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Action of mold inhibitors on dairy products

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ACTION OF MOLD INHIBITORS ON DAIRY PRODUCTS

by

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INTRODUCTION

Growth of molds on dairy products is of primary importance to the dairy industry. Molds are widely distributed and get into dairy products from many sources. Some of these products are suitable media for mold growth, and losses may result through the development of abnormal flavors, odors and colors.

The growth of molds in butter has been responsible, year after year, for a great deal of annoyance and financial loss to creameries and dealers in butter. The butter industry has constantly faced the possibility that molds might appear on butter, wrapper or package before the product reached the consumer. Molding of butter is by no means a new problem nor is there reason to believe that mold growth on butter is more common today than in the past, but interest has been aroused through the efforts of butter manufacturers to improve their products and through the demands of the consuming public.

In certain cheeses molds are important ripening and flavor imparting agents, but even with them abnormal molds may cause loss and inconvenience. Cheese plants often offer excellent conditions for mold growth, especially

in the curing rooms. Control of mold growth is one of the major problems of the cheese industry.

Milk and cream usually are contaminated with molds before delivery to the creameries and milk plants. These products are either used fresh or are pasteurized, and the molds do not develop enough to be of importance.

Molds are of little significance in the ice cream industry. Dairy products used in ice cream usually are contaminated with molds but these are destroyed during pasteurization. Ice cream mix may be contaminated with molds after pasteurization by equipment or by flavoring materials, but the storage temperatures used with the mix and finished ice cream are low enough to prevent growth.

STATEMENT OF THE PROBLEM

The studies herein reported were undertaken to obtain information on the mold inhibiting properties of various compounds, particularily from the standpoint of dairy products. Most of the work was done on propionic acid, calcium propionate and sodium propionate, but acetic acid, calcium acetate and various commercial materials also were considered.

HISTORICAL

Molds in Butter

Many types of molds are found in butter and contamination may come from various sources. Thom and Shaw (35) reported three general types of molds present in butter examined by them. These were (a) orange yellow areas produced by Oidium lactis, (b) smudged or dirty green areas produced by Alternaria and Cladosporium and (c) green surface colonies produced by Penicillium. They also reported that wet surfaces and wrappings and the presence of curd encouraged mold growth in butter but that 2.5 per cent salt was sufficient to inhibit it.

Hastings (5) stated that mold spores are widely distributed and are found on tubs, in cream, on parchment paper and on the finished butter. Food, moisture and air are necessary for mold growth, and these conditions are found in butter packed in tubs. The author noted that, if no mold growth has occurred in the cream, on the tubs or on the parchment, salted butter is not likely to show mold growth. Salt has a restraining influence on mold spores but does not prevent development of vegetative cells. Salt did not destroy spores since they germinated after 48 hours in saturated brine. Paraffining tubs or

treating them with salt aids in preventing mold growth but destruction of the spores was recommended. Boric acid, formalin and chloride of lime destroyed mold spores but were objectionable, whereas hot water was found to be the best agent for the destruction.

Later, Hastings (6) reported that molds in butter may come from milk, cream, factory equipment, salt, parchment and printing equipment. He suggested that careful plant methods would help keep down mold contamination and advocated treating tubs and liners with hot water or chloride of lime.

Macy and Combs (20) studied 15 creameries from the standpoint of the sources of yeasts and molds in butter. They reported the principal sources of molds are: (a) Milk and cream; milk may be contaminated by dust in the stable, milk pails and equipment, and cream may be contaminated by the separator, cans and other equipment and by the milk from which it is separated. (b) The creamery equipment, such as vats, churns, pipes and cans, the creamery walls and the water used to wash the butter. (c) Materials and supplies, such as tubs, liners, wrappers, boxes, starter and salt.

Lund (17) stated that butter made from properly pasteurized cream in a new churn usually was low in yeasts and molds and that the churn often was a source of mold

contamination. If the churn was not used for a few days, the first butter churned after the idle period was high in yeasts and molds. He recommended proper pasteurization of cream and proper cleaning and care of the churn and other equipment to keep down mold contamination.

Parchment as a source of molds in butter was studied by Macy and Pulkrabek (24). They made butter from sterile cream and wrapped it in regular parchment and also in parchment treated in the following ways: Boiling water, boiling aqueous supersaturated brine, cold aqueous saturated brine, cold aqueous saturated brine plus potassium nitrate and solutions of formalin, hypochlorite, sodium benzoate, benzoic acid and salicylic acid. They concluded that parchment definitely was a source of molds and recommended treating it with boiling water but also stated that treated parchment would not prevent mold growth on contaminated butter.

Six principal types of mold were found by Morgan (27) in unsalted butter manufactured in New Zealand. They were: Penicillium glaucum, Stemphylium, Fusarium lactis, Oidium lactis, Cladosporium herbarum and Cladosporium butyri. Morgan stated that the principal contamination comes from the air. The molds germinate in 2 to 3 weeks after butter is removed from storage; they are resistant to 6 to 7 per cent brine solution and to 4 per cent formalin solution.

Macy, Coulter and Combs (21) compared salted and unsalted butter before and after storing for 3 months and 9 months. During storage molds and yeasts tended to increase in unsalted butter and decrease in salted butter. The authors found that proper pasteurization destroyed molds 100 per cent and that the churn was the most prolific source of contamination.

Rogers (1) stated that molds are present in the air, on feed and on dairy utensils and that milk usually is contaminated with spores. Some molds grow readily in milk, some grow slowly and some do not grow at all. Rogers noted that most molds require abundant oxygen, plenty of moisture and relatively high temperatures (29.4° to 36.6°C.) for best growth. He suggested that mold growth might be partially prevented by low storage temperatures, exclusion of oxygen and control of moisture. Proper care of the dairy plant and equipment and treating wrappers and containers were recommended as aids in preventing growth.

Hammer (4) reported that bacteria are primarily responsible for the fermentation of milk and cream but that yeasts and molds are regularly present. The numbers of these organisms vary widely and depend on conditions of production and holding. With long holding at comparatively high temperatures, yeasts and molds are relatively numerous, due to their ability to grow in the presence of

acid. The molds are of various types. Oospora lactis is the mold most frequently found and when milk or cream is allowed to stand the organism occurs so regularly that it is regarded as the common mold of dairy products.

Molds may get into butter from various sources. Hammer (4) stated that raw cream, either sweet or sour, usually contains these organisms due to contamination from the air and utensils. The cream may have molds added to it in the butter plant because of improperly cleaned vats, pipes, churns and other utensils. Churns are very commonly a serious source of mold contamination. Packing materials, such as tub liners, tubs and wrappers and also cutting equipment, salt, starter, wash water and the person handling the butter often are sources of molds. Hammer recommended sanitary plant methods for preventing molds in butter and suggested thorough cleaning and sterilizing all equipment, especially the churn, treating of such materials as liners and tubs with boiling water or brine solutions and proper care of salt, starter and wash water.

Macy and Gibson (25) isolated 61 cultures of Oospora lactis from Canadian and domestic butter and studied their morphological, cultural and biochemical characteristics. Distinct differences were noted in colony formation, both in size and appearance, with great variations on several media. The size of the conidia fluctuated widely. Optimum

growth temperatures ranged from 15° to 25°C. Salt concentrations of 7.5 per cent or higher disturbed the development of the molds and 10 per cent inhibited growth. All strains produced indol, liquefied gelatin to some extent and showed lipolysis of cottonseed oil and butter fat. None produced an acid reaction in litmus milk nor hydrolyzed starch. Acid was formed in glucose broth, but maltose and sucrose were not attacked. No pronounced flavor was produced in skim milk, milk or cream.

Vernon (39) stated that molds found in butter thrive best under warm humid conditions and grow over a wide range of temperatures (-9.4° to 32.2°C.) but develop best at 21.1°C. The churn was found to be the most prolific source of mold contamination; and other sources were milk, cream, butter boxes, cartons, plant equipment, parchment paper and salt. Vernon recommended the use of a water soluble form of salicylanilide for preventing mold growth.

Various factors influence the growth of molds in butter. Macy (18) studied 10 species isolated from butter. The influence of food supply, moisture, temperature and salt concentration were investigated. Purified butterfat was not a suitable source of food unless water was present, and low humidities tended to check mold growth. The best mold growth occurred at 10°C. but no growth occurred when oxygen was exhausted from the atmosphere. Salt greatly

influenced mold growth; 5 per cent prevented growth of some molds, while others grew in the presence of 20 per cent.

Macy (19) reported data on 2,700 samples of market butter, giving yeast and mold counts and composition. He concluded that the moisture content apparently had little effect on yeast and mold counts, but salt concentration greatly affected them. The higher concentrations of salt influenced yeast growth much more than mold growth.

The effect of mold growth in cream on the quality of butter made from the cream was studied by Combs and Eckles (3). They concluded that: (a) Molds did not grow readily upon butter and their growth was influenced by the amounts of protein, salt and moisture that the butter contained and the amount of moisture in the surrounding atmosphere. (b) The quality of sweet cream, as judged by taste, was influenced to a marked degree by mold growth but with sour cream little difference was noted. (c) The growth of Oidium lactis or Penicillium in cream exerted a decidedly detrimental effect on the keeping quality of the butter. (d) Mold spores did not germinate or produce growth in butter and the mold growth was due to outside contamination. (e) Ordinary pasteurization temperatures greatly improved the keeping quality of butter made from cream upon which molds had grown but did not entirely check the action of the enzymes which the molds had produced; temperatures

high enough to check the action entirely are beyond the limit of commercial use. (f) The abnormal flavors which developed in butter due to mold growth in cream were caused by enzymes secreted by the molds. Yeast and mold counts have been suggested as an index of the keeping quality of butter.

Macy and Richie (23) concluded from a study on 597 lots of butter that yeast and mold counts did not furnish a basis for judging keeping quality but did serve as a check on manufacturing practices. The types of molds found are more important than the numbers.

From his studies Stiritz (34) concluded that yeast and mold counts were not an effective method of determining whether butter was made from pasteurized cream. He stated that the churn may be the greatest source of contamination when not properly cared for and that cream could be recontaminated by starter. The source of yeasts and molds had little effect on the quality of the finished butter.

Molds in Cheese

Although molds are necessary in the curing of some cheeses, abnormal molds can be the cause of loss and annoyance with them, and all molds are objectionable with other cheeses.

With cheddar cheese, curing-rooms appear to be the place where most trouble with molds is experienced. Van Slyke and Price (38) reported that, when the temperature and humidity of the curing-rooms favor growth, molds invariably attack the cheese. They also noted that the prevention of mold growth can be accomplished: (a) By ventilating the curing-rooms enough to dry the surface of the cheese within a short period after it is removed from the press. (b) By keeping shelves and walls dry and clean. (c) By turning the cheese daily and wiping the cheese and shelves with a clean, dry cloth. (d) By paraffining the cheese. When the cheese curing-room is badly infected, it should be scrubbed, dried and sprayed with a 10 per cent solution of formaldehyde.

Hammer (4) stated that cheese provides materials that are satisfactory for mold nutrition and, since spores are widely distributed, mold development occurs when the requirements as to air, humidity and temperature are met. Mold growth usually occurs at the surface due to air requirements and may be partially controlled by paraffining the cheese, but cracks in the paraffin, often due to moisture beneath, allow molds to enter and spread rapidly. Molds also may follow openings down into the cheese and plugging of cheese commonly results in mold development along the plug. He noted that the humidity

and temperature of storage rooms are usually satisfactory for mold growth so that air supply is the principal controlling factor .

Cottage cheese usually is sold 2 or 3 days after manufacture, and molds do not present much of a problem if the product is properly manufactured. Hammer (4) reported that cottage cheese would deteriorate because of mold growth, even when made from properly pasteurized milk, if recontaminated after heating. The organisms may gain entrance to milk or cheese from utensils, equipment, air and various other sources. Drain cloths are a serious source of contamination if not properly treated.

Prevention of Mold Growth

The prevention of mold growth on food products has become of major importance to producers and dealers. Various materials have been employed in attempts to inhibit mold growth, and some have proved successful. Use of mold inhibiting materials on food products presents new problems, since they must be safe from the standpoint of health and must not add undesirable flavors.

Perry and Beal (32), from their investigations of the amounts of preservatives needed to inhibit growth of Saccharomyces cerevisiae and Penicillium glaucum in 2 per

cent dextrose broth, reported the following amounts were necessary:

absolute alcohol	8.00 per cent
sodium salicylate	3.00 per cent
sodium benzoate	0.25 per cent
sodium acid sulphite	0.25 per cent
formaldehyde	0.25 per cent

Peck and Rosenfield (31) investigated the effects of hydrogen-ion concentration, fatty acids and their salts and vitamin C on the growth of Trichophyton gypseum and Epidermaphyton inguinale. When sodium hydroxide or hydrochloric acid was used to obtain the initial pH value, the organisms grew over a pH range of 4.0 to 10.0. For the work with fatty acids the authors used the acids from formic with one carbon atom to capric with 10 carbon atoms, the unsaturated undecylinic acid and the salts of most of these acids. They concluded that, with the possible exception of formic acid, the pH produced by the acids would allow growth. As a rule the salts were less fungicidal than the corresponding acids. They found the following concentrations necessary with the more effective of the acids and salts:

formic acid	0.03 per cent
sodium formate	0.50 per cent
acetic acid	0.03 per cent
sodium acetate	5.00 per cent
propionic acid	0.03 per cent
sodium propionate	0.03 per cent
butyric acid	0.01 per cent
sodium butyrate	0.05 per cent
valeric acid	0.003 per cent
caproic acid	0.009 per cent
capric acid	0.009 per cent
undecylinic acid	0.005 per cent

Vitamin C had no effect on the growth of fungi.

The effect of various acids and acid salts on Aspergillus niger was studied by Kiesel (10). He concluded that: (a) Physiological activity of the various acids and acid salts on Aspergillus niger did not correspond, in most cases, to the chemical activity of these substances. (b) Although there was a certain resemblance between the physiological activity and the chemical activity, there was a particular toxicity which did not correspond to the degree of dissociation of the acids, the property of being assimilated or the presence of specifically toxic atoms. (c) Fatty acids had a very great toxicity, even though they are less dissociated and assimilable, and the toxicity increased with the carbon atoms. The acids of the iso series were more toxic than the acids of the normal series. (d) Toxicity of acetic acid decreased with substitution of chlorine atoms, and trichloroacetic acid was much less toxic than acetic. (e) Substitution of a hydrogen by a hydroxyl group strongly reduced the activity of propionic acid. (f) Toxicity appeared to be due to the ability of the substance to permeate the protoplasm of the cell. Mold spores were not protected by neutralizing the added acid, and there was an increase in the acidity of the medium at the start but sometimes a decrease later. (g) Aspergillus niger was definitely affected by most of the acids.

Kirby, Atkin and Frey (11) investigated the influence

of acidity on the growth of Aspergillus niger. They noted that: (a) The mold grew equally well between pH 3.5 and 6.0. (b) Acetic acid had a marked toxicity for the mold and this was a function of pH. (c) The inhibiting action of acetic acid increased with an increase in the initial hydrogen-ion concentration of the medium. (d) The undissociated acetic acid molecule was the active agent in retarding or preventing the growth of mold and not the acetate ion.

The effect of acetic acid on certain micro-organisms related to food spoilage was studied by Levine and Fellers (15). The organisms used were: Salmonella aertrycke Staphylococcus aureus, Phytomonas phaseoli, Bacillus cereus, Bacillus mesentericus, Saccharomyces cerevisiae and Aspergillus niger. Acetic acid and hydrochloric acid were used and hydrogen-ion activity and total acidity were closely observed. The authors noted that: (a) An increase in acidity or in hydrogen-ion concentration inhibited growth of microorganisms. (b) Acetic acid was more effective in inhibiting growth of microorganisms. (c) The toxicity of acetic acid was not due to the organic nature and slight dissociation, since lactic acid was less toxic. (d) Acetic acid not only inhibited or destroyed the microorganisms but lowered the thermal death points. The authors suggested that the toxic effect of acetic acid might be due in part to the undissociated molecule.

Levine and Fellers (16) also studied the inhibiting effect of acetic acid in the presence of sodium chloride and sucrose. They concluded that acetic acid inhibited bacterial growth in almost direct proportion to the amount present. Bacteria were less resistant than molds and yeasts. Other than altering the pH, the added sodium chloride and sugar had little influence on the toxic effect of the acid and the toxic effect was not due to the hydrogen-ion concentration alone. The authors also found that the growth of Aspergillus niger was inhibited by a concentration of acetic acid giving a pH of 4.1 and a total acidity of 0.27 per cent. The addition of 5 per cent sodium chloride or 20 per cent sucrose did not significantly change the limits of growth. Acetic acid in non-toxic amounts served as a source of energy for molds and 20 sucrose stimulated growth. At acetic acid concentrations of 0.10 to 0.17 per cent no inhibition of mold growth resulted from the use of 5 per cent sodium chloride.

The fungistatic properties of the fatty acids were investigated by Hoffman, Schweitzer and Dalby (7). They used all the normal saturated fatty acids containing from 1 to 14 carbon atoms, isomeric forms (such as isobutyric), the 3 isomeric forms of n-valeric and some of the saturated acids (such as beta-iodopropionic). The medium used consisted of standard nutrient agar of one and one-half

times normal strength with 1 per cent added sucrose; it was buffered and a pH range of 2 to 8 was used. The authors concluded that many of the acids exhibited remarkable effectiveness in the inhibition of mold growth. The effectiveness varied according to the chain length, the concentration of the acid and the pH of the medium. A branched chain acid was less effective than the corresponding straight chain acid. Unsaturation of the acids tended to increase the effectiveness as a mold inhibitor.

Hoffman, Schweitzer and Dalby (8) studied the effect of chlorine substitution on the fungistatic properties of acetic and propionic acids. The introduction of chlorine on the alpha carbon atom of propionic acid sharply decreased its fungistatic properties; monochloroacetic acid was much less effective than acetic acid, except in the very high pH range. A chlorine atom on the carbon next to the carbonyl affected the fungistatic properties of the acid, but the same substituent on the beta carbon atom had no effect. The theory is rather widely held that the biological activity of an acid is dependent on the dissociation constant. The relative fungistatic properties of acetic and monochloroacetic acids and of propionic and alpha-chloropropionic acids, when compared with the dissociation constants, are in agreement with the theory. This is not the case with propionic and beta-chloropropionic acids, since they have essentially

identical fungistatic properties and the dissociation constants 1.33×10^{-5}, and 8.59×10^{-5} respectively.

Krog and Marshall (14) worked with a new compound, alkyl-dimethyl-benzyl-ammonium chloride, for sterilizing utensils used in eating places. A 1:5,000 solution of the compound showed marked action against bacteria, detergents and soaps had little effect on the potency of the compound, the stability of the compound was satisfactory and could be easily checked colorimetrically in the field, temperature did not affect the stability or bactericidal efficiency adversely above the temperature of 21.1°C. and an exposure of 1 minute apparently was sufficient to reduce bacterial counts below 100 per tumbler rim.

Kirby, Atkin and Frey (13) concluded that mold growth could be prohibited by use of vinegar spray or wash on the crust of baked bread or by spraying with ethylene oxide. Heat kills mold spores but mere acidity does not affect growth.

Molds frequently found on bread were studied by Kirby, Atkin and Frey (12) who reported that: (a) The effect of acidity on the growth of bread mold must be considered not only from the standpoint of hydrogen-ion concentration but from the specific effect of the kind of acid used. Fatty acids are much more toxic to molds than mineral acids or other organic acids. (b) Molds have a very wide growth optimum with respect to hydrogen-ion concentration. They

grow equally well at all hydrogen-ion concentration within which commercial bread is produced. (c) Acetic acid, either in C.P. form or in the form of vinegar, has a marked influence on the growth of bread mold. Similar activity is shown by formic, propionic and butyric acids. With pH values of 5.5 to 6.0 acetic acid retarded initial growth of molds but was found to have little effect on ultimate growth.

