ABSTRACT

THE EFFECT OF POTASSIUM SORBATE ON ORGANISMS COMMONLY ASSOCIATED WITH COTTAGE CHEESE SPOILAGE

by Robert L. Bradley, Jr.

Cottage cheese dressings containing several concentrations of potassium sorbate were inoculated with Pseudomonas fragi, Alcaligenes metalcaligenes, Geotrichum candidum, Penicillium frequentans, Rhodotorula mucilaginosa or Torulopsis candida. The dressings were then mixed with the curd and stored at 50°F. Cheese samples inoculated with psychrophiles were analysed daily and those inoculated with yeasts or molds were analysed on alternate days. At each examination interval analyses were performed on each sample for total count, psychrophile count, yeast and mold count, pH, and percentage of potassium sorbate.

The presence of potassium sorbate in the cheese in concentrations of 0.050, 0.075, and 0.100 per cent adversely affected the growth of the spoilage organisms. Potassium sorbate was both fungicidal and fungistatic. The fungicidal property was evidenced when the organism populations decreased as the concentrations of potassium sorbate were increased. Fungistatic activity was shown by extended lag phases and retarded growth. Potassium sorbate appeared to be bactericidal and bacteriostatic when used against small concentrations of psychrophiles.

In duplicate trials involving different populations of inocula, a longer lag phase was associated with a lower population. No off-flavor was noticed in creamed cottage cheese containing 0.100 per cent potassium sorbate. There was no evidence that any of the organisms metabolized potassium sorbate as a source of carbon.

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By

ROBERT L. BRADLEY, Jr.

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TABLE OF CONTENTS

TABLES

FIGURES

INTRODUCTION

Creamed cottage cheese, a protein concentrate containing about 475 to 500 calories per pound, is becoming an important food item with our diet conscious public. The per capita consumption has increased from 1.9 pounds in 1940 to 5.1 pounds in 1958. Continued improvement in the quality of cottage cheese is necessary to maintain increased consumption.

Improper handling during manufacture, storage, and delivery together with adverse temperatures in grocery store and household refrigerators are detrimental to a long shelf-life. Research studies have proved that high quality acid foods will have a longer shelf-life when sorbic acid is added. Also, sorbic acid imparts no toxicity to the food.

The scope of this study is to demonstrate the efficacy of potassium sorbate against surface spoilage organisms in creamed cottage cheese and to determine whether these organisms utilize sorbic acid during normal storage periods.

REVIEW OF LITERATURE

Cottage cheese with a relatively low microorganism content may become unsalable in a short time unless it is constantly refrigerated at 40° F. or lower. Harmon et al. (11) reported on the relationship of temperature to keeping quality of cottage cheese. By lowering the storage temperature from 50° to 42° F., they demonstrated that shelf-life could be increased by 12 to 100 per cent. Another section of their study revealed that temperatures of display cabinets in a cross section of retail markets ranged from 43° to 60° F. at the top of the cabinet and 38° to 55° F. at the bottom.

Adverse storage temperatures have caused cheese manufacturers to consider the advantages of using sorbic acid and its potassium salt to increase the shelf-life of their product. The Federal Food and Drug Administration (28) has approved sorbic acid for use in cheese and cheese products in amounts up to 0.2 per cent sorbic acid by weight.

The Antimycotic Acitivity of Fatty Acids

Sorbic acid (2,4-hexadienoic acid) is an , -unsaturated acid containing the same number of carbon atoms as caproic acid, but exhibiting greater fungicidal properties than caproic acid. Clark (3) was one of the first to report that the un-ionized portion of the fatty acid molecule was toxic to microorganisms. He demonstrated that acetic acid was much more toxic than the mineral acids.

Kiesel (14) revealed many factors about the antimycotic characteristics of the fatty acids, most important of which are: a) the activity of the saturated fatty acids increases with the carbon chain length, and b) branched chain fatty acids are less active than those with straight chains and an equal number of carbons. Wyss et al. (29) also contributed to the knowledge of the inhibitory effect of the fatty acids and showed that: a) the activity of a fatty acid increases with decreasing pH, b) unsaturated fatty acids are slightly more active than saturated fatty acids, c) the point of unsaturation has little effect on the activity, d) multiple unsaturation gives about the same activity as mono-unsaturation and e) optimum chain length is determined by the resistance of the organism and the solubility of the fatty acid.

Cowles (6) showed that fatty acids are bactericidal at pH 4.7 and lower. Keeney (13) reported fungicidal properties for the 5, 6, 8, 10, and 11 carbon fatty acids, whereas, the shorter chain acids exhibited only fungistatic properties.

