iodophor and the quaternary compounds.

Only a small number of the organisms tested was destroyed at pH 5.2 and above with concentrations of 0.05, 0.10, and 0.25 percent sorbic acid, while at pH 5.0 and 4.8, the viability of the

cultures was decreased considerably as the sorbic acid content increased from 0.05 to 0.25 percent.

The data presented indicate that acetic, citric, and sorbic acids added to the wash water increased the shelf-life of cottage cheese significantly when spoilage was caused by various organisms.

The data also show that the addition of sorbic acid and starter to cottage cheese dressing increased the shelf-life of the product significantly when the cheese was inoculated with various spoilage organisms.

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