In an attempt to find something that would inhibit mold on citrus fruit during storage and would not injure the fruit, Tompkins and Trout (37) tried several compounds. Acetaldehyde proved satisfactory for the inhibition of mold. Green rot of citrus due to Penicillium digitatum was greatly reduced by storing in an atmosphere with a small regulated amount of ammonia. The ammonia concentration was obtained by damp crystals of ammonium carbonate.

Blue-mold has been the cause of considerable loss to the apple growers of the country. Baker and Heald (2) conducted a series of tests in an attempt to reduce loss from this mold. They noted that contamination from packing boxes and equipment was greatest during the harvest season. Copper sulphate was ineffective for control of blue-mold, and chemically treated wraps, sodium bicarbonate and sodium tetraborate also were ineffective. Sodium hypochlorite solution, with 0.4 per

cent available chlorine, was recommended for rinsing the packing boxes, equipment and apples.

Neill (28) worked on the control of mold in dairy plants and used the following materials: Copper sulphate, sodium carbonate, trisodium phosphate, borax, formalin, soft soap and common chlorine disinfectants. Results showed the common chlorine solutions were ineffective. Copper sulphate and formalin were somewhat effective, but hot water, soap and scrubbing gave best results.

Much of the butter manufactured in New Zealand is exported in wooden boxes and mold growth on these boxes is important in the butter industry. Riddet and Neill (33) studied the growth of mold on butter boxes in an effort to find a method of prevention. They concluded that: (a) Mold from boxes contaminated butter in the boxes. (b) Double layers of parchment did not prevent mold mycelia from penetrating into butter from the boxes. (c) Mold growth was stimulated on boxes by defrosting at high atmospheric temperature and humidity, admission of air, use of soft timber and treatment which rendered lumber more susceptible to mold growth. (d) White pine boxes were very susceptible to mold. (e) Immersion of boxes in an aqueous solution of sodium salicylanilide proved to be a very effective means of preventing mold. (f) Aluminum foil linings prevented mold from penetrating from infected boxes to the butter.

General experiments were conducted by Neill (29) to find a method of preventing mold growth on timber for butter boxes. The wood was immersed for 10 minutes in a 0.1 per cent solution of Shirlan W. S. at 13.3°C. before making up the boxes. The boxes were air dried for 4 days at -10°C., removed to a room at 21.1° to 26.6°C., held for 10 days and then filled with butter. The boxes were dropped from a height of 3 feet to simulate shipping and the contents examined for mold. A degree of resistance sufficient for most purposes was obtained by the treatment.

Thom and Ayres (36) tested various molds to determine (a) whether pasteurization killed them and (b) the effect of dry heat on them. They used spores from pure cultures of Penicillium, Aspergillus, Oidium lactis and one strain of Fusarium and heated with the holder type of pasteurization (60° to 62.8°C. for 30 minutes) and with the flash process (73.9° to 79.5°C. for 30 seconds followed by quick cooling); the dry heat tests were made by heating mold cultures in glass tubes to 87.8° to 121.1°C. Both types of pasteurization destroyed the molds commonly found in milk but heating in dry air below 121.1°C. did not destroy the organisms.

Preliminary investigations on the use of sodium propionate and calcium propionate for mold prevention were reported by Macy and Olson (22). They employed salted

and unsalted butter. The butter was exposed to mold contaminated air and then wrapped in parchment treated with 8, 16 or 20 per cent aqueous solutions of sodium prppionate or calcium propionate and stored at 10°C. All control samples were very moldy after 11 to 12 weeks. Marked inhibition of mold development was noted with both salts, especially the higher concentrations, and inhibition was more pronounced with salted butter than with unsalted.

Ingle (9) tested the effectiveness of propionic acid, sodium propionate and calcium propionate as mold inhibitors for dairy products. Fresh cut blocks of natural cheese were wrapped in tin foil, moisture-proof cellophane or pliofilm, the samples being dipped in aqueous solutions of the compounds or the wrappers being sponged with the solutions. Sponging the wrappers did not prevent mold growth. Samples dipped in 8 per cent propionic acid showed growth in about twice as long as the control samples. Solutions of 8 per cent sodium propionate and 8 per cent calcium propionate gave some inhibition but were less effective than propionic acid. Incorporating the propionates into cream cheese was ineffective for inhibiting mold growth. The best method for controlling surface mold on cream cheese was waxing the wrapper with wax containing propionic acid. Surface mold on unsalted butter, stored at 15.6°C. and 100 per cent humidity, was greatly inhibited by using wrappers containing 12 per cent sodium propionate or 6 per cent

calcium propionate. Wetting the impregnated wrappers increased their effectiveness. There appeared to be little difference between treating the parchment with sodium propionate or with calcium propionate.

Olson and Macy (30) used propionic acid, calcium propionate and sodium propionate to inhibit the growth of various species of molds on the surface of butter and in culture media. Calcium propionate was more effective than sodium propionate, but propionic acid was most effective. The final pH of media containing the salts was found to be an extremely important factor in restraining mold growth. A 2 per cent concentration of sodium propionate in potato dextrose agar gave a reaction of about pH 7.0, and mold growth was relatively rapid and abundant. When the pH was adjusted to 6.1 with lactic acid no mold growth occurred in 5 days. Some molds were relatively tolerant of propionic acid and the propionates and Penicillium showed the greatest tolerance. In potato dextrose agar to which propionic acid was added to give 0.009 M concentration, the growth of Hormodendrum cladosporioides was relatively abundant, but when 0.4 gm. of sodium propionate per 100 ml. of the medium was added mold growth was checked. When 0.4 gm. sodium propionate alone was used growth was abundant. The investigators concluded that possibly the undissociated propionic acid is responsible

for the mold inhibition.

The retarding of mold on cheese by use of various compounds was studied by Miller (26). The cheese was cut in pieces, dipped in solutions of the inhibitors, wrapped in cellophane and stored in air tight containers at 14.4° to 15.6°C. The compounds used were propionic acid, calcium propionate, and sodium propionate. Miller concluded that the compounds were effective as mold retarding agents, propionic acid being the most effective, sodium propionate second and calcium propionate third; the compounds had little effect on color and flavor of the cheese. He recommended care in the preparation of the cheese to prevent contamination, use of closely fitting wrappers and use of proper concentrations of the compounds. The period of immersion and proper draining of the cheese also are important.

MATERIALS USED

With a few exceptions the dairy products used in the trials were obtained from the laboratories operated by the Dairy Industry Department, Iowa State College. The raw cream and milk were from routes or were delivered by patrons. The butter and cheese were the regular products manufactured by the laboratories; the salted butter contained about 2 per cent salt.

Butter made for the trials was churned in a 6-quart Dazey hand churn. Raw sweet cream from the butter laboratory was used. The butter was washed with sterile water, worked by hand in sterile equipment and made into prints with a sterile form. In some cases all the butter was made into prints without salting, while in other cases a churning was divided and half made into a print without salt and half with 2 per cent salt.

Propionic acid, calcium propionate and sodium propionate were obtained from the E. I. du Pont de Nemours & Co. Acetic acid and calcium acetate were regular commercial products. The solutions of propionic acid, calcium propionate, sodium propionate, acetic acid and calcium acetate were made by dissolving the compounds in distilled water; neither the water nor the containers were sterilized but the containers

were well washed. The solutions were used for dipping samples and for treating parchment. When the compounds were used directly in liquids or in cottage cheese, they were added to the material to be treated and distributed with a sterile glass rod.

The various parchment were obtained from the Kalamazoo Vegetable Parchment Co. They included regular parchment, parchment treated with calcium propionate, pliowax parchment and a special wax parchment. The parchment treated with calcium propionate commercially contained different amounts of the salt, 6, 10, 25 and 30 per cent.

Czapek's medium was prepared in the following manner: 2.0 grams sodium nitrate, 1.0 gram monopotassium phosphate, 0.5 grams potassium chloride, 0.5 gram magnesium sulphate, 0.01 gram ferrous sulphate and 30 grams sucrose were dissolved in enough distilled water to make 1000 ml.; the solution was sterilized in the autoclave for 30 minutes at 15 pounds pressure.

The mixed mold cultures used for inoculation were obtained from moldy butter or cheese, from the walls and shelves of cheese curing-rooms, from plates poured for mold counts and from butter made in various creameries. Mold suspensions were prepared by shaking the materials in sterile distilled water, and the suspensions were used for inoculations.

The pure cultures of molds were obtained from the stock cultures in the dairy bacteriology laboratories of the Iowa Agricultural Experiment Station.

EXPERIMENTAL

Action of Propionic and Acetic Acids and Their Salts

Action on raw cream

The action of the various compounds on the molds in raw cream was studied by weighing 230 gram portions of cream into sterile pint milk bottles, adding and distributing the compounds, stoppering the bottles with sterile cotton and holding them at room temperature. The portions of cream were treated with various amounts of the different compounds, the concentrations being: Propionic acid 0.04, 0.05, 0.1, 0.16, 0.4, 0.5 and 1.0 per cent; calcium propionate 0.01, 0.05, 0.1, 0.2, 0.5, 0.8 and 1.0 per cent; sodium propionate 1 per cent; acetic acid 0.1, 0.4, 0.5, 0.8 and 1 per cent; and calcium acetate 0.2, 0.5 and 1.0 per cent. An untreated portion (the control) was included in each trial. The portions were observed daily for mold growth. The data are presented in Table 1.

All the control samples developed mold growth very rapidly; two showed mold growth after 1 day, eleven after 2 days, two after 3 days and four after 4 days.

With propionic acid solutions of 0.04, 0.05, 0.1 and

Table 1. Inhibition of Molds in Raw Cream by Propionic Acid, Calcium Propionate, Sodium Propionate, Acetic Acid and Calcium Acetate

Compound used	Trial Number																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
	Days until mold was evident macroscopically																		
None-control	4	3	2	2	2	2	2	2	2	4	3	2	4	1	2	2	4	2	1
Propionic acid:																			
1.0%	175	165	150	138	165	150	155	138	92	96	93	87	184	160	120	152	118	121	140
0.5										104	97	106	98	138	104	106	103	115	90
0.4	8	10	10	12	18	18	12	18											
0.16	3	4	3	5	3	4	3	5											
0.1	3	3	2																
0.05	2	4	3																
0.04	2	4	2																
Ca propionate																			
1.0%	184	160	120	152	92	96	93	87	138	175	165	150	155	120	108	92	102	88	84
0.8														89	92	96	92	90	84
0.5	6	4	4	4	6	4	5												
0.2	3	3	4	4	4	3	3												
0.1	4	3	4	6	4	4	3												
0.05	2	2	2	2	4	4	3												
0.01	4	3	3	4	4	3	3												
Na propionate																			
1.0%														86	76	82	86	76	78
Acetic acid																			
1.0%	12	8	10																
0.8	8	12	8																
0.5	5	4	4																
0.4	3	8	7																
0.1	5	4	3																
Ca acetate																			
1.0%	5	3	4																
0.5	5	3	4																
0.2	3	3	3																

0.16 per cent, mold growth was evident after 2 to 5 days; with a solution of 0.4 per cent, after 8 to 18 days; with a solution of 0.5 per cent, after 90 days to 138 days; and with a solution of 1 per cent, after 87 to 184 days. With calcium propionate solutions of 0.01, 0.05, 0.1, 0.2 and 0.5 per cent, mold growth was evident after 2 to 6 days; with a solution of 0.8 per cent, after 84 to 96 days; and with a solution of 1 per cent, after 84 to 184 days. Sodium propionate in a 1 per cent concentration prevented mold growth for 76 to 86 days.

With acetic acid solutions of 0.1, 0.4 and 0.5 per cent, mold growth was evident after 3 to 7 days; and with solutions of 0.8 and 1 per cent, after 8 to 18 days. With calcium acetate solutions of 0.2, 0.5 and 1 per cent, mold growth was evident after 3 to 5 days.

Action on raw milk

The action of the various compounds on the molds in raw milk was studied by measuring the milk into sterile test tubes, adding and thoroughly distributing the compounds, plugging the tubes with sterile cotton and holding at room temperature. The compounds were added so that the following concentrations were obtained in the milk: Propionic acid 0.5 and 1 per cent; calcium propionate 1, 1.5, 2 and 5 per cent; and sodium propionate 5 per cent. An untreated

sample (the control) was included in each trial. Daily observations were made for mold growth. Table 2 gives the results.

The control samples regularly showed mold growth very quickly; three showed mold growth after 2 days, six after 3 days, nineteen after 4 days and two after 5 days.

Propionic acid was used in all 30 trials in concentrations of 0.5 and 1 per cent. The samples containing 0.5 per cent acid were held for 60 days and those containing 1 per cent were held for 90 days without mold growth being evident in any of them. Calcium propionate was used in concentrations of 1.0, 1.5 and 2 per cent in 12 trials. With 1 per cent calcium propionate, three samples showed mold growth after 3 days, eight after 4 days and one after 5 days; with 1.5 per cent, one sample showed mold growth after 3 days, six after 4 days, two after 5 days and three after 6 days; and with 2 per cent, three samples showed mold growth after 5 days, three after 6 days, four after 7 days and two after 8 days.

Calcium and sodium propionates were each used in 5 per cent concentrations in 18 trials. With the calcium propionate, one sample showed mold growth after 7 days, two after 8 days, four after 10 days, four after 12 days, three after 14 days and four after 16 days. With the sodium propionate, four samples showed mold growth after 6 days, three after 7 days, four after 8 days, two after

Table 2. Inhibition of Molds in Raw Milk by Propionic Acid, Calcium Pro

Compound used	Trial Number 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	Days until mold was evide															
None-control	2	2	3	2	4	4	4	4	4	4	3	4	4	3	3	4
Propionic acid																
0.5%	>60	>60	>60	>60	>60	>60	>60	>60	>60	>60	>60	>60	>60	>60	>60	>60
1.0	>90	>90	>90	>90	>90	>90	>90	>90	>90	>90	>90	>90	>90	>90	>90	>90
Ca propionate:																
1.0%	3	3	3	4	4	4	5	4	4	4	4	4				
1.5	4	3	4	6	4	4	5	6	4	4	5	6				
2.0	5	7	5	7	6	8	7	6	8	7	5	6				
5.0													10	12	12	10
Na propionate:																
5.0%													10	9	9	7

Propionic Acid, Calcium Propionate and Sodium Propionate

Trial Number																			
10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Days until mold was evident macroscopically																				
4	3	4	4	3	3	4	4	4	3	3	4	4	5	5	4	4	4	4	4	4
>60	>60	>60	>60	>60	>60	>60	>60	>60	>60	>60	>60	>60	>60	>60	>60	>60	>60	>60	>60	>60
>90	>90	>90	>90	>90	>90	>90	>90	>90	>90	>90	>90	>90	>90	>90	>90	>90	>90	>90	>90	>90
4	4	4																		
4	5	6																		
7	5	6																		
			10	12	12	10	10	16	10	8	14	12	8	14	7	16	14	12	16	16
			10	9	9	7	7	12	6	8	8	6	8	8	7	6	6	18	16	18

9 days, one after 10 days, one after 12 days, one after 16 days and two after 18 days.

Action on Czapek's medium

Czapek's medium is very satisfactory for growing molds, and trials were conducted to determine whether propionic acid, calcium propionate and sodium propionate would inhibit mold growth in it.

Sterile Czapek's medium was measured into sterile test tubes with a sterile pipette. The compounds were added and thoroughly distributed, the following concentrations being employed: Propionic acid 1 per cent; calcium propionate 0.001, 0.01, 0.2, 0.4, 0.6, 0.8 and 5 per cent; and sodium propionate 5 per cent. The tubes were inoculated with a mold suspension prepared from a moldy cheese, held at room temperature and observed daily for mold growth. Each trial included a tube (the control) that was not treated with one of the compounds.

In the preliminary trials calcium propionate was used in the following concentrations: 0.001, 0.01, 0.1, 0.2, 0.4, 0.6 and 0.8 per cent. These concentrations were ineffective for inhibiting mold growth since growth was observed in many samples after 2 days and in all samples after 4 days; control samples showed mold growth after 2 days.

In a series of 40 trials propionic acid was used in a 1 per cent concentration and calcium and sodium propionates each in 5 per cent concentrations. The data are presented in Table 3.

The control samples showed mold growth after 2 to 3 days. The samples containing the propionic acid were held for 60 to 65 days without mold growth being evident. With the calcium propionate mold growth was evident after 12 to 30 days, and with the sodium propionate mold growth was evident after 4 to 14 days.

Action on butter

Since the presence of salt significantly affects the growth of molds on butter, the results obtained with butter are divided into three parts. These are: (a) Unsalted butter, (b) salted butter and (c) comparison of salted and unsalted butter. The pieces of butter were cut from prints with a knife that was clean but not sterile.

Unsalted butter. Five series of trials were conducted. Inoculations were made by spreading a mold suspension over a considerable portion of one face of each piece of butter. The data are given in Table 4.

Series 1. Each pound print of butter was cut into eight equal pieces, and these were inoculated with a mold suspension made from a portion of moldy butter. Two sets of samples were prepared, one to be held at 10°C. and one

Table 3. Inhibition of Molds in Czapek's Medium by Propionic Acid.