Physiological Properties of Sorbic Acid

One of the most efficient fungistats known today is sorbic acid and its potassium salt. The toxicity of these compounds as dietary constituents was investigated by Deuel et al. (8). They proved that sorbic acid would not interfere with the digestion of foods and that α,β-unsaturated fatty acids were readily metabolizable. In toxicity tests with rats, it was found that tissue changes did not occur when 0 to 8 per cent sorbic acid was included in the feed. However, a slight increase in liver weight was reported at the 8 per cent level. When an 8 per cent concentration of

benzoic acid was fed to rats, there was a 50 per cent mortality and an increase in liver and kidney weight. These workers concluded that sorbic and benzoic acids fed to rats at a 4 per cent concentration showed no harmful effects in a 90 day trial. Demaree et al. (7) investigated the effects of feeding sorbic acid at a 10 per cent level to two generations of rats. Their results showed that the parents and only the male offspring had increased liver weights. The males in both generations showed an increase in depot fat. None of the test rats appeared to decrease in reproductive capacity.

Physical and Chemical Characteristics of Sorbic Acid

Melnick and Luckmann (16) proved that migration of sorbic acid occurred in six varieties of cheese at a rate proportional to the moisture content of the cheese. One surface of a wrapped block of cheddar cheese was covered with a wrapper treated with 2.5 grams of sorbic acid per 1,000 square inches of surface. The concentration of sorbic acid at the treated surface decreased during the aging period. To determine the depth of migration the cheese was sliced into one-eighth inch strips, homogenated, and analysed for sorbic acid content. Some sorbic acid was actually lost from the wrapped cheese. The possibilities of sublimation through the wrapper and auto-oxidation were disproved. Melnick et al. (17) found that mold enzymes, concentrated near the surface of the cheese, accomplish metabolic degradation of the sorbic acid by reducing it to carbon dioxide and water; whereupon, the migrated sorbic acid attempts to re-establish the concentration equilibrium by re-migrating toward the lesser concentration at the outer surface.

The work of Mukherjee (20, 21, 22) elucidated the mechanism by which sorbic acid inhibits microorganisms. β-oxidation of unsaturated fatty acids occurs according to the sequence in Figure 1. The intermediates formed are β-keto acids. In the degradation of butyric acid by the dehydrogenase enzymes of Aspergillus niger, hydration of the β-unsaturate produced β-hydroxybutyric acid. This compound was always present during the reaction, usually in small quantities. Continual oxidation prevented an accumulation of β-hydroxybutyric acid. In the later stages of the degradation, acetone accumulation and the absence of the unsaturated compound were noted. The presence of acetone indicated that the end-products were the same as in human metabolism. Accumulation of increased amounts of the β-hydroxybutyric acid inhibited dehydrogenation which would produce the β-unsaturate. This β-hydroxy acid inactivated the dehydrogenase enzymes of the mold. Since this inhibition is a function of the amount of the β-hydroxy acid present, the continual production of dehydrogenase would eventually overcome the inhibitor after which the mold utilizes the inhibitor as a source of carbon.

Sorbic acid, with the formula CH_3-CH=CH-CH=CH-COOH is an α,β -unsaturated acid. Melnick et al. (17) noted that a β-hydroxy acid occurred two times during the degradation of sorbic acid, once after hydration, and again after it passed through a dehydration, oxidation, and hydration sequence.

Samson et al. (26) observed that the effectiveness of fatty acids as fungistats was a function of pH. The inhibitory effect increased with decreasing pH because the acid was permeable to the cell in the undissociated