Compound used	Trial 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
	Days until mold was ev																
None-control	2	2	2	3	2	2	2	2	2	2	2	2	2	2	2	2	2
Propionic acid																	
1.0%	>65	>65	>65	>65	>65	>65	>65	>63	>63	>63	>63	>63	>63	>63	>63	>63	>6
Ca propionate:																	
5.0%	18	24	24	16	18	16	12	14	16	18	14	16	16	16	14	16	1
Na propionate:																	
5.0%	4	8	9	6	4	2	8	4	4	4	4	4	6	6	5	6	

y Propionic Acid, Calcium Propionate and Sodium Propionate

	Trial Number																					
13:	14:	15:	16:	17:	18:	19:	20:	21:	22:	23:	24:	25:	26:	27:	28:	29:	30:	31:	32:	33:		
s until mold was evident macroscopically																						
2 :	2 :	2 :	2 :	2 :	2 :	2 :	2 :	2 :	2 :	2 :	2 :	2 :	2 :	2 :	2 :	2 :	2 :	2 :	2 :	2 :	2 :	2 :
>63:>63:>63:>63:>63:>63:>63:>60:>60:>60:>60:>60:>60:>60:>60:>60:>60:>60:>60:>60:>60:>60:>60:>60:																						
16:	16:	14:	16:	16:	14:	24:	20:	20:	18:	19:	22:	20:	20:	26:	24:	30:	26:	20:	24:	26:		
6:	6:	5:	6:	6:	4:	12:	9:	8:	6:	6:	8:	7:	7:	12:	9:	14:	12:	6:	7:	7:		

onate and Sodium Propionate

21:	22:	23:	24:	25:	26:	27:	28:	29:	30:	31:	32:	33:	34:	35:	36:	37:	38:	39:	40
optically																			
2 :	2 :	2 :	2 :	2 :	2 :	2 :	2 :	2 :	2 :	2 :	2 :	2 :	2 :	2 :	2 :	2 :	2 :	2 :	2
>60:	>60:	>60:	>60:	>60:	>60:	>60:	>60:	>60:	>60:	>60:	>60:	>60:	>60:	>60:	>60:	>60:	>60:	>60:	>60
20:	18:	19:	22:	20:	20:	26:	24:	30:	26:	20:	24:	26:	28:	28:	30:	26:	26:	28:	28
8:	6:	6:	8:	7:	7:	12:	9:	14:	12:	6:	7:	7:	7:	9:	9:	7:	7:	7:	7

Table 4 — Inhibition of Molds on U

Series Number	Butter Treatment	Holding Temperature	1	2	3	4	5	6	7	8	9
											Days un
1	None = control	10°C.	15	14	22	22	20	18	17	18	21
	Dipped in 18% sol.	10°C.	34	27	33	28	22	30	30	34	26
	Dipped in 25% sol.	10°C.	36	33	38	28	22	33	32	36	38
	In treated parchment	10°C.	25	21	22	22	20	20	17	20	23
	None = control	15°C.	11	13	9	12	11	8	12	16	15
	Dipped in 18% sol.	15°C.	15	17	19	16	17	13	23	22	24
	Dipped in 25% sol.	15°C.	29	28	27	18	25	28	26	27	33
	In treated parchment	15°C.	15	21	13	16	13	15	14	18	17
2	None = control	10°C.	30	29	33	33	22	21	22	24	15
	Dipped in 18% sol.	10°C.	68	75	74	63	44	34	31	34	23
	Dipped in 25% sol.	10°C.	77	77	76	63	56	36	24	36	36
	None = control	15°C.	22	22	24	22	22	21	16	15	8
	Dipped in 18% sol.	15°C.	50	41	36	33	37	22	20	19	17
	Dipped in 25% sol.	15°C.	55	60	38	35	27	24	20	21	23
3	None = control	10°C.	18	22	37	41	32	24	26	24	
	Dipped in 12.8% sol.	10°C.	26	34	54	41	43	24	32	24	
	Dipped in 19.0% sol.	10°C.	38	46	56	51	46	27	36	28	
	Dipped in 25.0% sol.	10°C.	42	50	58	60	52	29	36	34	
4	None = control	10°C.	24	26	35	21	32	24	23	28	25
	In parchment dipped in 12.5% sol.	10°C.	24	56	68	21	73	24	23	33	25
	In parchment dipped in 19.0% sol.	10°C.	26	72	111	132	131	27	23	43	87
	In parchment dipped in 25.0% sol.	10°C.	26	72	112	136	131	27	23	73	87
5	None = control	10°C.	15	14	14	9	25	10	32	22	26
	In treated parchment	10°C.	20	14	29	9	25	10	32	25	26
	In parchment dipped in 12.5% sol.	10°C.	29	26	53	14	33	22	45	30	36
	In parchment dipped in 20.0% sol.	10°C.	42	28	63	14	49	25	48	34	39
	In parchment smeared with 20.0% sol.	10°C.	21	17	42	17	28	12	45	26	36

ibition of Molds on Unsalted Butter by Calcium Propionate

Trial Number																			
5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	2
Days until mold was evident macroscopically																				
20	18	17	18	21	14	15	26	20	17	18	13	12	11	12	11	12	14	15	14	
22	30	30	34	26	32	24	37	29	36	30	22	21	22	21	18	31	21	22	21	
22	33	32	36	38	34	36	39	32	43	32	22	30	22	31	27	31	23	25	28	
20	20	17	20	23	18	15	28	22	19	23	17	14	11	17	13	27	19	20	17	
11	8	12	16	15	14	15	16	18	17	18	13	12	11	10	9	10	14	13	14	
17	13	23	22	24	27	24	26	25	28	27	22	19	20	21	23	25	21	22	19	
25	28	26	27	33	30	30	35	27	28	37	29	26	22	26	23	45	23	25	28	
13	15	14	18	17	14	20	16	20	19	18	20	12	20	14	16	30	14	15	17	
22	21	22	24	15	18	17	22	19	23	20	19	16	15	13						
44	34	31	34	23	24	46	33	38	37	42	41	38	31	36						
56	36	24	36	36	33	51	46	46	49	44	45	40	43	43						
22	21	16	15	8	14	13	16	13	16	13	12	9	15	13						
37	22	20	19	17	22	21	22	19	26	31	31	24	30	25						
27	24	20	21	23	24	28	30	27	26	34	31	28	33	36						
32	24	26	24																	
43	24	32	24																	
46	27	36	28																	
52	29	36	34																	
32	24	23	28	25	24	26	20	35	35	27	41									
73	24	23	33	25	29	44	20	53	50	42	41									
131	27	23	43	87	84	62	44	86	92	96	89									
131	27	23	73	87	92	86	88	86	126	112	89									
25	10	32	22	26	26															
25	10	32	25	26	26															
33	22	45	30	36	30															
49	25	48	34	39	34															
28	12	45	26	36	28															

ed Butter by Calcium Propionate

Trial Number																	
1:	12:	13:	14:	15:	16:	17:	18:	19:	20:	21:	22:	23:	24:	25:	26:	27:	28:	29
old was evident macroscopically																		
5:	26:	20:	17:	18:	13:	12:	11:	12:	11:	12:	14:	15:	14:	16:	13:	12:	15:	17
4:	37:	29:	36:	30:	22:	21:	22:	21:	18:	31:	21:	22:	21:	23:	22:	28:	24:	28
6:	39:	32:	43:	32:	22:	30:	22:	31:	27:	31:	23:	25:	28:	30:	27:	33:	27:	31
5:	28:	22:	19:	23:	17:	14:	11:	17:	13:	27:	19:	20:	17:	18:	17:	14:	17:	21
5:	16:	18:	17:	18:	13:	12:	11:	10:	9:	10:	14:	13:	14:	18:	15:	16:	15:	14
4:	26:	25:	28:	27:	22:	19:	20:	21:	23:	25:	21:	22:	19:	27:	20:	21:	20:	37
0:	35:	27:	28:	37:	29:	26:	22:	26:	23:	45:	23:	25:	28:	41:	27:	39:	27:	37
0:	16:	20:	19:	18:	20:	12:	20:	14:	16:	30:	14:	15:	17:	20:	17:	19:	17:	19
7:	22:	19:	23:	20:	19:	16:	15:	13:										
6:	33:	38:	37:	42:	41:	38:	31:	36:										
1:	46:	46:	49:	44:	45:	40:	43:	43:										
3:	16:	13:	16:	13:	12:	9:	15:	13:										
1:	22:	19:	26:	31:	31:	24:	30:	25:										
8:	30:	27:	26:	34:	31:	28:	33:	36:										
6:	20:	35:	35:	27:	41:													
4:	20:	53:	50:	42:	41:													
2:	44:	86:	92:	96:	89:													
6:	88:	86:	126:	112:	89:													

at 15°C. One sample of each set was held as a control and others were dipped in 18 and 25 per cent solutions of calcium propionate; these samples were wrapped in regular parchment. The remaining sample in each set was wrapped in commercially treated parchment (containing 6 per cent calcium propionate). All samples were observed daily for mold growth.

At 10°C. the control samples showed mold growth after 11 to 26 days. Samples dipped in 18 per cent calcium propionate solution showed mold growth after 18 to 37 days; samples dipped in 25 per cent calcium propionate solution, after 22 to 43 days; and samples wrapped in commercially treated parchment, after 11 to 28 days. At 15°C. the control samples showed mold growth after 8 to 18 days. Samples dipped in 18 per cent calcium propionate solution showed mold growth after 13 to 37 days; samples dipped in 25 per cent calcium propionate solution, after 18 to 41 days; and samples wrapped in commercially treated parchment, after 13 to 30 days.

Series 2. The samples were prepared and held in the same manner as in Series 1 except that each pound print of butter was cut into six equal pieces and commercially treated parchment was not used.

At 10°C. control samples showed mold growth after 13 to 33 days; samples dipped in 18 per cent calcium propionate

solution, after 24 to 75 days; and samples dipped in 25 per cent calcium propionate solution, after 24 to 77 days. At 15°C. control samples showed mold growth after 8 to 24 days; samples dipped in 18 per cent calcium propionate solution, after 17 to 50 days; and samples dipped in 25 per cent calcium propionate solution, after 20 to 60 days.

Series 3. Each pound print of butter was cut into four equal pieces and inoculated with a mold suspension prepared from a plate poured for a mold count on butter. One sample of each set was held as a control and others were dipped in 12.5, 19 and 25 per cent solutions of calcium propionate. All samples were wrapped in regular parchment. The samples were held at 10°C. and observed daily for mold growth.

The control samples showed mold growth after 18 to 41 days. Samples dipped in 12.5 per cent calcium propionate solution showed mold growth after 24 to 54 days; samples dipped in 19 per cent calcium propionate solution, after 27 to 56 days; and samples dipped in 25 per cent calcium propionate solution, after 29 to 60 days.

Series 4. Each pound print of butter was cut into four equal pieces and inoculated with a mold suspension prepared with mold from the shelves in a cheese curing-room. One sample of each set was held as a control. Parchments were dipped in solutions of 12.5, 19 and 25 per cent calcium propionate, squeezed as dry as possible and used

to wrap the other samples. All the samples were held at 10°C. and observed daily for mold growth.

The control samples showed mold growth after 20 to 41 days. Samples wrapped in parchment dipped in 12.5 per cent calcium propionate solution showed mold growth after 20 to 73 days; samples wrapped in parchment dipped in 19 per cent calcium propionate solution, after 23 to 132 days; and samples wrapped in parchment dipped in 25 per cent calcium propionate solution, after 23 to 136 days.

Series 5. In order to have butter naturally contaminated with relatively small numbers of molds, raw sweet cream was churned in a Dazey churn. The butter was made into pound prints and each was cut into five equal pieces. One sample was held as a control. One sample was wrapped in commercially treated parchment (containing 6 per cent calcium propionate), one was wrapped in parchment dipped in a 15 per cent solution of calcium propionate, one was wrapped in parchment dipped in a 25 per cent solution of calcium propionate and one was wrapped in regular parchment that was smeared with a 20 per cent solution of calcium propionate. The samples were held at 10°C. and observed daily for mold growth.

Control samples showed mold growth after 9 to 32 days. Samples wrapped in commercially treated parchment showed growth after 9 to 32 days; samples wrapped in parchment

dipped in 15 per cent calcium propionate solution, after 14 to 53 days; samples wrapped in parchment dipped in 25 per cent calcium propionate solution, after 14 to 63 days; and samples wrapped in parchment smeared with 20 per cent calcium propionate solution, after 12 to 45 days.

The inhibition of molds on unsalted butter by calcium propionate is illustrated in Figure 1.

Salted butter. The trials were arranged in two series; the results of these are given in Table 5.

Series 1. Each pound print of butter was cut into four equal pieces and inoculated with a mold suspension prepared from a portion of moldy butter. One sample was held as a control and the others were dipped in 12.5, 19 and 25 per cent solutions of calcium propionate. All the samples were wrapped in regular parchment, held at 10°C. and observed daily for mold growth.

The control samples showed mold growth in 16 to 40 days. Samples dipped in 12.5 per cent calcium propionate solution showed mold growth after 21 to 50 days; samples dipped in 19 per cent calcium propionate solution, after 28 to 60 days; and samples dipped in 25 per cent calcium propionate solution, after 33 to 66 days.

Series 2. The pound prints of butter were each cut into four equal pieces and inoculated with a mold suspension prepared from a plate poured for a mold count on butter. One sample was held as a control. Parchments

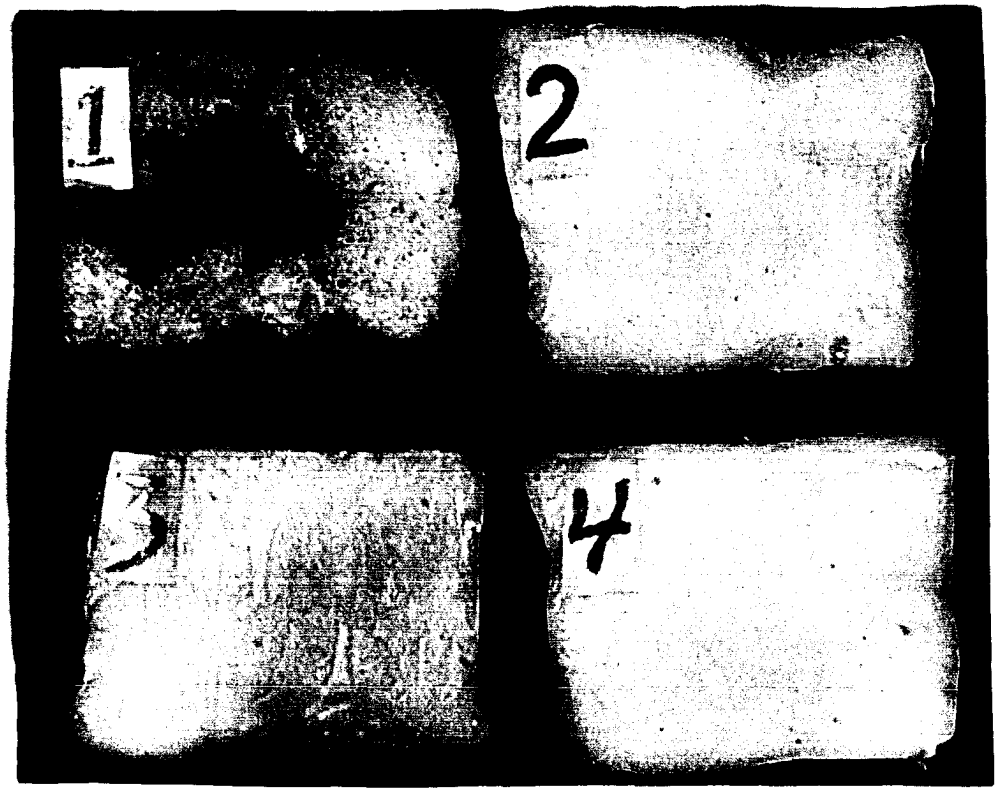

Figure 1. The inhibition of molds on unsalted butter by calcium propionate. The pieces of butter were inoculated; 1 was untreated, 2 was dipped in a 12.5 per cent solution of the salt, 3 was dipped in a 19 per cent solution and 4 was dipped in a 25 per cent solution. The butter was held 37 days at 10°C.

Table 5. Inhibition of Molds on Salted Butter by Calcium Propionate
Holding Temperature 10°C.

Series Number	Butter Treatment	Trial Number 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
		Days until mold was evident macroscopically														
1	None = control	28	27	26	26	34	40	31	21	20	23	20	26	20	28	16
	Dipped in 12.5% sol.	28	36	50	35	54	40	42	21	23	30	32	38	32	28	22
	Dipped in 19.0% sol.	58	57	56	54	60	51	48	33	34	36	28	42	40	42	28
	Dipped in 25.0% sol.	47	57	66	58	68	53	56	33	36	34	36	38	46	40	40
2	None = control	28	28	26	26	47	36	41	21	30						
	In parchment dipped in 12.5% sol.	28	29	62	26	59	38	53	24	30						
	In parchment dipped in 19.0% sol.	89	145	88	65	115	44	128	123	32						
	In parchment dipped in 25.0% sol.	86	145	88	89	115	44	128	132	32						

were dipped in 12.5, 19 and 25 per cent solutions of calcium propionate, squeezed as dry as possible and used to wrap the other samples. All the samples were held at 10°C. and observed daily for mold growth.

Control samples showed mold growth after 21 to 47 days. Samples wrapped in parchment dipped in 12.5 per cent calcium propionate solution showed mold growth after 24 to 62 days; samples wrapped in parchment dipped in 19 per cent calcium propionate solution, after 32 to 145 days; and samples wrapped in parchment dipped in 25 per cent calcium propionate solution, after 32 to 145 days.

Figure 2 illustrates the inhibition of molds on salted butter by calcium propionate.

Comparison of salted and unsalted butter. Four series of trials were conducted. Tables 6 and 7 give the results.

Series 1. Butter was churned from raw sweet cream in a Dazey churn and a pound print of salted butter and a pound print of unsalted butter were prepared from it. Each print was cut into five equal pieces. With both the salted and unsalted butter, one sample was held as a control while the others were dipped in 18 and 25 per cent calcium propionate solutions and 18 and 25 per cent sodium propionate solutions. All the samples were wrapped in regular parchment. They were held at 10°C. and observed daily for mold growth.

Control samples showed mold growth with salted butter

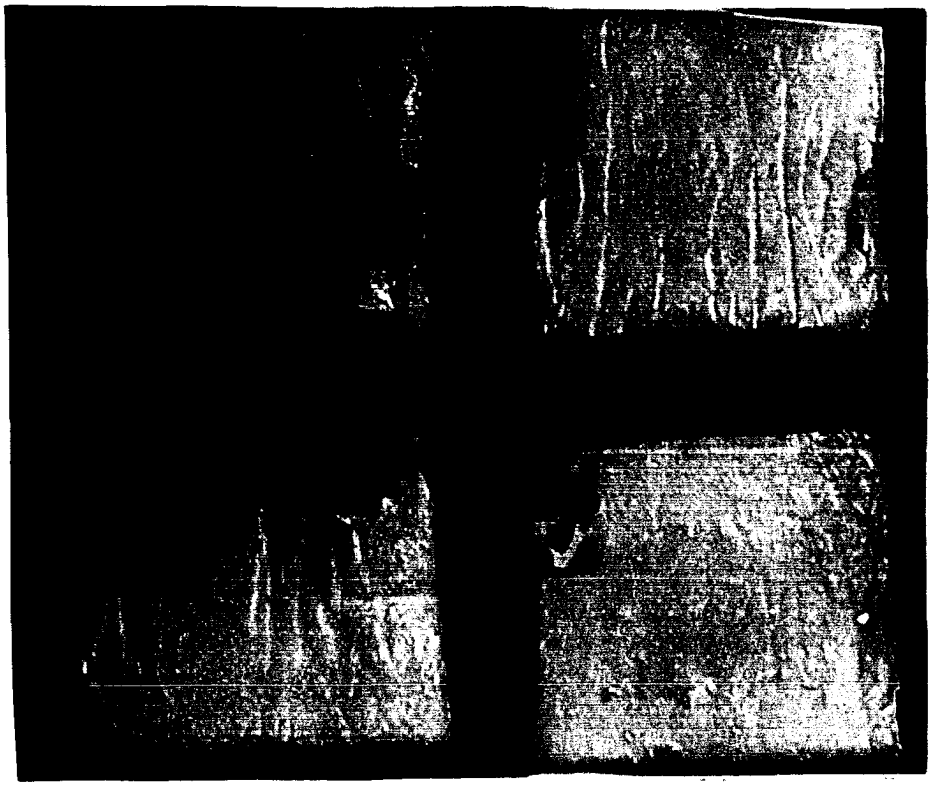

Figure 2. The inhibition of molds on salted butter by calcium propionate. The pieces of butter were inoculated; 1 was untreated, 2 was dipped in a 12.5 per cent solution of the salt, 3 was dipped in a 19 per cent solution and 4 was dipped in a 25 per cent solution. The butter was held 37 days at 10°C.

Table 6. Inhibition of Molds on Salted and Unsalted Butter by Propionic Acid, Calcium Propionate, Sodium Propionate and Treated Parchment Holding Temperature 10°C.

Series Number	Butter Treatment	Type of Butter	Trial Number 1	2	3	4	5	6	7	8	9	10
			Days until mold was evident macroscopically									
1	None = control	Salted	23	22	21	23	24	27	19	20	20	20
		Unsalted	23	22	21	21	19	15	19	17	15	17
	Dipped in 18% sol. Ca propionate	Salted	27	32	33	34	29	32	29	24	23	29
		Unsalted	27	29	33	31	29	32	24	25	20	27
	Dipped in 25% sol. Ca propionate	Salted	33	37	39	45	32	34	31	30	29	31
		Unsalted	33	37	37	45	32	41	29	30	27	31
	Dipped in 18% sol. Na propionate	Salted	23	32	33	33	29	30	29	24	23	25
		Unsalted	23	22	33	31	29	32	21	25	25	25
	Dipped in 25% sol. Na propionate	Salted	33	37	39	35	32	34	31	29	23	33
		Unsalted	27	32	39	45	32	34	27	27	20	27
2	None = control	Salted	28	23	24	29	36	37	42	32	29	24
		Unsalted	7	14	18	7	6	14	12	19	21	18
	Dipped in 5% sol. propionic acid	Salted	73	67	59	68	64	61	60	48	52	59
		Unsalted	12	17	23	12	33	51	56	46	35	36
	Dipped in 18% sol. Ca propionate	Salted	71	64	59	63	64	61	60	48	38	55
		Unsalted	21	17	23	10	16	51	18	37	30	30
	In treated parchment (6%)	Salted	52	62	51	27	46	42	44	32	38	24
		Unsalted	12	12	20	9	7	18	14	16	20	16
	In treated parchment (25%)	Salted	52	60	48	42	42	44	48	32	36	24
		Unsalted	11	14	18	7	11	18	14	16	20	16
	In treated parchment (30%)	Salted	48	62	46	42	46	40	42	36	38	24
		Unsalted	12	12	16	7	7	18	16	16	16	18
3	None = control	Salted	41	56	64	67	61					
		Unsalted	20	32	35	11	21					
	Dipped in 6% sol. Ca propionate	Salted	59	63	88	86	65					
		Unsalted	26	46	46	26	29					
	Dipped in 12% sol. Ca propionate	Salted	62	67	88	88	74					
		Unsalted	31	48	42	24	36					
	Dipped in 18% sol. Ca propionate	Salted	69	83	96	98	77					
		Unsalted	36	58	66	28	44					
	In treated parchment (6%)	Salted	46	58	72	72	63					
		Unsalted	20	34	37	18	23					

Table 7. Inhibition of Molds on Salted and Unsalted Butter by Calcium Propionate and Propionate Treated Parchment with Different Inoculation Holding Temperature 15°C.

Butter Treatment	Type of Butter	Inoculation	Trial Number 1	Trial Number 2
			Days until mold was evident macroscopically	
None = control	Salted	1X	30	15
	Unsalted	0.01X	17	15
	Salted	0.001X	30	15
	Unsalted	1X	24	22
	Salted	0.01X	40	18
	Unsalted	0.001X	24	19
Dipped in 18% sol.	Salted	1X	30	17
	Unsalted	0.01X	18	15
	Salted	0.001X	30	22
	Unsalted	1X	24	15
	Salted	0.01X	48	20
	Unsalted	0.001X	24	18
Dipped in 25% sol.	Salted	1X	52	34
	Unsalted	0.01X	28	26
	Salted	0.001X	56	31
	Unsalted	1X	42	26
	Salted	0.01X	52	36
	Unsalted	0.001X	45	25
In treated parchment	Salted	1X	49	26
	Unsalted	0.01X	26	24
	Salted	0.001X	45	28
	Unsalted	1X	30	22
	Salted	0.01X	48	26
	Unsalted	0.001X	24	25

after 19 to 27 days and with unsalted butter after 15 to 23 days. Samples dipped in 18 per cent calcium propionate solution showed mold growth with salted butter after 23 to 30 days and with unsalted butter after 20 to 33 days; samples dipped in 25 per cent calcium propionate solution showed growth with salted butter after 29 to 45 days and with unsalted butter after 30 to 45 days; samples dipped in 18 per cent sodium propionate solution showed growth with salted butter after 23 to 33 days and with unsalted butter after 21 to 33 days; and samples dipped in 25 per cent sodium propionate showed growth with salted butter after 23 to 39 days and with unsalted butter after 20 to 45 days.

Series 2. A portion of butter was taken from the churn in the butter laboratory before the salt was added and made into a pound print with the butter printer used in the laboratory. A pound print of salted butter from the same churning was obtained after the churning was printed. Each of the prints was cut into six equal pieces and inoculated with a mold suspension prepared from a portion of moldy butter. With each type of butter one sample was wrapped in regular parchment and held as a control; one sample was wrapped in parchment dipped in a 5 per cent solution of propionic acid; one sample was wrapped in parchment dipped in an 18 per cent solution of calcium propionate; three samples were wrapped

in commercially treated parchment (containing 10, 25 and 30 per cent calcium propionate). All the samples were held at 15°C. and observed daily for mold growth.

Control samples showed mold growth with salted butter after 23 to 42 days and with unsalted butter after 7 to 21 days. Samples wrapped in parchment dipped in 5 per cent propionic acid solution showed mold growth with salted butter after 48 to 73 days and with unsalted butter after 12 to 56 days; samples wrapped in parchment dipped in 18 per cent calcium propionate solution showed growth with salted butter after 38 to 71 days and with unsalted butter after 10 to 37; samples wrapped in commercially treated parchment containing 10 per cent calcium propionate showed mold growth with salted butter after 24 to 62 days and with unsalted butter after 7 to 20 days; samples wrapped in commercially treated parchment containing 25 per cent calcium propionate showed growth with salted butter after 24 to 60 days and with unsalted butter after 7 to 20 days; and samples wrapped in commercially treated parchment containing 30 per cent calcium propionate showed growth with salted butter after 24 to 62 days and with unsalted butter after 7 to 18 days.

Series 3. Prints of salted and unsalted butter, obtained in the same manner as those for Series 2, were each cut into five equal pieces. One sample of each type of butter was wrapped in regular parchment and held as a

control, others were dipped in 6, 12 and 18 per cent solutions of calcium propionate and wrapped in regular parchment and one sample was wrapped in commercially treated parchment (containing 6 per cent calcium propionate). The samples were held at 15°C. and observed daily for mold growth.

Control samples showed mold growth with salted butter after 41 to 67 days and with unsalted butter after 11 to 35 days. Samples dipped in 6 per cent calcium propionate solution showed mold growth with salted butter after 59 to 88 days and with unsalted butter after 26 to 46 days; samples dipped in 12 per cent calcium propionate solution showed growth with salted butter after 62 to 88 days and with unsalted butter after 24 to 48 days; samples dipped in 18 per cent calcium propionate solution showed mold growth with salted butter after 69 to 98 days and with unsalted butter, after 28 to 66 days; and samples wrapped in commercially treated parchment showed growth with salted butter after 46 to 72 days and with unsalted butter after 18 to 37 days.

Series 4. Six pound prints of salted butter and six of unsalted butter were obtained in the same manner as those for Series 2. The prints were cut in half and the portions treated as follows: (a) Four salted and four unsalted one-half pound samples were inoculated with 1

ml. of a mold suspension prepared from a portion of moldy butter. One sample of each type of butter was wrapped in regular parchment and held as a control; others were dipped in 15 and 25 per cent solutions of calcium propionate and wrapped in regular parchment; and one sample was wrapped in commercially treated parchment (containing 6 per cent calcium propionate). (b) Samples were prepared and treated exactly as in (a) except that they were inoculated with 1 ml. of a 0.01 dilution of the mold suspension. (c) Samples were prepared and treated exactly as in (a) except that they were inoculated with 1 ml. of a 0.001 dilution of the mold suspension. All the samples were held at 10°C. and observed daily for mold growth.

Diluting the mold suspension used for inoculation had no definite effect (Table 7) on the development of mold on the butter. If the results with the different inoculations are grouped together, control samples showed mold growth with salted butter after 15 to 40 days and with unsalted butter after 15 to 24 days; samples dipped in 15 per cent calcium propionate solution showed mold growth with salted butter after 17 to 48 days and with unsalted butter after 15 to 24 days; samples dipped in 25 per cent calcium propionate solution showed growth with salted butter after 31 to 56 days and with unsalted butter after 25 to 45 days; and samples wrapped in commercially treated parchment

showed growth with salted butter after 26 to 49 days and with unsalted butter after 22 to 30 days.

Action on cheddar cheese

Since the growth of mold in some types of cheese is undesirable and the cause of considerable loss, four series of trials were conducted with portions of cheddar cheese.

The portions were cut from longhorn cheese with a knife that was clean but not sterile, care being taken to eliminate all of the outer surface of the original cheese. In three series of trials the cheese was cut into portions approximately 3 inches square and 0.5 inch thick and in the fourth series the portions were approximately 5 inches square and 1 inch thick. Inoculations were made by spreading a mold suspension over a considerable part of one surface of each portion of cheese. The data are given in Tables 8 and 9.

Series 1 (Table 8). Samples of cheese were inoculated with a mold suspension from a piece of moldy cheese. Two sets of samples were prepared, one to be held at 10°C. and one at 15°C. One sample of each set was held as a control, and others were dipped in 10, 18 and 25 per cent solutions of calcium propionate. All the samples were wrapped in tinfoil. Observations for mold growth were made daily.

Table 8. Inhibition of Molds on Cheddar Cheese t

Series Number	Cheese Treatment	Holding Temperature	Wrapped in	Tr: 1:	2:	3:	4:	5:	6:	7:	8:	9:	10:	11:
				Days until mold was										
1	None = control	10°C.	Tin foil	7:	7:	6:	5:	4:	6:	9:	12:	7:	10:	18:
	Dipped in 10% sol.	10°C.	Tin foil	17:	16:	12:	11:	12:	21:	12:	12:	7:	19:	18:
	Dipped in 18% sol.	10°C.	Tin foil	40:	36:	33:	39:	42:	39:	62:	36:	34:	41:	74:
	Dipped in 25% sol.	10°C.	Tin foil	40:	52:	62:	48:	53:	36:	34:	60:	74:	41:	74:
	None = control	15°C.	Tin foil	3:	4:	4:	4:	3:	5:	6:	8:	6:	6:	9:
	Dipped in 10% sol.	15°C.	Tin foil	8:	10:	10:	9:	9:	8:	9:	10:	8:	6:	9:
	Dipped in 18% sol.	15°C.	Tin foil	38:	36:	53:	44:	27:	32:	46:	50:	26:	24:	28:
	Dipped in 25% sol.	15°C.	Tin foil	52:	30:	43:	47:	37:	29:	37:	56:	26:	28:	28:
2	None = control	10°C.	Parchment	15:	14:	15:	12:	11:	8:	7:	6:	5:	6:	5:
	Dipped in 18% sol.	10°C.	Parchment	43:	58:	63:	33:	44:	29:	28:	22:	28:	25:	24:
	Dipped in 25% sol.	10°C.	Parchment	57:	72:	74:	40:	53:	34:	49:	32:	31:	37:	31:
	None = control	15°C.	Parchment	9:	10:	10:	6:	5:	4:	3:	6:	5:	4:	3:
	Dipped in 18% sol.	15°C.	Parchment	31:	57:	50:	33:	33:	27:	19:	22:	31:	27:	24:
	Dipped in 25% sol.	15°C.	Parchment	57:	59:	55:	22:	38:	41:	32:	27:	28:	27:	24:

lon of Molds on Cheddar Cheese by Calcium Propionate

Trial Number																										
1:	2:	3:	4:	5:	6:	7:	8:	9:	10:	11:	12:	13:	14:	15:	16:	17:	18:	19:	20:	21:	22:	23:	24:	25:	26:	27:	28:
Days until mold was evident macroscopically																											
7:	7:	6:	5:	4:	6:	9:	12:	7:	10:	18:	18:	16:	15:	12:	11:	13:	8:	7:	11:	12:	13:	12:	11:	12:	9:	11:	8
7:	16:	12:	11:	12:	21:	12:	12:	7:	19:	18:	27:	26:	15:	12:	21:	20:	10:	10:	21:	22:	19:	26:	32:	31:	30:	28:	29
0:	36:	33:	39:	42:	39:	62:	36:	34:	41:	74:	77:	35:	36:	34:	32:	39:	27:	26:	28:	44:	46:	45:	60:	69:	49:	63:	37
0:	52:	62:	48:	53:	36:	34:	60:	74:	41:	74:	77:	46:	51:	34:	62:	61:	42:	37:	42:	44:	52:	48:	72:	72:	52:	47:	53
3:	4:	4:	4:	3:	5:	6:	8:	6:	6:	9:	17:	16:	15:	15:	11:	10:	8:	6:	6:	5:	5:	6:	9:	8:	11:	11:	8
8:	10:	10:	9:	9:	8:	9:	10:	8:	6:	9:	17:	16:	15:	25:	11:	20:	10:	10:	8:	7:	29:	18:	26:	25:	22:	24:	19
8:	36:	53:	44:	27:	32:	46:	50:	26:	24:	28:	62:	61:	25:	28:	45:	35:	15:	14:	40:	33:	42:	49:	39:	60:	56:	34:	48
2:	30:	43:	47:	37:	29:	37:	56:	26:	28:	28:	62:	61:	25:	25:	60:	31:	19:	35:	42:	44:	42:	51:	55:	62:	61:	40:	60
5:	14:	15:	12:	11:	8:	7:	6:	5:	6:	5:	7:	6:	8:	7:	6:	9:	8:	7:	6:	3:	5:	4:	3:	2:	3:	5:	4
3:	58:	63:	33:	44:	29:	28:	22:	28:	25:	24:	33:	20:	25:	18:	27:	21:	22:	19:	27:	24:	21:	20:	21:	16:	22:	19:	18
7:	72:	74:	40:	53:	34:	49:	32:	31:	37:	31:	40:	32:	28:	37:	29:	56:	29:	21:	32:	27:	23:	27:	26:	30:	35:	28:	27
9:	10:	10:	6:	5:	4:	3:	6:	5:	4:	3:	5:	4:	5:	4:	4:	3:	4:	3:	4:	1:	2:	1:	3:	2:	4:	2:	4
1:	57:	50:	33:	33:	27:	19:	22:	31:	27:	24:	26:	25:	20:	16:	22:	26:	18:	21:	23:	22:	14:	20:	19:	18:	16:	16:	18
7:	59:	55:	22:	38:	41:	32:	27:	28:	27:	24:	35:	29:	22:	27:	36:	40:	27:	24:	32:	24:	23:	25:	24:	25:	25:	19:	27

Table 9. Inhibition of Molds on Cheddar Cheese by Propionic Acid, Calcium Propionate, Sodium Propionate and Propionate Treated Parchment
Holding Temperature 10°C.

Series Number	Compound Used	Wrapped in	Trial Number 1	2	3	4	5	6	7	8
			Days until mold was evident macroscopically							
1	None = control	Parchment	5	4	5	5	4	4	4	5
	Propionic acid									
	5%	Parchment	10	9	9	9	8	9	8	9
	7%	Parchment	12	13	12	12	11	13	12	14
	10%	Parchment	17	17	17	15	15	16	16	21
	Ca propionate									
	18%	Parchment	12	13	14	15	15	16	15	21
	Na propionate									
	18%	Parchment	13	13	14	15	15	16	15	16
	In treated parchment:									
	10%	Parchment	7	7	9	6	4	6	8	5
	25%	Parchment	7	6	9	6	4	7	6	5
	30%	Parchment	7	7	7	7	2	6	6	6
2	None = control	Parchment	2	3	4	3	2	3		
	Ca propionate									
	12%	Tin foil	5	10						
	12%	Cellophane	8	14	12	13	12	13		
	18%	Tin foil	10	12						
	18%	Cellophane	12	16	16	16	16	15		
	Na propionate									
	12%	Tin foil	5	8						
	12%	Cellophane	8	12	12	9	12	9		
	18%	Tin foil	8	8						
	18%	Cellophane	8	16	16	16	16	16		
	In treated parchment:									
	6%	Parchment	2	3						

At 10°C. control samples showed mold growth after 4 to 18 days. Samples dipped in 10 per cent calcium propionate solution showed mold growth after 7 to 32 days; samples dipped in 18 per cent calcium propionate solution, after 26 to 77 days; and samples dipped in 25 per cent calcium propionate solution, after 34 to 77 days. At 15°C. control samples showed mold growth after 3 to 17 days. Samples dipped in 10 per cent calcium propionate solution showed mold growth after 6 to 29 days; samples dipped in 18 per cent calcium propionate solution, after 14 to 62 days; and samples dipped in 25 per cent calcium propionate solution, after 19 to 62 days.

Series 2 (Table 8). Samples were prepared and held in the same manner as in Series 1 except that no inoculations were made, 10 per cent calcium propionate was not used and the portions were wrapped in parchment.

At 10°C. control samples showed mold growth after 2 to 15 days; samples dipped in 18 per cent calcium propionate solution, after 16 to 63 days; and samples dipped in 25 per cent calcium propionate solution, after 21 to 74 days. At 15°C. control samples showed mold growth after 1 to 10 days; samples dipped in 18 per cent calcium propionate solution, after 14 to 57 days; and samples dipped in 25 per cent calcium propionate solution, after 19 to 59 days.

Series 1 (Table 9). No inoculations were used. Only one set of samples was prepared and it was held at 10°C.

Each trial included a control sample and samples dipped in solutions of 5, 7 and 10 per cent propionic acid, 18 per cent calcium propionate and 18 per cent sodium propionate; these samples were wrapped in regular parchment. Samples also were wrapped in commercially treated parchment (containing 10, 25 and 30 per cent calcium propionate).

Control samples showed mold growth after 4 to 5 days. Samples dipped in 5 per cent propionic acid solution showed mold growth after 8 to 10 days; samples dipped in 7 per cent propionic acid solution, after 11 to 14 days; samples dipped in 10 per cent propionic acid solution, after 15 to 21 days; samples dipped in 18 per cent calcium propionate solution after 12 to 21 days; and samples dipped in 18 per cent sodium propionate solution, after 13 to 16 days. Samples wrapped in commercially treated parchment showed mold growth as follows: With 10 per cent calcium propionate, after 4 to 9 days; with 25 per cent calcium propionate, after 4 to 9 days; and with 30 per cent calcium propionate, after 2 to 7 days.

Series 2.(Table 9). No inoculations were used. All trials included a control sample; two trials included samples dipped in 12 and 18 per cent calcium propionate and 12 and 18 per cent sodium propionate solutions and wrapped in tin foil; all trials included samples similarly dipped but wrapped in cellophane; and two trials included

samples wrapped in commercially treated parchment (containing 6 per cent calcium propionate). The samples were held at 10°C. and observed daily.

Control samples showed mold growth after 2 to 4 days. Samples dipped in 12 and 18 per cent calcium and sodium propionate solutions and wrapped in tin foil showed mold growth after 5 to 12 days. Samples dipped in calcium and sodium propionate solutions and wrapped in cellophane showed mold growth as follows: 12 per cent calcium propionate, after 8 to 14 days; 18 per cent calcium propionate, after 12 to 16 days; 12 per cent sodium propionate, after 8 to 12 days; and 18 per cent sodium propionate, after 8 to 16 days. Samples wrapped in treated parchment showed mold growth after 2 to 3 days.

The inhibition of molds on cheddar cheese by propionic acid, calcium propionate and sodium propionate is illustrated in Figure 3.

Action on swiss-type cheese

The rather characteristic flavor of swiss cheese and some of the closely related types is due, in part, to salts of propionic acid, calcium propionate probably being the most important. Propionic acid is formed during the ripening process by organisms of the genus Propionibacterium, that are either naturally present

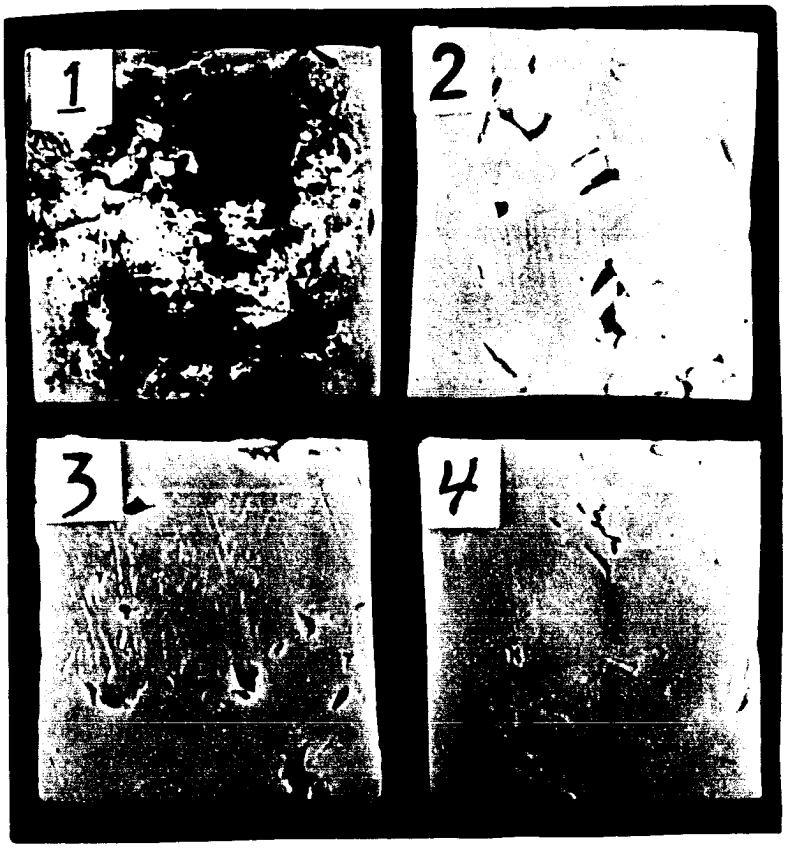

Figure 3. The inhibition of molds on cheddar cheese by propionic acid, calcium propionate and sodium propionate. The pieces of cheese were not inoculated; 1 was untreated, 2 was dipped in a 10 per cent solution of propionic acid, 3 was dipped in a 15 per cent solution of calcium propionate and 4 was dipped in a 15 per cent solution of sodium propionate. The cheese was held 21 days at 10°C.

in the milk or are added to it, and then largely combines to form salts.

Two trials were conducted with a swiss-type cheese made from pasteurized milk to which large numbers of propionic acid producing organisms had been added. In each trial the cheese was cut into portions approximately 5 inches square and 1 inch thick, using a knife that was clean but not sterile; the outer surface of the original cheese was always eliminated. One sample was held as a control and other samples were dipped in solutions of 10 per cent propionic acid and 18 per cent calcium propionate. The samples were wrapped in parchment, held at 10°C. and observed daily for mold growth.

Control samples showed mold growth after 2 days; samples dipped in 10 per cent propionic acid solution, after 9 to 10 days; and samples dipped in 18 per cent calcium propionate solution, after 8 days.

Action on cottage cheese

Cottage cheese usually is sold before there is time for molds to develop on it to any great extent, but this product furnishes a good medium for mold growth. A series of 16 trials was conducted with it.

Samples of 100 grams of freshly creamed cottage cheese

Table 10. Inhibition of Molds on Cottage Cheese by Propionic Acid, Calcium Propionate and Sodium Propionate

Compound Used	Trial Number															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	Days until mold was evident macroscopically															
None = control	5	3	3	3	5	3	2	2	4	6	4	4	5	4	2	2
Propionic acid																
7% sol. spread on cheese	7	5	6	5	6	3	4	6	6	7	7	6	6	4	3	5
7% sol. mixed with cheese*	22	29	26	18	25	25	11	22	22	28	27	19	25	24	12	26
Ca propionate																
18% sol. spread on cheese	9	7	6	5	6	7	6	5	8	9	7	6	6	9	6	5
18% sol. mixed with cheese*	22	24	21	18	18	25	12	15	22	23	22	19	18	24	12	14
Na propionate																
18% sol. spread on cheese	9	17	6	4	4	4	5	6	8	8	7	6	6	4	4	4
18% sol. mixed with cheese*	20	22	19	16	16	16	10	15	20	21	20	17	16	16	10	12

* 10 ml. per 100 gm. cheese

were weighed into 4 ounce paper ice cream cups. Neither the cups nor the spoon used for dipping the cheese were sterile. The samples of cheese were treated with solutions of 7 per cent propionic acid, 18 per cent calcium propionate and 18 per cent sodium propionate. With one set of samples the surfaces of the cheese were smeared with the solutions by means of sterile cotton dipped into them, while with another set 10 ml. quantities of the solutions were thoroughly distributed through the cheese. Samples were held at room temperature and observed daily for mold growth. The data are presented in Table 10.

The control samples showed mold growth after 2 to 5 days. Samples smeared on top with 7 per cent solution of propionic acid showed mold growth after 3 to 7 days and samples in which the solution was mixed with cheese after 11 to 29 days. Samples smeared on top with 18 per cent solution of calcium propionate showed mold growth after 5 to 9 days and samples in which the solution was mixed with the cheese after 12 to 25 days. Samples smeared on top with 18 per cent solution of sodium propionate showed mold growth after 4 to 17 days and samples in which the solution was mixed with the cheese after 10 to 22 days.

Action of Special Compounds

With the increased interest in the prevention of mold

growth on food products, various compounds have been suggested for use as mold inhibitors. At least some of these have a destructive action on bacteria which indicates that they also may destroy or inhibit molds. Action of a number of the compounds was investigated in studies on cream, Czapek's medium, salted butter and cheddar cheese.

Methyl, ethyl, propyl, butyl and benzyl derivatives of para-hydroxy benzoic acid and servex emulsion (a mixture of the five), known as paracepts, were obtained from the Hayden Chemical Co. An industrial solution of higher molecular alkyl-dimethyl-benzyl-ammonium chlorides, known as zephiran, was obtained from the Alba Pharmaceutical Co. Sodium benzoate was a regular commercial product. Calcium and sodium propionates were included for comparisons.

Action on raw cream

Samples of raw cream of 50 grams each were weighed into sterile 2-ounce, cork stoppered bottles. One sample was held as a control. The compounds were added and distributed so that the cream contained: 0.1 per cent of each of the paracepts; 5 per cent zephiran, 0.5 and 1 per cent sodium benzoate, 1 per cent calcium propionate and 1 per cent sodium propionate. The samples were held at room temperature and observed daily for mold growth. Table 11 gives the results of eight trials.

Table 11. Inhibition of Molds on Raw Cream by Various Compounds

Compound used	Trial Number							
	1	2	3	4	5	6	7	8
	Days until mold was evident macroscopically							
None = control	2	2	2	2	2	2	3	2
Paracepts								
Methyl 1%	12	20	7	6	9	7	7	12
Ethyl 1%	4	4	5	4	3	4	5	4
Propyl 1%	4	3	4	4	5	4	5	5
Butyl 1%	4	3	4	4	3	4	4	5
Benzyl 1%	4	2	4	2	3	3	5	4
Servex emulsion 1%	4	2	4	2	3	3	5	2
Zephiran 5%	4	5	5	5	4	2	3	4
Na benzoate 0.5%	27	26	27	24	24	20	27	20
Na benzoate 1%	27	26	30	24	28	22	28	26
Ca propionate 1%	21	26	32	26	32	28	24	26
Na propionate 1%	8	8	20	16	8	10	14	13

Control samples showed mold growth after 2 to 4 days. Samples treated with 0.1 per cent methyl paracept showed growth after 6 to 20 days; samples treated with 0.1 per cent ethyl, propyl, butyl, benzyl and servex emulsion paracepts, after 2 to 5 days; samples treated with 5 per cent zephiran, after 2 to 5 days; samples treated with 0.5 and 1 per cent sodium benzoate after 20 to 30 days; samples treated with 1 per cent calcium propionate, after 21 to 32 days; and samples treated with 1 per cent sodium propionate, after 8 to 20 days.

Action on Czapek's medium

Sterile Czapek's medium was measured into sterile cotton stoppered test tubes with a sterile pipette. A control sample was held for each set of trials. The compounds used with raw cream were added and distributed to give the same concentrations in the medium as were used with the cream. The tubes were inoculated with a mold suspension prepared from a portion of moldy cheese. All samples were held at room temperature and observed daily for mold growth. The results are given in Table 12.

Control samples showed mold growth after 1 to 3 days. Samples treated with 0.1 per cent methyl paracept showed mold growth after 8 to 18 days; samples treated with 0.1 per cent ethyl, propyl, butyl, benzyl and servex emulsion

Table 12. Inhibition of Molds on Czapek's Medium by Various Compounds

Compound used	Trial Number							
	1	2	3	4	5	6	7	8
	Days until mold was evident macroscopically							
None = control	2	2	1	2	3	3	3	2
Paracepts								
Methyl 1%	18	10	12	10	12	10	8	12
Ethyl 1%	2	6	6	6	6	6	6	6
Propyl 1%	6	6	6	4	6	6	4	4
Butyl 1%	4	4	6	4	4	3	3	2
Benzyl 1%	4	4	3	4	5	4	3	2
Servex emulsion 1%	2	4	3	4	4	3	3	3
Zephiran 1%	4	6	4	2	4	4	4	2
Na benzoate 0.5%	40	42	28	22	15	28	24	22
Na benzoate 1%	44	47	32	33	24	24	24	18
Ca propionate 1%	38	34	44	43	28	37	32	27
Na propionate 1%	14	14	16	14	16	8	15	7

paracepts, after 2 to 6 days; samples treated with 5 per cent zephiran, after 2 to 6 days; samples treated with 0.5 and 1 per cent sodium benzoate, after 15 to 47 days; samples treated with 1 per cent calcium propionate, after 28 to 44 days; and samples treated with 1 per cent sodium propionate, after 8 to 16 days.

Action on salted butter

Six series of trials were conducted using only the paracepts. In each trial a pound print of salted butter was cut into seven equal pieces with a knife that was clean but not sterile and a large portion of one surface of each piece was inoculated with a mold suspension prepared from a portion of moldy butter. One sample was wrapped in parchment and held as a control, while the remaining samples were dipped in 0.1 per cent solutions of the paracepts and wrapped in parchment. The samples were held at 10°C. and daily observations were made for mold growth.

The control samples showed mold growth after 6 to 10 days. The treated samples also showed mold growth in 6 to 10 days; in some trials a treated sample showed mold growth a little later than the control but the difference in time was never more than 2 days.

Action on cheddar cheese

Cheddar cheese was employed in six series of trials and it was treated in the same manner as the butter except that the samples were not inoculated. The portions were cut from longhorn cheese with a knife that was clean but not sterile, care being taken to eliminate all of the outer surface of the original cheese.

The control samples showed mold growth after 3 to 5 days. All of the treated samples also showed mold growth after 3 to 5 days.

Use of Special Parchment in Addition to Mold Inhibitors

Since the effectiveness of propionic acid and its salts as mold inhibitors appears to be due largely to the free acid, added as the acid or freed from the salts by hydrolysis, the use of a parchment that would reduce evaporation of moisture and the loss of the acid might increase the effectiveness of these mold inhibitors.

Trials were conducted using pliowax parchment and a special wax parchment in addition to regular parchment. The mold inhibitors employed were 10 per cent propionic acid solution, 15 per cent calcium propionate solution and 15 per cent sodium propionate solution.

Table 13. Inhibition of Molds on Salted Butter by Special Parchments in Addition to Mold Inhibitors Holding Temperature 15°C.

Butter Treatment	Type of Parchment	Trial Number 1	2	3	4	5	6
		Days until mold was evident macroscopically					
Dipped in sterile water	Regular	9	13	27	15	16	20
	Pliowax	14	13	17	9	10	12
	Special wax	9	13	17	9	16	20
Dipped in 10% sol. Propionic acid	Regular	38	36	37	32	29	30
	Pliowax	34	34	37	26	38	33
	Special wax	34	31	37	25	38	33
Dipped in 15% sol. Ca propionate	Regular	34	27	37	23	27	29
	Pliowax	34	27	37	23	31	29
	Special wax	27	31	34	23	31	33
Dipped in 15% sol. Na propionate	Regular	34	19	35	23	27	29
	Pliowax	27	19	23	11	20	19
	Special wax	27	19	37	15	38	20

Action on salted butter

Each of three 1-pound prints of salted butter from the same churning was cut into four equal pieces with a knife that was clean but not sterile. The 12 pieces were inoculated by spreading a mold suspension, prepared from a plate poured for a mold count on butter, over a large part of one surface of each piece.

Three of the samples which constituted the controls, were dipped in sterile distilled water: three in 10 per cent propionic acid solution; three in 15 per cent calcium propionate solution; and three in 15 per cent sodium propionate solution. One sample from each set of three was wrapped in regular parchment; one in pliowax parchment; and one in special wax parchment. All the samples were held at 15°C. and observed daily for mold growth. The results are given in Table 13.

Control samples wrapped in regular parchment showed mold growth after 9 to 27 days; those in pliowax parchment, after 9 to 17 days; and those in special wax parchment, after 9 to 20 days. Samples dipped in 10 per cent propionic acid solution and wrapped in regular parchment showed mold growth after 29 to 38 days; those in pliowax parchment, after 26 to 38 days; and those in special wax parchment, after 25 to 38 days. Samples dipped in 15 per cent calcium propionate solution and wrapped in regular parchment showed

mold growth after 23 to 37 days; those in pliowax parchment, after 23 to 37 days; and those in special wax parchment, after 23 to 34 days. Samples dipped in 15 per cent sodium propionate solution and wrapped in regular parchment showed mold growth after 19 to 35 days; those in pliowax parchment, after 11 to 27 days; and those in special wax parchment paper, after 15 to 38 days.

Action on cheddar cheese

Pieces of cheddar cheese 3 inches square and 0.5 inch thick were prepared and held in the same manner as the butter, except that the samples were not inoculated. The portions were cut from longhorn cheese with a knife that was clean but not sterile, care being taken to eliminate the outer surface of the original cheese. Table 14 presents the data.

Control samples wrapped in regular parchment showed mold growth after 4 to 6 days; those in pliowax, after 4 to 7 days; and those in special wax parchment, after 4 to 5 days. Samples dipped in 10 per cent propionic acid solution and wrapped in regular parchment showed mold growth after 13 to 17 days; those in pliowax parchment, after 13 to 18 days; and those in special wax parchment, after 11 to 17 days. Samples dipped in 15 per cent calcium propionate solution and wrapped in regular parchment showed

Table 14. Inhibition of Molds on Cheddar Cheese by Special Parchment in Addition to Mold Inhibitors Holding Temperature 15°C.

Butter Treatment	Type of Parchment	Trial Number 1	2	3	4	5	6
		Days until mold was evident macroscopically					
Dipped in sterile water	Regular	4	5	6	4	5	4
	Pliowax	7	5	6	6	4	4
	Special wax	4	5	5	4	4	4
Dipped in 10% sol. Propionic acid	Regular	13	15	15	15	17	16
	Pliowax	13	17	15	16	17	18
	Special wax	11	15	14	15	17	16
Dipped in 15% sol. Ca propionate	Regular	13	15	12	9	12	16
	Pliowax	11	15	12	9	12	16
	Special wax	7	8	12	15	10	12
Dipped in 15% sol Na propionate	Regular	11	8	12	9	10	8
	Pliowax	13	8	8	9	9	8
	Special wax	11	8	12	9	10	10

mold growth after 9 to 16 days; those in pliowax, after 8 to 13 days, and those in special wax parchment, after 7 to 15 days. Samples dipped in 15 per cent sodium propionate solution and wrapped in regular parchment showed mold growth after 8 to 12 days; those in pliowax parchment, after 8 to 13 days; and those in special wax parchment, after 8 to 12 days.

Use of Wetting Agents With Mold Inhibitors

With various sprays a wetting agent is usually used to make them more effective. It has been suggested that these agents would increase the effectiveness of mold inhibitors. Trials were carried out using calcium and sodium propionates on raw cream, Czapek's medium, salted butter and cheddar cheese. The compounds employed as wetting agents were obtained from E. I. Du Pont de Nemours Co.

Action on raw cream

For each trial a 1200 gram portion of raw cream was used. Two-ounce, wide-mouth, cork-stoppered bottles were used as containers. Two samples of cream, of 50 grams each, were held as controls. The remainder of the cream was divided into 550 gram portions in sterile containers.

To one portion calcium propionate was added to give a 1 per cent solution, and to the other portion sodium propionate was added to give the same concentration. The two lots of treated cream were weighed into the bottles, 50 grams in each. One sample of each lot was held without adding a wetting agent and others of each set were treated with 1 per cent of the various wetting agents. All samples were held at room temperature and observed daily for mold growth. The data are presented in Table 15.

The controls showed mold growth after 3 days or less; samples treated with 1 per cent calcium propionate solution, after 6 to 25 days; and samples treated with 1 per cent sodium propionate solution, after 6 to 25 days. Samples containing 1 per cent calcium propionate solution and a wetting agent showed mold growth after 16 to 48 days and samples containing 1 per cent sodium propionate and a wetting agent, after 7 to 48 days. The wetting agents extended the period during which molds were not evident macroscopically from 2 to 35 days with calcium propionate and from 1 to 39 days with sodium propionate.

Action on Czapek's medium

Six series of trials were made with Czapek's medium. The sterile medium was inoculated with a mold suspension prepared from a portion of moldy cheese and then was

Table 15. Inhibition of Molds on Raw Cream by Calcium Propionate and Sodium Propionate with Wetting Agents Present

Compound Used	Trial Number					
	1	2	3	4	5	6
	Days until mold was evident macroscopically					
None = control	2	1	2	3	2	3
Ca propionate 1%						
Alone	13	25	6	8	6	16
With 1% tergitol 7	18	41	16	18	16	36
With 1% tergitol 4	29	43	16	22	18	46
With 1% tergitol 3	29	45	18	39	16	25
With 1% aerosol O.T.	29	45	22	39	18	46
With 1% aerosol A.Y.	48	30	19	36	18	46
With 1% aerosol O.S.	29	41	17	20	21	40
With 1% gardinol L.S.	48	30	19	42	35	21
With 1% Du Pont B.C.	29	46	18	33	35	27
With 1% avirol A.W.	32	46	24	39	35	27
With 1% duponol A.W.	48	48	23	20	35	32
Na propionate 1%						
Alone	9	25	6	8	6	13
With 1% tergitol 7	29	41	7	46	25	27
With 1% tergitol 4	29	43	8	36	28	32
With 1% tergitol 3	15	45	8	36	26	22
With 1% aerosol O.T.	29	45	7	34	35	34
With 1% aerosol A.Y.	29	44	7	39	30	36
With 1% aerosol O.S.	48	46	8	17	30	28
With 1% gardinol L.S.	48	46	8	39	35	32
With 1% Du Pont B.C.	48	42	7	39	35	36
With 1% avirol A.W.	48	30	13	39	30	26
With 1% duponol A.W.	48	41	28	36	35	32

treated and held in the same manner as the cream except that 10 ml. portions were employed and sterile cotton stoppered test tubes were used as containers. The data are presented in Table 16.

Untreated samples showed mold growth in 4 days or less; samples treated with 1 per cent calcium propionate solution, after 4 to 14 days; and samples treated with 1 per cent sodium propionate solution, after 3 to 9 days. Samples containing 1 per cent calcium propionate solution and a wetting agent showed mold growth after 23 to 52 days and samples containing 1 per cent sodium propionate solution and a wetting agent, after 12 to 52 days. The wetting agents extended the period during which molds were not evident macroscopically from 18 to 48 days with calcium propionate and from 9 to 44 days with sodium propionate.

Action on salted butter

Action of wetting agents in addition to mold inhibitors was studied in six series of trials with salted butter. Each 1-pound print of butter was cut into seven equal pieces with a knife that was clean but not sterile. The pieces were inoculated by spreading a mold suspension, prepared from a portion of moldy butter, over a considerable portion of one surface of each piece. A control sample was dipped in sterile distilled water; other samples were dipped in

Table 16. Inhibition of Molds on Czapek's Medium by Calcium Propionate and Sodium Propionate with Wetting Agents Present

Compound used	Trial Number					
	1	2	3	4	5	6
	Days until mold was evident macroscopically					
None = control	2	2	2	3	4	3
Ca propionate 1%						
Alone	4	5	4	12	10	14
With 1% tergitol 7	52	49	43	31	29	34
With 1% tergitol 4	50	49	43	33	29	32
With 1% tergitol 3	52	49	40	31	27	30
With 1% aerosol O.T.	51	43	36	29	28	26
With 1% aerosol A.Y.	52	40	43	31	29	28
With 1% aerosol O.S.	50	49	40	37	29	24
With 1% gardinol L.S.	52	40	36	34	33	34
With 1% Du Pont B.C.	50	36	38	36	32	34
With 1% avirol A.W.	46	40	43	40	30	32
With 1% duponol A.W.	48	42	40	38	36	30
Na propionate 1%						
Alone	8	9	4	5	8	3
With 1% tergitol 7	52	49	43	31	29	14
With 1% tergitol 4	52	47	43	31	27	14
With 1% tergitol 3	52	42	43	36	27	12
With 1% aerosol O.T.	52	25	34	25	20	24
With 1% aerosol A.Y.	24	47	38	31	29	14
With 1% aerosol O.S.	28	42	46	33	27	14
With 1% gardinol L.S.	36	40	38	36	25	12
With 1% Du Pont B.C.	41	36	38	34	28	12
With 1% avirol A.W.	50	48	46	42	26	14
With 1% duponol A.W.	52	46	42	38	24	14

15 per cent calcium propionate and 15 per cent sodium propionate solutions; and still others were dipped in 15 per cent calcium propionate and 15 per cent sodium propionate solutions each containing 1 per cent of the wetting agents. The samples were wrapped in parchment, held at 10°C. and observed daily for mold growth.

The control samples showed mold growth after 11 to 17 days; samples dipped in 15 per cent calcium propionate solution, after 22 to 38 days; and samples dipped in 15 per cent sodium propionate solution, after 18 to 32 days. Samples dipped in 15 per cent calcium propionate solution containing a wetting agent showed mold growth after 20 to 34 days; and samples dipped in 15 per cent sodium propionate solution containing a wetting agent, after 17 to 28 days.

Action on cheddar cheese

The portions of cheddar cheese were cut from longhorn cheese with a knife that was clean but not sterile and were 3 inches square and 0.5 inch thick. They were prepared and held in the same manner as the pieces of butter except that no inoculation was used. Six series of trials were carried out.

Control samples showed mold growth after 2 to 8 days; samples dipped in 15 per cent calcium propionate

solution, after 9 to 17 days; and samples dipped in 15 per cent sodium propionate solution, after 7 to 12 days. Samples dipped in 15 per cent calcium propionate solution containing a wetting agent showed mold growth after 9 to 17 days; and samples dipped in 15 per cent sodium propionate solution containing a wetting agent, after 9 to 14 days.

Inhibition of Pure Cultures of Molds by Propionic Acid and Its Salts

The action of propionic acid and its salts on pure cultures of molds was studied using milk and Czapek's medium. The molds employed were Penicillium roqueforti, Penicillium camemberti and three strains of Oospora lactis. Inoculations were made by growing the molds in Czapek's medium, vigorously agitating a well developed culture and then adding a portion of the material to a test tube of milk or Czapek's medium.

Action on milk

Eight series of trials were conducted. Sterile milk was measured into sterile, cotton-stoppered test tubes with a sterile pipette and inoculated with the mold suspensions. One tube was held as a control while solutions

Table 17. Inhibition of Pure Cultures of Molds on Milk by Propionic Acid, Calcium Propionate and Sodium Propionate

Cream Treatment	Species of Mold	Trial Number 1	2	3	4	5	6	7	8
		Days until mold was evident macroscopically							
None = control	P. roqueforti	4	3	2	3	2	2	1	2
	P. camemberti	4	1	2	5	2	4	1	2
	O. lactis A.	2	1	2	2	2	2	1	2
	O. lactis B.	2	1	2	2	2	2	1	1
	O. lactis C.	2	1	2	1	2	2	1	1
Propionic Acid 0.5%	P. roqueforti	14	26	24	18	12	16	9	12
	P. camemberti	36	18	7	23	22	20	9	4
	O. lactis A.	36	19	23	23	11	24	16	16
	O. lactis B.	36	10	16	12	11	7	6	10
	O. lactis C.	14	7	18	23	9	17	12	7
Propionic Acid 1%	P. roqueforti	23	35	30	34	26	20	26	22
	P. camemberti	36	20	30	32	30	24	26	24
	O. lactis A.	36	35	31	34	23	24	26	24
	O. lactis B.	36	35	30	32	22	24	27	22
	O. lactis C.	36	35	31	30	23	24	21	24
Ca propionate 1%	P. roqueforti	6	3	2	4	5	4	3	2
	P. camemberti	6	7	4	5	5	4	2	2
	O. lactis A.	4	4	7	3	5	4	4	2
	O. lactis B.	2	1	4	2	2	2	4	2
	O. lactis C.	4	2	4	2	2	4	2	2
Ca propionate 5%	P. roqueforti	8	7	12	8	12	8	6	6
	P. camemberti	29	28	13	18	12	6	4	8
	O. lactis A.	14	10	13	15	12	8	6	12
	O. lactis B.	14	7	12	10	8	6	8	6
	O. lactis C.	16	10	6	4	8	4	4	4
Na propionate 1%	P. roqueforti	4	3	2	2	2	2	2	2
	P. camemberti	4	5	2	6	4	2	2	4
	O. lactis A.	4	1	4	2	2	4	4	4
	O. lactis B.	2	3	2	2	2	4	4	2
	O. lactis C.	2	3	4	2	2	2	4	2
Na propionate 5%	P. roqueforti	6	3	12	12	4	6	4	4
	P. camemberti	29	7	12	11	4	8	6	7
	O. lactis A.	6	10	6	6	4	6	6	8
	O. lactis B.	4	6	3	4	3	4	4	5
	O. lactis C.	4	6	8	6	4	6	2	4

of the compounds were added to the other tubes to give the following concentrations: Propionic acid 0.5 and 1 per cent, calcium propionate 1 and 5 per cent and sodium propionate 1 and 5 per cent. All the tubes were held at room temperature and daily observations were made for mold growth. The data are presented in Table 17.

With P. roqueforti control tubes showed mold growth after 1 to 4 days. Tubes treated with 0.5 per cent propionic acid showed mold growth after 9 to 26 days; tubes treated with 1 per cent propionic acid, after 20 to 35 days; tubes treated with 1 per cent calcium propionate, after 2 to 6 days; tubes treated with 5 per cent calcium propionate, after 8 to 12 days; tubes treated with 1 per cent sodium propionate, after 2 to 4 days; and tubes treated with 5 per cent sodium propionate, after 4 to 12 days.

With P. camemberti control tubes showed mold growth after 1 to 5 days. Tubes treated with 0.5 per cent propionic acid showed mold growth after 4 to 36 days; tubes treated with 1 per cent propionic acid, after 20 to 36 days; tubes treated with 1 per cent calcium propionate, after 2 to 7 days; tubes treated with 5 per cent calcium propionate, after 4 to 29 days; tubes treated with 1 per cent sodium propionate, after 2 to 5 days; and tubes treated with 5 per cent sodium propionate, after 4 to 29 days.

With O. lactis control tubes showed mold growth after 1 to 2 days. Tubes treated with 0.5 per cent propionic acid showed mold growth after 6 to 36 days; tubes treated with 1 per cent propionic acid, after 21 to 36 days; tubes treated with 1 per cent calcium propionate, after 1 to 7 days; tubes treated with 5 per cent propionic acid, after 4 to 16 days; tubes treated with 1 per cent sodium propionate, after 1 to 4 days; and tubes treated with 5 per cent sodium propionate, after 2 to 10 days.

Action on Czapek's medium

Eight series of trials were conducted with Czapek's medium. The tubes were prepared, treated and held in the same manner as with milk except that only one strain of O. lactis was used. Table 18 gives the results.

With P. roqueforti control tubes showed mold growth after 2 to 3 days. Tubes treated with 0.5 per cent propionic acid showed growth after 12 to 22 days; tubes treated with 1 per cent propionic acid, after 23 to 38 days; tubes treated with 1 per cent calcium propionate, after 6 to 8 days; tubes treated with 5 per cent calcium propionate, after 14 to 26 days; tubes treated with 1 per cent sodium propionate, after 2 to 4 days; and tubes treated with 5 per cent sodium propionate, after 6 to 14 days.

Table 18. Inhibition of Pure Cultures of Molds on Czapek's Medium by Propionic Acid, Calcium Propionate and Sodium Propionate

Medium Treatment	Species of Mold	Trial Number 1	2	3	4	5	6	7	8
		Days until mold was evident macroscopically							
None = control	P.roqueforti	3	2	2	3	3	3	2	3
	P.camemberti	1	2	2	3	3	2	3	2
	O.lactis	1	1	2	1	2	2	2	2
Propionic Acid 0.5%	P.roqueforti	22	18	16	14	16	12	18	16
	P.camemberti	46	42	38	31	24	18	16	12
	O.lactis	42	38	43	48	41	28	24	18
Propionic Acid 1%	P.roqueforti	38	30	22	27	34	23	27	29
	P.camemberti	72	70	77	62	52	44	46	41
	O.lactis	67	70	61	72	62	54	50	42
Ca propionate 1%	P.roqueforti	6	8	8	6	8	6	7	6
	P.camemberti	12	16	18	12	8	10	8	7
	O.lactis	9	7	9	11	8	10	8	7
Ca propionate 5%	P.roqueforti	22	20	18	24	26	20	18	14
	P.camemberti	38	32	30	24	21	18	16	18
	O.lactis	40	36	29	26	24	26	20	18
Na propionate 1%	P.roqueforti	2	2	2	3	5	4	3	4
	P.camemberti	6	2	3	6	5	7	7	6
	O.lactis	3	4	3	2	3	6	5	6
Na propionate 5%	P.roqueforti	6	7	6	7	12	14	10	8
	P.camemberti	18	6	14	10	11	10	12	10
	O.lactis	8	7	5	6	9	12	14	10

With P. camemberti control tubes showed mold growth after 1 to 3 days. Tubes treated with 0.5 per cent propionic acid showed growth after 12 to 46 days; tubes treated with 1 per cent propionic acid, after 41 to 77 days; tubes treated with 1 per cent calcium propionate, after 8 to 18 days; tubes treated with 5 per cent calcium propionate after 16 to 38 days; tubes treated with 1 per cent sodium propionate, after 2 to 5 days; and tubes treated with 5 per cent sodium propionate, after 6 to 18 days.

With O. lactis control tubes showed mold growth after 1 to 2 days. Tubes treated with 0.5 per cent propionic acid showed mold growth after 18 to 48 days; tubes treated with 1 per cent propionic acid, after 42 to 72 days; tubes treated with 1 per cent calcium propionate after 7 to 11 days; tubes treated with 5 per cent calcium propionate, after 18 to 40 days; tubes treated with 1 per cent sodium propionate, after 2 to 6 days; and tubes treated with 5 per cent sodium propionate, after 5 to 14 days.

Figures 4, 5 and 6 illustrate the inhibition of pure cultures of molds on Czapek's medium by propionic acid, calcium propionate and sodium propionate.

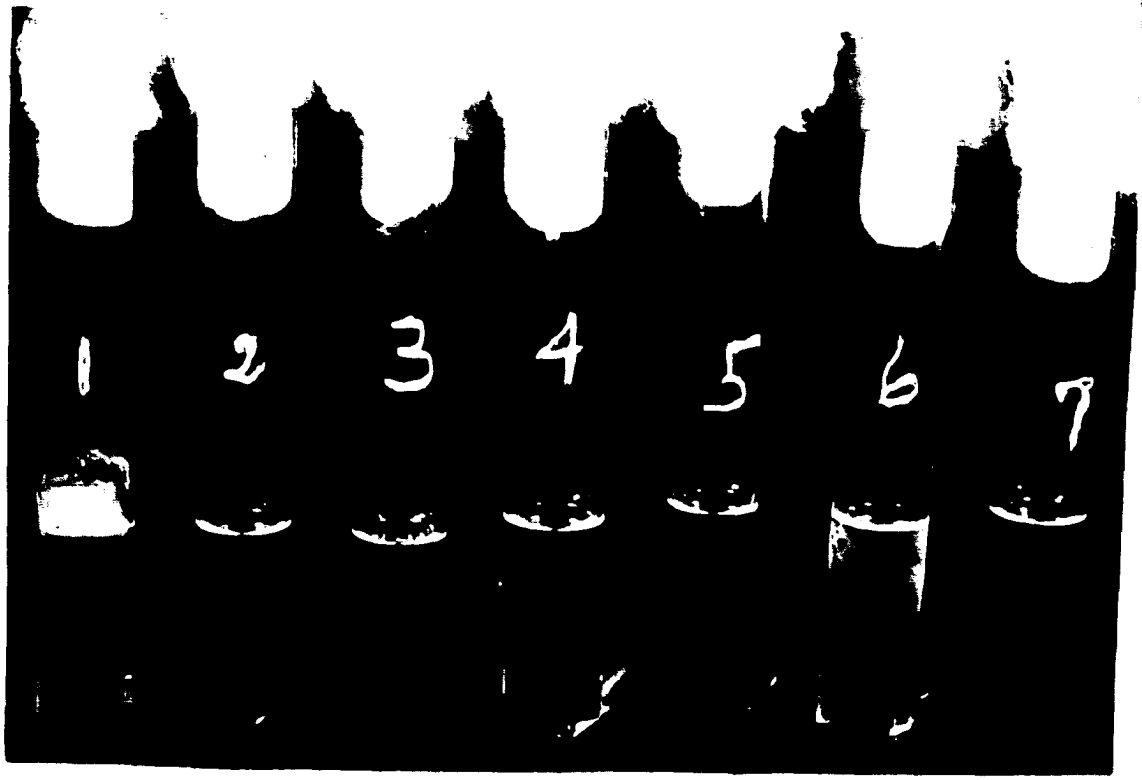

Figure 4. The inhibition of O. lactis on Csapek's medium by propionic acid, calcium propionate and sodium propionate. The tubes were inoculated; 1 was untreated, 2 contained 0.5 per cent propionic acid, 3 contained 1 per cent propionic acid, 4 contained 1 per cent calcium propionate, 5 contained 5 per cent calcium propionate, 6 contained 1 per cent sodium propionate and 7 contained 5 per cent sodium propionate. The tubes were held 14 days at room temperature.

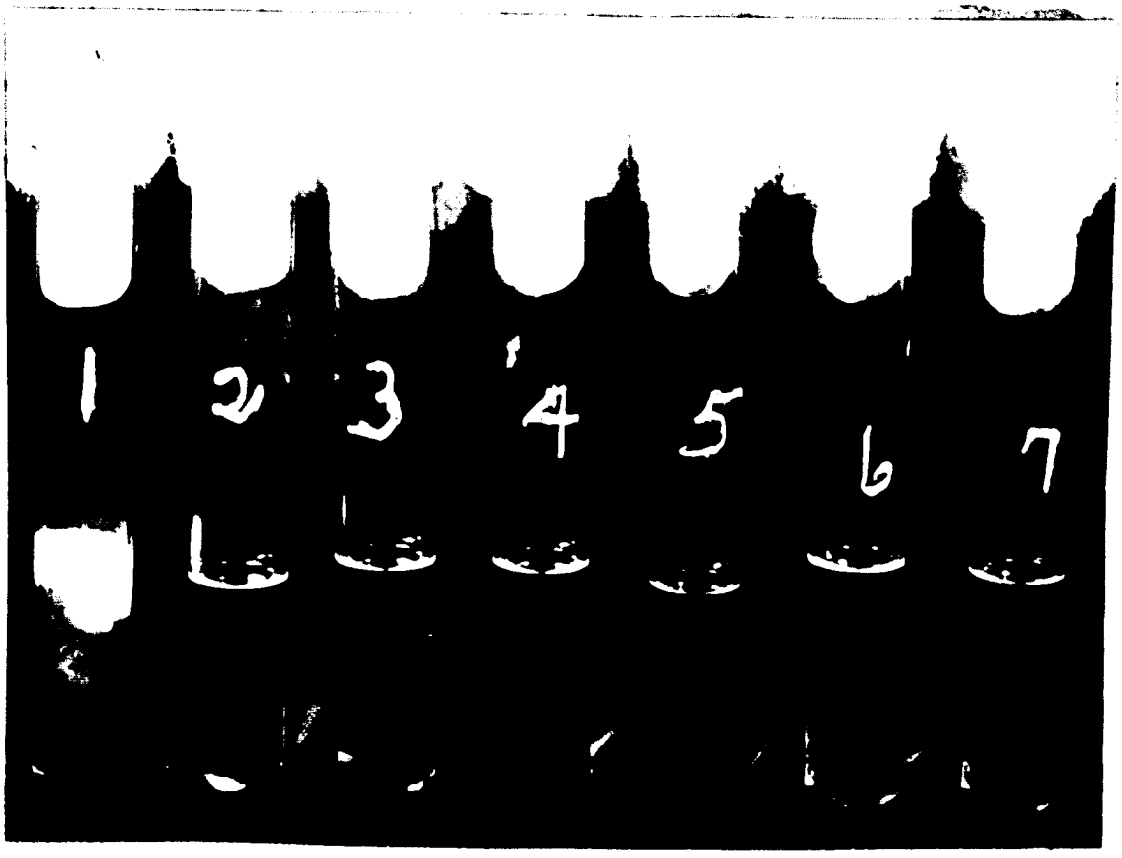

Figure 5. The inhibition of P. camemberti on Czapek's medium by propionic acid, calcium propionate and sodium propionate. The tubes were inoculated; 1 was untreated, 2 contained 0.5 per cent propionic acid, 3 contained 1 per cent propionic acid, 4 contained 1 per cent calcium propionate, 5 contained 5 per cent calcium propionate, 6 contained 1 per cent sodium propionate and 7 contained 5 per cent sodium propionate. The tubes were held 18 days at room temperature.

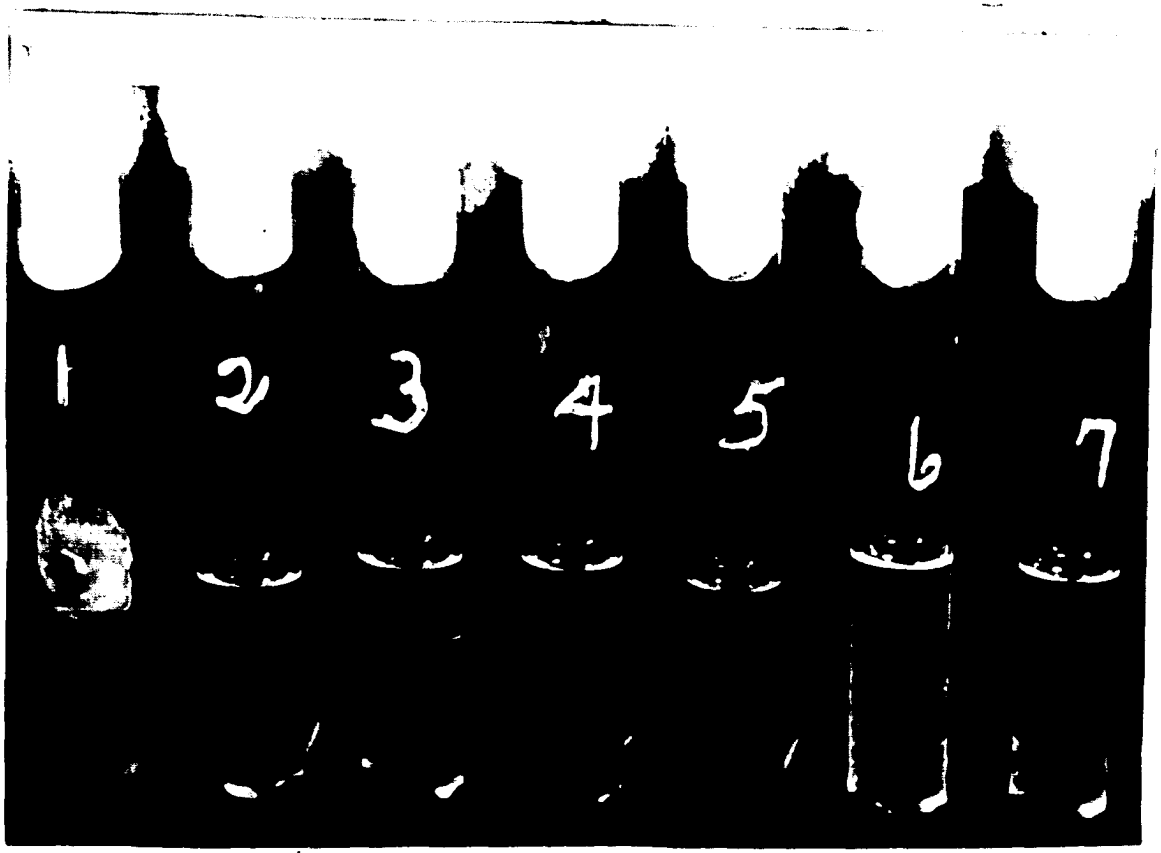

Figure 6. The inhibition of P. roqueforti on Czapek's medium by propionic acid, calcium propionate and sodium propionate. The tubes were inoculated; 1 was untreated, 2 contained 0.5 per cent propionic acid, 3 contained 1 per cent propionic acid, 4 contained 1 per cent calcium propionate, 5 contained 5 per cent calcium propionate, 6 contained 1 per cent sodium propionate and 7 contained 5 per cent sodium propionate. The samples were held 14 days at room temperature.

Comparative Growth of Mold Spores Dusted on The Surface And Distributed Through a Medium Containing Propionic Acid or a Salt of It.

The comparative growth of mold spores dusted on the surface and distributed through milk containing propionic acid or a salt of it was studied in four series of trials.

For each trial two sets of samples were prepared. The milk was weighed into 2-ounce bottles with cotton stoppers and sterilized in the autoclave at 15 pounds pressure for 25 minutes. One bottle in each set was held as a control and the compounds were added to the other bottles so that the following concentrations in the milk were obtained: Propionic acid 0.5 per cent, calcium propionate 1 per cent and sodium propionate 1 per cent. A portion of mold powder prepared for use in the manufacture of blue cheese was used to inoculate the milk. For each bottle an inoculating loop was dipped into the mold powder and the material adhering to the needle was dusted into it. Each set included two bottles containing 0.5 per cent propionic acid, and one of these was left with the cotton stopper while the other was stoppered with a sterile cork to avoid excessive loss of the acid. With one set of bottles the mold spores were thoroughly distributed by shaking the milk and with the other set the mold spores were left undisturbed on the surface. The samples were held at room temperature and observed daily for mold

growth.

Control samples in which the mold spores were distributed showed growth after 1 to 3 days; and samples with the mold spores on the surface, after 3 to 6 days. Cotton stoppered samples containing 0.5 per cent propionic acid in which the mold spores were distributed showed growth after 3 to 7 days; and samples with mold spores on the surface, after 3 to 8 days. Cork stoppered samples containing 0.5 per cent propionic acid in which the mold spores were distributed showed growth after 2 to 10 days; and samples with mold spores on the surface, after 2 to 10 days. Samples treated with 1 per cent calcium propionate in which the mold spores were distributed showed growth after 2 to 7 days; and samples with mold spores on the surface, after 2 to 7 days. Samples treated with 1 per cent sodium propionate in which the mold spores were distributed showed growth after 2 to 6 days; and samples with mold spores on the surface, after 4 to 7 days.

Microscopic Observations on Mold Spores and Mold Growth in a Medium Containing Propionic Acid or Calcium Propionate.

The effect of propionic acid and calcium propionate on mold spores and mold growth was studied microscopically by preparing slides from inoculated milk, and also from raw cream, treated with the compounds. The slides were stained

with the Newman-Lampert stain and examined under the oil immersion lens (X900). In the studies the affect of pH on the inhibition of molds was given some attention.

Observations on Inoculated Milk

For each of six trials 100 ml. portions of milk were placed in 2-ounce, cork-stoppered bottles and sterilized at 15 pounds pressure for 30 minutes. These portions were treated in the following manner: One portion was held as a control; to four portions propionic acid was added so as to give concentrations of 0.01, 0.1, 0.5 and 1 per cent; and to nine portions calcium propionate was added so as to give concentrations of 1, 2 and 5 per cent (three portions with each). For each concentration of calcium propionate one portion was untreated, the pH of one portion was adjusted with lactic acid to 5.5 to 5.8 and the pH of one portion was adjusted with lactic acid to 4.5 to 4.8. The pH of each portion was taken at the beginning and the end of the holding. All portions were inoculated with a mixed mold suspension prepared from a moldy cheese and held at room temperature. Immediately after inoculation a smear of each portion was made on a glass slide and smears were made at 5 day intervals. The number of mold segments in 20 fields was counted with each portion and the average taken as the count on the portion. The data are presented in Table 19 to 24 inclusive.

Table 19. Number of Mold Segments Per Microscopic Field in Milk Treated With Mold inhibitors

Milk Sterilized and Inoculated With Material Prepared by Grinding Moldy Cheese

Compound Used	pH Original	pH Final	*Average number of segments per microscopic field: At once	After 5 days	After 10 days	After 15 days	After 20 days	After 25 days	After 30 days	After 35 days	After 40
None = control	6.02	3.97	0.0	3.0	130.0	150.2	280.3	400.0	180.0	140.2	7.6
Propionic Acid											
1.0%	4.63	4.56	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.5%	4.98	4.27	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8.1	21.6
0.1%	5.73	3.57	0.0	0.0	0.0	0.0	0.0	0.0	0.0	14.2	38.2
0.01%	5.98	3.82	0.0	0.0	0.0	0.0	0.0	0.0	0.0	12.6	14.1
Ca propionate 1%											
Alone	5.92	4.12	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.0	28.2
With lactic acid	5.62	4.28	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.6	62.8
With lactic acid	4.78	4.23	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.1	3.6
Ca propionate 2%											
Alone	5.92	4.09	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.7	18.2
With lactic acid	5.52	4.51	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.1
With lactic acid	4.65	4.70	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.0	61.8
Ca propionate 5%											
Alone	5.94	5.12	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.6
With lactic acid	5.48	4.97	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	18.2
With lactic acid	4.76	4.67	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

*Values based on 20 fields.

Table 20. Number of Mold Segments Per Microscopic Field in Inoculated Milk Treated With Mold Inhibitors

Milk Sterilized and Inoculated With Material Prepared by Grinding Moldy Cheese

Compound Used	pH Original	pH Final	*Average number of segments per microscopic field: At once	After 5 days	After 10 days	After 15 days	After 20 days	After 25 days	After 30 days	After 35 days	After 40 days
None = control	6.45	4.31	0.0	40.0	62.0	120.0	195.2	280.5	130.2	82.5	60.2
Propionic Acid											
1.0%	4.17	4.30	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.5%	4.46	4.50	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	20.2
0.1%	5.21	4.30	0.0	0.0	0.0	0.0	20.0	40.0	60.3	88.0	91.0
0.01%	6.32	4.12	0.0	0.0	8.0	28.0	40.0	58.0	71.2	92.0	60.2
Ca propionate 1%											
Alone	5.97	4.28	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.2	14.2
With lactic acid	5.62	4.43	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.0	8.2
With lactic acid	4.42	4.50	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.0
Ca propionate 2%											
Alone	5.48	4.70	0.0	0.0	0.0	0.0	0.0	8.0	23.2	31.0	42.1
With lactic acid	4.73	4.43	0.0	0.0	0.0	0.0	0.0	0.0	3.0	8.0	16.1
With lactic acid	4.92	4.45	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.2	6.2
Ca propionate 5%											
Alone	6.02	4.61	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.1	12.3
With lactic acid	5.58	4.83	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.1
With lactic acid	4.78	4.91	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.2

*Values based on 20 fields.

Table 21. Number of Mold Segments Per Microscopic Field in Inoculated Milk Treated With Mold Inhibitors

Milk Sterilized and Inoculated With Material Prepared by Grinding Moldy Cheese

Compound Used	pH Original	pH Final	*Average number of segments per microscopic field: At once	After 5 days	After 10 days	After 15 days	After 20 days	After 25 days	After 30 days	After 35 days	After 40 days
None = control	6.33	4.06	0.0	5.0	16.0	32.5	45.2	108.0	202.0	180.6	102.5
Propionic Acid											
1.0%	4.72	4.76	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.5%	5.05	3.95	0.0	1.0	5.0	9.0	16.0	21.2	43.6	50.0	42.1
0.1%	5.88	4.20	0.0	3.0	9.0	11.2	18.2	28.2	36.7	41.2	30.6
0.01%	6.18	4.50	0.0	4.0	16.0	32.2	30.0	36.1	35.2	41.6	38.2
Ca propionate 1%											
Alone	5.91	4.05	0.0	0.0	0.0	0.0	4.0	12.2	16.8	27.2	29.0
With lactic acid	5.70	3.72	0.0	0.0	2.0	7.0	16.0	21.2	27.6	38.2	36.7
With lactic acid	4.52	3.82	0.0	0.0	0.0	0.0	2.5	12.6	21.3	30.6	27.2
Ca propionate 2%											
Alone	5.91	4.02	0.0	5.5	12.0	21.2	23.5	36.8	32.4	30.7	20.8
With lactic acid	5.54	4.32	0.0	3.4	9.0	17.1	21.2	28.2	26.7	36.0	30.7
With lactic acid	4.67	4.76	0.0	0.0	0.0	0.0	0.0	2.0	6.7	12.7	15.3
Ca propionate 5%											
Alone	5.91	4.41	0.0	0.0	2.0	7.0	11.2	21.2	26.3	28.4	36.1
With lactic acid	5.52	4.41	0.0	0.0	0.0	0.0	0.0	3.1	6.7	12.3	20.1
With lactic acid	4.90	4.91	0.0	0.0	0.0	0.0	0.0	2.1	3.0	7.6	9.4

*Values based on 20 fields.

Table 22. Number of Mold Segments Per Microscopic Field in Inoculated Milk Treated With Mold Inhibitors

Milk Sterilized and Inoculated With Material Prepared by Grinding Moldy Cheese

Compound Used	pH Original	pH Final	*Average number of segments per microscopic field: At once	After 5 days	After 10 days	After 15 days	After 20 days	After 25 days	After 30 days	After 35 days	After 40 days
None = control	6.23	5.39	0.0	12.6	128.2	37.0	6.8	3.0	7.0	9.0	6.0
Propionic Acid											
1.0%	4.45	4.52	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.5%	4.84	4.98	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0
0.1%	5.54	5.43	0.0	0.0	70.0	83.1	32.1	8.3	3.2	5.3	21.3
0.01%	6.15	4.45	0.0	0.0	0.0	0.0	8.0	61.2	8.2	3.6	4.0
Ca propionate 1%											
Alone	5.87	4.42	0.0	0.0	38.0	42.3	30.8	23.0	2.6	1.1	7.2
With lactic acid	5.70	4.89	0.0	0.0	14.2	18.2	26.8	38.3	12.3	3.2	38.6
With lactic acid	4.77	4.79	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.2
Ca propionate 2%											
Alone	5.85	4.58	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.0	12.2
With lactic acid	5.50	4.69	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.2	13.1
With lactic acid	4.78	4.69	0.0	0.0	0.0	3.1	8.4	16.2	27.0	8.1	18.3
Ca propionate 5%											
Alone	6.00	4.81	0.0	0.0	0.0	4.0	18.3	26.7	31.2	8.6	3.0
With lactic acid	5.51	4.68	0.0	0.0	2.0	6.0	9.6	16.2	22.6	16.2	18.3
With lactic acid	4.91	4.90	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.6

* Values based on 20 fields.

Table 23. Number of Mold Segments Per Microscopic Field in Inoculated Milk Treated With Mold Inhibitors

Milk Sterilized and Inoculated With Material Prepared by Grinding Moldy Cheese

Compound Used	pH Original	pH Final	*Average number of segments per microscopic field: At once	After 5 days	After 10 days	After 15 days	After 20 days	After 25 days	After 30 days	After 35 days	After 40 days
None = control	6.28	4.50	0.0	13.2	32.8	48.3	70.0	90.1	81.3	70.2	27.2
Propionic Acid											
1.0%	4.45	3.99	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.5%	4.74	3.89	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	9.5
0.1%	5.41	3.98	0.0	0.0	0.0	0.0	0.0	0.0	2.1	2.0	4.0
0.01%	6.26	4.50	0.0	0.0	0.0	4.1	7.0	18.2	5.9	6.2	3.2
Ca propionate 1%											
Alone	5.87	4.13	0.0	0.0	0.0	0.0	0.0	10.8	12.1	14.3	16.0
With lactic acid	5.51	4.40	0.0	0.0	0.0	0.0	0.0	2.0	6.2	22.6	12.0
With lactic acid	4.73	4.55	0.0	0.0	0.0	0.0	0.0	0.0	3.7	16.2	17.0
Ca propionate 2%											
Alone	5.91	4.75	0.0	2.0	14.0	27.8	53.2	30.2	12.6	18.3	270.0
With lactic acid	5.52	4.87	0.0	0.0	0.0	0.0	0.0	0.0	12.2	24.8	29.0
With lactic acid	4.78	4.67	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.0
Ca propionate 5%											
Alone	5.93	4.25	0.0	12.0	16.8	23.2	4.0	22.0	27.2	15.0	11.5
With lactic acid	5.41	4.55	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.2
With lactic acid	4.83	4.85	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.0

*Values based on 20 fields.

Table 24. Number of Mold Segments Per Microscopic Field in Inoculated Milk Treated With Mold Inhibitors

Milk Sterilized and Inoculated With Material Prepared by Grinding Moldy Cheese

Compound Used	pH Original	pH Final	*Average number of segments per microscopic field: At once	After 5 days	After 10 days	After 15 days	After 20 days	After 25 days	After 30 days	After 35 days	After 40 days
None = control	6.34	4.07	0.0	0.0	0.0	18.0	56.0	103.0	116.0	13.4	2.0
Propionic Acid											
1.0%	4.35	4.33	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.5%	4.76	3.81	0.0	0.0	0.0	0.0	0.0	0.5	1.3	8.4	21.6
0.1%	5.53	4.21	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	12.0
0.01%	6.21	3.61	0.0	0.0	0.0	0.0	0.0	0.0	0.0	43.8	44.0
Ca propionate 1%											
Alone	5.95	4.48	0.0	0.0	0.0	0.0	8.2	8.0	13.3	16.3	12.6
With lactic acid	5.58	4.04	0.0	0.0	0.0	4.2	12.7	89.2	94.0	300.0	54.0
With lactic acid	4.86	4.77	0.0	0.0	0.0	5.1	17.2	21.0	13.2	18.6	10.3
Ca propionate 2%											
Alone	5.95	4.12	0.0	0.0	0.0	2.0	6.3	27.2	21.6	8.3	2.6
With lactic acid	5.52	4.80	0.0	0.0	0.0	1.5	7.2	38.4	31.0	36.2	10.2
With lactic acid	4.79	4.67	0.0	0.0	0.0	0.0	0.0	0.0	6.2	9.6	3.2
Ca propionate 5%											
Alone	5.96	3.71	0.0	0.0	0.0	0.0	0.0	8.1	183.9	102.0	8.2
With lactic acid	5.48	4.02	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	7.2
With lactic acid	4.84	4.73	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.0

*Values based on 20 fields.

The control samples had initial pH values of 6.02 to 6.45 and final values of 4.06 to 5.39. Mold growth was evident microscopically after 5 to 15 days and in most samples after 5 days.

Samples treated with 1 per cent propionic acid had low initial pH values (4.05 to 4.72) and there was little change in pH during the time the samples were held. The final values were from 3.99 to 4.76. No mold growth was evident in the samples microscopically, even after 40 days. With 0.5 per cent propionic acid the initial pH values were slightly higher (4.46 to 5.05) than with 1 per cent and the final values were from 3.81 to 4.98; mold growth was evident microscopically after 30 to 40 days in 5 trials and after 5 days in 1 trial. Samples treated with 0.1 per cent propionic acid had initial pH values of 5.21 to 5.73 and final values of 3.57 to 4.30; mold growth was evident microscopically after 5 to 30 days. Samples treated with 0.01 per cent propionic acid had initial pH values of 5.73 to 6.26 and final values of 3.61 to 4.50; mold growth was evident microscopically after 5 to 30 days.

Samples containing 1, 2 and 5 per cent calcium propionate had initial pH values slightly lower (5.48 to 6.02) than the control samples. Increased concentration of the salt had little effect on the pH. The pH values decreased during the holding and the final values were 3.71 to 5.12. Samples containing calcium propionate, (1, 2 and 5 per cent) with the

initial pH adjusted to 5.5 to 5.8 decreased in pH during the holding; those with the initial pH adjusted to 4.5 to 4.8 showed little change in pH during the holding.

Samples containing 1 per cent calcium propionate alone showed mold growth microscopically after 10 to 35 days; those with the pH adjusted to 5.5 to 5.8, after 10 to 35 days; and those with the pH adjusted to 4.5 to 4.8, after 20 to 40 days. Samples containing 2 per cent calcium propionate alone showed mold growth microscopically after 5 to 35 days; those with the pH adjusted to 5.5 to 5.8, after 5 to 40 days; and those with the pH adjusted to 4.5 to 4.8, after 15 to 40 days. Samples containing 5 per cent calcium propionate alone showed mold growth after 5 to 40 days; those with the pH adjusted to 5.5 to 5.8, after 10 to 40 days; and those with the pH adjusted to 4.5 to 4.8, after 25 to 40 days.

With the control samples the slides prepared at once showed many normal mold spores, slides prepared after 5 days commonly showed mold segments and slides prepared later all showed mold segments. In many of the trials large numbers of mold segments were present and in some instances these segments decreased in numbers and spores again appeared.

With 1 per cent propionic acid the slides showed very few spores and these were usually distorted or broken; no segments were present in any of the slides. With lower concentration of propionic acid the slides showed fewer mold.

spores than with the control samples. Mold segments were present in slides prepared after 5 days and later but usually were much lower in numbers than with the control samples.

With 1, 2 and 5 per cent calcium propionate the slides showed fewer mold spores and segments than slides prepared from control samples but showed more spores than samples treated with 1 per cent propionic acid. Increasing the concentration of the salt usually increased the period before mold segments were present and decreased the numbers of segments. In most trials adjusting the pH of the samples containing calcium propionate to 4.5 to 4.8 and to 5.5 to 5.8 increased the time before mold segments were present and reduced the numbers of segments.

All the slides prepared from the samples treated with the mold inhibitors contained materials that appeared to be broken cell walls and the growth suggested a lack of vigor.

Observations on Raw Cream

One hundred ml. portions of raw cream were placed in the 2-ounce, cork-stoppered bottles and treated in the following manner: One portion was held as a control; propionic acid was added to four portions to give concentrations of 0.01, 0.1, 0.5 and 1 per cent; and calcium propionate was added to three portions to give concentrations of 1, 2 and 5 per cent. The

pH of each sample was taken at the beginning and the end of the holding; no pH adjustments were made. Slides were prepared and counted in the same way as with the milk samples. The results are given in Tables 25 to 30.

The control samples had initial pH values of 5.00 to 6.77 and final values of 3.34 to 4.55; mold growth was evident microscopically after 5 to 10 days.

Samples treated with 1 per cent propionic acid had initial pH values of 4.05 to 4.37 and showed little change in pH during the test period, the final values being 3.50 to 4.07; no mold growth was evident microscopically, even after holding the samples for 40 days. With 0.5 per cent propionic acid the initial pH values were slightly higher (4.25 to 4.94) than with 1 per cent, but there was little change in pH during the holding; mold growth was evident microscopically after 25 to 40 days. Samples treated with 0.1 per cent propionic acid had initial pH values of 4.57 to 5.73 and final values of 3.32 to 4.82; mold growth was evident microscopically after 10 to 30 days. Samples treated with 0.01 per cent propionic acid had initial pH values of 4.87 to 6.63 and final values of 3.30 to 5.50; mold growth was evident microscopically after 5 to 30 days.

Samples treated with 1, 2 and 5 per cent calcium propionate had initial pH values slightly lower (5.11 to 6.30) than the control samples, and the increased concentrations of the salt had little effect on the pH. The pH values were

Table 25. Number of Mold Segments Per Microscopic Field in Raw Cream Treated With Mold Inhibitors

Compound Used	pH Original	pH Final	*Average number of segments per microscopic field: At once	After 5 days	After 10 days	After 15 days	After 20 days	After 25 days	After 30 days	After 35 days	After 40 days
None = control	6.77	3.34	0.0	8.0	40.0	36.0	34.2	91.8	92.7	82.1	21.6
Propionic Acid											
1.0%	4.37	3.83	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.5%	4.76	3.32	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	7.0
0.1%	5.73	3.42	0.0	0.0	0.0	0.0	0.0	0.0	4.0	28.0	48.6
0.01%	6.63	3.30	0.0	0.0	0.0	0.0	0.0	0.0	7.0	42.2	68.3
Ca propionate											
1%	6.13	3.93	0.0	0.0	0.0	0.0	0.0	0.0	0.0	12.0	5.0
2%	6.13	4.22	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.0	20.2
5%	6.25	4.60	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.0	26.1

*Values based on 20 fields.

Table 26. Number of Mold Segments Per Microscopic Field in Raw Cream Treated With Mold Inhibitors

Compound Used	pH Original	pH Final	*Average number of segments per microscopic field: At once	After 5 days	After 10 days	After 15 days	After 20 days	After 25 days	After 30 days	After 35 days	After 40 days
None = control	6.45	3.34	0.0	3.0	45.0	60.3	63.7	67.2	73.8	26.2	21.8
Propionic Acid											
1.0%	4.32	3.83	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.5%	4.67	3.38	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.0	12.2
0.1%	5.68	3.32	0.0	0.0	0.0	0.0	6.0	5.8	21.2	38.2	46.7
0.01%	6.17	4.23	0.0	0.0	0.0	0.0	0.0	17.0	34.2	84.1	96.2
Ca propionate											
1%	6.07	3.80	0.0	0.0	0.0	0.0	0.0	0.0	18.0	22.0	32.7
2%	6.02	4.15	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.2	16.2
5%	6.09	4.51	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.5	7.6

*Values based on 20 fields.

Table 27. Number of Mold Segments Per Microscopic Field in Raw Cream Treated With Mold Inhibitors

Compound Used	pH Original	pH Final	*Average number of segments per microscopic field: At once	After 5 days	After 10 days	After 15 days	After 20 days	After 25 days	After 30 days	After 35 days	After 40 days
None = control	6.35	4.47	0.0	18.0	62.0	127.0	280.0	325.7	207.6	180.2	28.3
Propionic Acid											
1.0%	4.23	4.07	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.5%	4.94	4.82	0.0	0.0	0.0	0.0	1.0	12.0	18.4	40.2	41.8
0.1%	5.52	4.82	0.0	0.0	0.0	0.0	6.0	10.2	16.2	18.4	40.3
0.01%	5.96	5.50	0.0	0.0	0.0	0.0	4.2	8.0	27.6	36.2	51.8
Ca propionate											
1%	5.86	4.14	0.0	0.0	0.0	0.0	2.0	12.4	16.2	21.7	36.7
2%	5.90	4.45	0.0	0.0	0.0	0.0	2.6	8.6	21.2	23.4	32.1
5%	5.92	5.33	0.0	0.0	0.0	0.0	4.0	6.8	10.2	12.7	21.8

*Values based on 20 fields.

Table 28. Number of Mold Segments Per Microscopic Field in Raw Cream Treated With Mold Inhibitors

Compound Used	pH Original	pH Final	*Average number of segments per microscopic field: At once	After 5 days	After 10 days	After 15 days	After 20 days	After 25 days	After 30 days	After 35 days	After 40 days
None = control	5.00	4.55	0.0	4.0	12.0	28.0	88.3	72.0	43.8	25.2	26.0
Propionic Acid											
1.0%	4.05	3.68	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.5%	4.25	3.70	0.0	0.0	0.0	0.0	0.0	4.0	9.1	17.2	19.3
0.1%	4.57	3.35	0.0	0.0	3.2	6.3	16.2	18.4	21.7	41.2	4.2
0.01%	4.87	3.62	0.0	0.0	1.5	14.3	36.8	63.4	52.6	16.1	26,2
Ca propionate											
1%	5.11	4.75	0.0	0.0	1.0	19.1	21.6	18.4	36.2	27.8	12.0
2%	5.62	4.35	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.1	7.8
5%	5.82	4.68	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.1

*Values based on 20 fields.

Table 29. Number of Mold Segments Per Microscopic Field in Raw Cream Treated With Mold Inhibitors

Compound Used	pH Original	pH Final	*Average number of segments per microscopic field							
			At once	After 5 days	After 10 days	After 15 days	After 20 days	After 25 days	After 30 days	After 35 days	After 40 days
None = control	6.56	3.65	0.0	63.5	82.0	43.0	18.2	70.2	160.2	62.7	50.2
Propionic Acid											
1.0%	4.36	3.50	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.5%	4.68	3.58	0.0	0.0	0.0	0.0	0.0	0.0	0.0	7.2	18.1
0.1%	5.58	3.43	0.0	0.0	4.2	8.3	16.2	27.1	32.8	16.8	18.2
0.01%	6.42	4.15	0.0	20.0	62.0	76.0	92.3	50.2	42.6	5.3	7.3
Ca propionate											
1%	6.07	3.95	0.0	10.0	26.1	28.1	36.2	32.8	48.0	2.2	6.2
2%	6.10	4.98	0.0	0.0	4.0	8.3	21.6	29.2	41.2	4.5	3.0
5%	5.95	5.10	0.0	0.0	0.0	0.0	0.0	31.0	36.2	20.2	14.6

*Values based on 20 fields.

Table 30. Number of Mold Segments Per Microscopic Field in Raw Cream Treated With Mold Inhibitors

Compound Used	pH Original	pH Final	*Average number of segments per microscopic field								
			At once	After 5 days	After 10 days	After 15 days	After 20 days	After 25 days	After 30 days	After 35 days	After 40 days
None = control	6.70	3.77	0.0	0.0	8.2	14.3	31.7	73.8	36.2	22.8	7.2
Propionic Acid											
1.0%	4.30	3.97	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.5%	4.55	3.92	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.1	16.2
0.1%	5.50	3.68	0.0	0.0	3.0	7.2	12.0	38.2	32.7	23.0	17.2
0.01%	6.52	3.76	0.0	0.0	5.0	18.1	20.0	32.6	8.6	4.7	3.2
Ca propionate											
1%	6.10	4.00	0.0	0.0	0.0	0.0	0.0	0.0	6.2	16.2	18.4
2%	6.15	4.25	0.0	0.0	0.0	0.0	0.0	0.0	10.2	18.3	26.4
5%	6.30	4.58	0.0	0.0	0.0	0.0	0.0	0.0		2.6	8.3

*Values based on 20 fields.

lowered during the test period and the final pH values were from 3.80 to 5.33. Samples treated with 1 per cent calcium propionate showed mold growth microscopically after 5 to 35 days; those treated with 2 per cent calcium propionate, after 10 to 35 days; and those treated with 5 per cent calcium propionate, after 20 to 40 days.

With the control samples the slides prepared at once showed large numbers of normal mold spores; the slides prepared after 5 to 10 days showed mold segments and the slides prepared later often showed very large numbers of mold segments. After the samples were held for several days, the numbers of segments tended to decrease.

With 1 per cent propionic acid the slides showed very few spores and no mold segments were present in any of them. The slides prepared from samples containing lower concentrations of propionic acid showed more spores than with 1 per cent and more segments were present. Decreasing the concentration of the acid decreased the period before mold segments were present and increased the numbers of segments.

Samples treated with 1, 2 and 5 per cent calcium propionate showed fewer mold spores and segments than control samples but more spores than with 1 per cent propionic acid. An increase in concentration increased the period before mold segments were present and decreased the numbers of segments.

Effect of the Mold Inhibitors on Odor, Flavor and Color of Various Dairy Products

Although no systematic study of the effect of the mold inhibitors on the odor, flavor and color of various dairy products was attempted, general observations were made with most of the trials.

Propionic acid has a rather conspicuous odor, but the odor is not as objectionable as that of some of the higher fatty acids. The odors of such acids as caproic and caprylic are so objectionable that they commonly can not be used as mold inhibitors on food products.

Milk and cream treated with propionic acid in concentrations of less than 1 per cent had a slight odor of the acid; with a concentration of 1 per cent the odor was pronounced and persisted throughout the test period. Butter and cheese dipped in propionic acid solutions of 5 per cent and above had a very definite odor of the acid. The products also had a propionic acid flavor which was not objectionable but was pleasant and agreeable, especially with cheddar and swiss-type cheeses in which the natural flavors were enhanced by the propionic acid flavor. Dipping the parchments for butter and cheese in propionic acid solutions had much the same effect on odor and flavor as dipping the products.

Calcium propionate and sodium propionate also imparted a propionic acid odor and flavor to the dairy products. With

milk and cream in concentrations of less than 1 per cent, the odor was very slight but in concentrations of 1 per cent or more the odor was quite pronounced. With butter and cheese the odor and flavor were similar to those produced by the acid but were less pronounced. The odor and flavor were much less pronounced after the samples were held for several weeks. Dipping the parchment for butter and cheese in calcium propionate or sodium propionate solutions had much the same effect on the odor and flavor as dipping the products.

Acetic acid gave a slight odor to the materials treated but it was not objectionable. Sodium benzoate, zephiran and calcium acetate did not produce any noticeable odor in the products. The paraceptS were used in very weak concentrations and no odors were noticed when they were employed.

Propionic acid in concentrations of 10 per cent or more, when used to dip butter and cheese, often caused a white discoloration on the surface, especially with butter. When the wrapper was wrinkled a white streak sometimes followed the crease across the surface of the butter or cheese. The discolorations disappeared after a few days. Calcium propionate and sodium propionate caused white discolorations in only a few instances and these were with 25 per cent solutions and on surfaces where creases were present in the parchment. The other compounds studied as mold inhibitors did not produce any noticeable discoloration.

DISCUSSION

Propionic acid and its calcium and sodium salts showed significant mold inhibiting properties in many of the trials with different dairy products. Commonly, relatively high concentrations were required. This is a disadvantage from the standpoint of cost when the large volumes of dairy products on which molds can grow is considered. The presence of propionic acid and its salts in various food products, such as cheeses, indicates that there should be no objection to the use of this general group of compounds as mold inhibitors.

The different compounds varied in their effectiveness in preventing mold growth in the various trials. The dairy products used often were from different lots and the molds naturally present varied in species and numbers. Mold suspensions employed for inoculations were prepared from materials likely to contain molds commonly found in dairy products, but here also the molds varied in species and numbers.

Propionic acid, calcium propionate and sodium propionate were very effective in preventing mold growth in raw cream. The lactic acid producing organisms naturally present produce lactic acid from the lactose and the acid lowers the pH, thus producing conditions which, presumably, are favorable

for the action of the mold inhibiting compounds. The mold suspensions used for inoculating Czapek's medium also contained bacteria and probably the action of these organisms lowered the pH, making the mold inhibitors more effective. Many samples of cream and inoculated Czapek's medium treated with propionic acid did not show mold growth when held for extended periods.

Propionic acid was very effective in preventing mold growth in raw milk but calcium and sodium propionates were much less effective than with raw cream, even when used in stronger concentrations. The cream contained approximately 64 per cent moisture and the milk contained approximately 87 per cent moisture. The increased moisture content of the milk may have lowered the effectiveness of the mold inhibitors, but the differences in the percentages of the salts added to the cream and milk should have compensated for the differences in the moisture contents. Another possible factor is that the increased fat percentages in the cream may have had the same effect as a wetting agent and thus increased the effectiveness of the mold inhibitors.

Although cream for buttermaking is pasteurized, large numbers of lactic acid producing organisms often are added as one of the types in the butter culture. With salted butter, the action of these organisms does not significantly affect the pH of the butter; with unsalted butter, however, the pH

may be lowered to a significant degree. Salt definitely affects mold growth and appears to have more effect on the mold inhibiting properties of propionic acid and its salts than the lower pH of the unsalted butter. The salt concentrations used in commercial butter are too low to definitely inhibit mold growth alone.

Freshly made cheese contains large numbers of lactic acid producing organism but mold growth usually occurs at the surface of the cheese where reaction changes may have been influenced by various factors.

Dipping butter and cheese in solutions of the mold inhibiting compounds was an effective method of preventing mold growth on these products. Under commercial conditions it is impractical to dip butter in solutions of mold inhibitors due to the consistency of the product. Dipping the parchments, used for wrapping butter and for lining butter tubs, in a solution of one of the mold inhibiting compounds immediately before using should prove a satisfactory method of limiting mold growth since this method was only slightly less effective than dipping the butter. Various cheese, such as cheddar, swiss and similar types, could very easily be dipped in solutions of mold inhibitors.

The paracepts were only slightly soluble in water and were used in 0.1 per cent solutions or solutions and suspensions. The methyl paracept was more soluble in water than

the other paracepts and was definitely more effective in preventing mold growth. The paracepta higher in the series, especially benzyl, were very difficult to get into solution and were ineffective as mold inhibitors.

The effectiveness of mold inhibitors in liquid media was greatly increased by the addition of a wetting agent. In this type of media the moisture provides a continuous phase and the wetting agents aid in keeping the compounds in contact with the mold spores. The wetting agents did not increase the effectiveness of the mold inhibitors on solid materials, such as butter and cheese. With the butter and cheese there was not enough moisture present to provide a continuous phase and this may have prevented the wetting agent from being effective.

Since the conditions in many of the trials were quite similar to conditions in commercial plants, it appears that propionic acid and its salts would be effective and useful commercially for preventing mold growth on dairy products. The possibilities apparently are greatest in connection with butter and various cheese. The demand for consumer-size packages of natural cheese has presented a problem to the industry in that these small pieces of cheese have often developed mold in retail stores and homes.

SUMMARY AND CONCLUSIONS

1. In raw cream, propionic acid in a 0.5 per cent solution inhibited molds for 90 to 138 days and in a 1 per cent solution for 87 to 184 days; lower concentrations were ineffective. Calcium propionate in a 0.8 per cent solution inhibited molds for 84 to 96 days and in a 1 per cent solution for 87 to 184 days; lower concentrations were ineffective. Sodium propionate in a 1 per cent solution inhibited molds for 76 to 86 days. Acetic acid and calcium acetate in solutions of 1 per cent or less were ineffective.

2. In raw milk containing propionic acid in 0.5 and 1 per cent solutions, no mold growth was evident during holding periods of 60 to 90 days. Calcium propionate in a 5 per cent solution inhibited molds for 7 to 16 days; lower concentrations were ineffective. Sodium propionate in a 5 per cent solution inhibited molds for 6 to 18 days.

3. In Czapek's medium inoculated with mixed mold suspensions and containing propionic acid in a 1 per cent solution, no mold growth was evident during holding periods of 60 and 65 days. Calcium propionate in a 5 per cent solution inhibited molds for 12 to 30 days; lower concentrations were

ineffective. Sodium propionate in a 5 per cent solution inhibited molds for 4 to 9 days.

4. With unsalted butter inoculated with mixed mold suspensions, samples held at 15°C. usually showed mold growth before samples held at 10°C. Dipping the butter in 12.5, 18, 19 and 25 per cent solutions of calcium propionate was effective in inhibiting mold growth; a 12.5 per cent solution was least effective and a 25 per cent solution was slightly more effective than an 18 or 19 per cent solution. Dipping regular parchments in 12.5, 15, 19 and 25 per cent solutions of calcium propionate and using them to wrap the unsalted butter was effective in inhibiting mold growth; smearing parchments with a 20 per cent solution was less effective than dipping in 12.5, 15 and 19 per cent solutions. Commercially treated parchment, containing 6 per cent calcium propionate, gave some inhibition but was less effective than dipping the butter or regular parchment in the more concentrated solutions.

5. With salted butter inoculated with mixed mold suspensions, dipping the butter, or dipping regular parchment for wrapping the butter, in 12.5, 19 and 25 per cent solutions of calcium propionate proved effective in inhibiting mold growth. The 12.5 per cent solution was least effective and 19 and 25 per cent solutions were about equally effective.

6. In the comparisons of salted and unsalted butter inoculated with mixed mold suspensions, the unsalted samples usually showed mold growth before the salted samples. The use of different dilutions of a mold suspension to inoculate the butter made little difference in the period before mold growth appeared. Dipping parchments in a 5 per cent solution of propionic acid was effective in inhibiting mold growth. Dipping the butter or parchments in 6, 12, 15, 18 and 25 per cent solutions of calcium propionate was effective in inhibiting mold growth; the 6, 12 and 15 per cent concentrations were slightly less effective than the higher concentrations.

Dipping the butter in 18 and 25 per cent solutions of sodium propionate was effective in preventing mold growth. Commercially treated parchment, containing 6, 10, 25 and 30 per cent calcium propionate, gave little inhibition with either the salted or unsalted butter.

7. With cheddar cheese that, except for one series of trials, was uninoculated with mold suspensions, samples held at 15°C. usually showed mold growth before samples held at 10°C. Dipping in propionic acid solutions of 5, 7 and 10 per cent was effective in inhibiting mold growth. Dipping in calcium propionate solutions of 10 and 12 per cent gave some inhibition; 18 and 25 per cent solutions were very effective. Dipping in a sodium propionate solution of 12

per cent was only slightly effective; an 18 per cent solution was effective. Commercially treated parchment, containing 6, 10, 25 and 30 per cent calcium propionate, gave slight inhibition. Cellophane, when used to wrap cheese, increased the effectiveness of the mold inhibitors compared to tin foil and parchment.

With uninoculated swiss-type cheese, dipping in a 10 per cent propionic acid solution and an 18 per cent calcium propionate solution inhibited mold for 8 to 10 days.

8. With uninoculated cottage cheese, smearing the surface with 7 per cent propionic acid, 18 per cent calcium propionate and 18 per cent sodium propionate solutions was ineffective in preventing mold growth. When these solutions were mixed with the cheese in relatively large volumes, they were effective as mold inhibitors.

9. In raw cream, and also in Czapek's medium inoculated with mixed mold suspensions, 1 per cent sodium benzoate was effective as a mold inhibitor. One-tenth per cent methyl paracept gave some inhibition. A 5 per cent solution of zephiran and 0.1 per cent solution (or suspension and solution) of ethyl, propyl, butyl, benzyl and servex emulsion paracepts were ineffective.

The paracepts, when used in 0.1 per cent solutions (or suspension and solution) for dipping salted butter and cheddar cheese were ineffective.

10. Special parchments used to wrap salted butter and cheddar cheese did not increase the effectiveness of propionic acid, calcium propionate and sodium propionate as mold inhibitors.

11. In raw cream, and also in Czapek's medium inoculated with mixed suspensions, the use of 1 per cent of various wetting agents increased the effectiveness of calcium and sodium propionate as mold inhibitors; when salted butter and cheddar cheese were dipped in solutions of the mold inhibitors with the wetting agents present, these agents did not increase the effectiveness of the inhibitors.

12. With pure cultures of P. roqueforti, P. camemberti and O. lactis in milk and Czapek's medium, propionic acid in 0.5 and 1 per cent solutions was very effective in preventing growth. Calcium propionate in a 5 per cent solution inhibited growth; a 1 per cent solution was ineffective. Sodium propionate in 1 and 5 per cent solutions was relatively ineffective except with P. camemberti.

13. Dusting mold spores on the surface or distributing them through a medium containing propionic acid, calcium propionate or sodium propionate made little difference in the period before mold growth was evident.

14. With 1 per cent propionic acid in inoculated milk and raw cream, the effect of the mold inhibitor was evident microscopically by the disappearance of the spores from the

microscopic preparations. With lower concentrations of propionic acid and with 1, 2 and 5 per cent calcium propionate, there was a delay in the germination of the spores, compared to the controls, and often there was a disappearance of some of the spores; eventually some spores germinated so that segments of mold were evident. The rate and extent of segment formation was dependent on the concentration of the inhibitors. With calcium propionate in inoculated milk and raw cream, lowering the pH with lactic acid increased the inhibition of the molds.

15. Propionic acid often imparted a slight odor and flavor to the dairy products used, but neither was objectionable and with cheese the flavor was rather pleasing. White discolorations on butter and cheese were sometimes caused by the acid, but these disappeared after a few days. Calcium propionate and sodium propionate imparted a slight odor and flavor of propionic acid, but they were less pronounced than those from the acid. These salts in higher concentrations occasionally produced white discolorations on butter and cheese. Acetic acid produced a slight odor, but the other compounds produced no noticeable odors, flavors or discolorations.

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