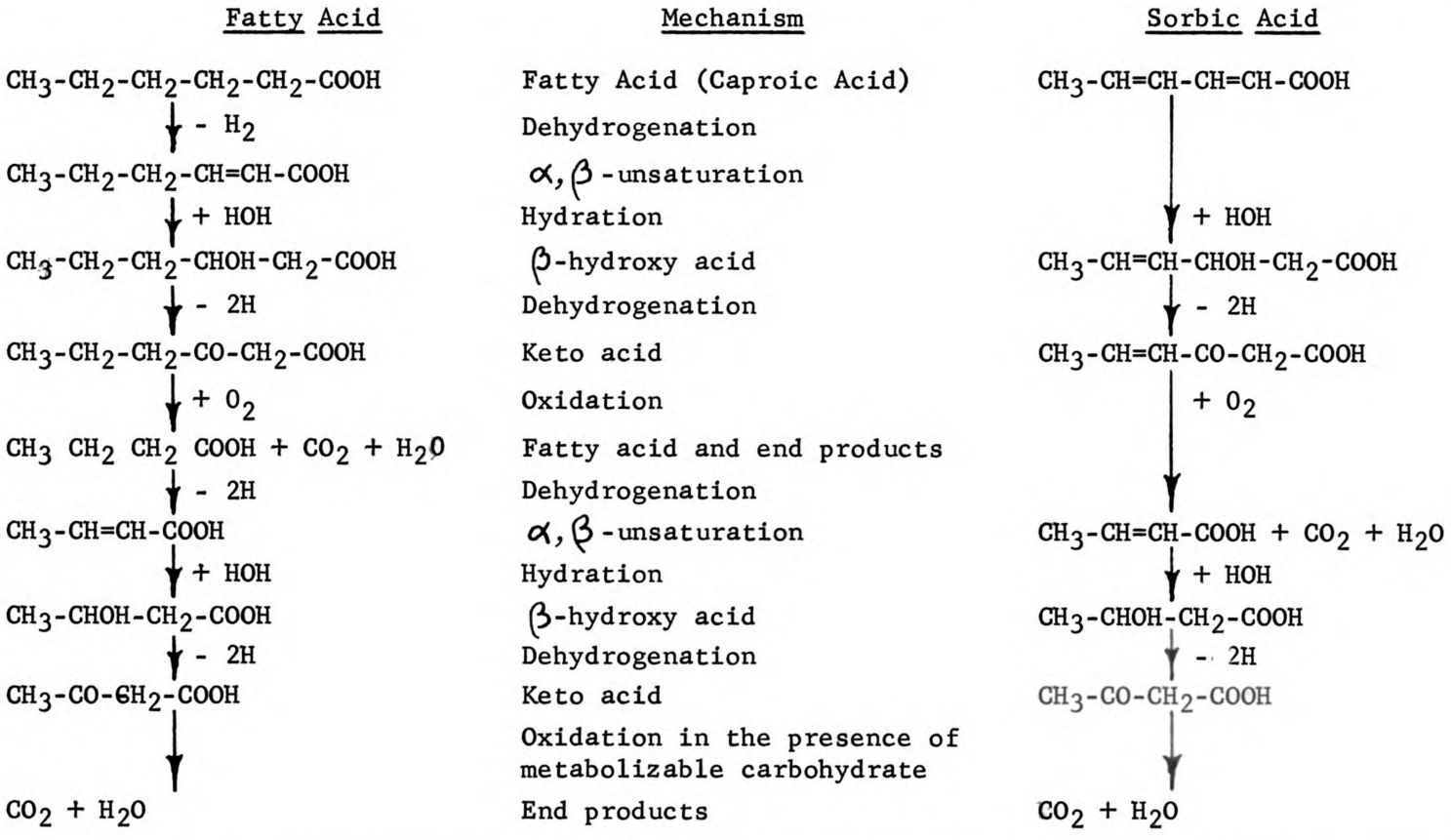

Figure 1. Wieland's dehydrogenation - hydration - dehydrogenation scheme[a]

[a]Melnick, D. et al. Metabolic degradation of Sorbic Acid in Cheese by Molds and the Mechanism of Mold Inhibition. Food Research 19:44.1954.

form only. According to O'Neill (22), any food product with a pH of 6.5 or lower and a potassium sorbate concentration of 0.1 per cent or less would have all the sorbate hydrolyzed to sorbic acid. Pfizer (24, 25) indicates the following solubilities for sorbic acid and potassium sorbate:

	Sorbic Acid	Potassium Sorbate
Solubility in H_2O at 20° C. at pH 4.4	0.22%	*
Solubility in H_2O at 20° C. at pH 5.9	1.02%	*
Solubility in H_2O at 20° C. at pH 7.0	7.00%	>40%

*Hydrolyzed to the acid form at this pH.

Cowles (6) reported that the germicidal activity of the salts of saturated fatty acids varies according to Traube's law which states that the lowering of surface tension is directly proportional to the length of the carbon chain. Hartman (12) stated that substances become absorbed at the cell interfaces by lowering the surface tension of the solvent.

Sensitivity of Organisms to Sorbic Acid

Bell et al. (1) reported on the influence of sorbic acid at several pH levels on many species of bacteria, yeasts, and molds commonly associated with cucumber fermentations. Thirty two species representing 12 genera of yeasts grew in 0.1 per cent sorbic acid at pH 7.0 but not at pH 4.5. Candida krusei was the most tolerant yeast of the 32 species tested. Sixty six species representing 32 genera of filamentous fungi would not grow in 0.1 per cent sorbic acid at pH 4.5. Six species representing three genera of lactic bacteria were inhibited by 0.1 per cent sorbic acid at pH 3.5 but all grew at pH 5.0. These tests suggest that the efficiency

of sorbic acid as an inhibitor of certain microorganisms is based on the concentration of the undissociated molecule and not the total concentration of the acid.

Costilow et al. (4) reported that neither the lactic bacteria nor a large contamination of a yeast (Torulopsis holmii) metabolized sorbic acid in cucumber brines. Another study by Costilow et al. (5) demonstrated that sorbic acid concentrations of 0.01 to 0.1 per cent in an 8 per cent sodium chloride brine were inhibitory to yeasts and molds.

Perry and Lawrence (23) stated that sorbic acid would not suppress heavy contaminations but would effectively retard small contaminations of microorganisms. They also noted that samples containing up to 0.07 per cent sorbic acid by weight were similar in flavor to the controls. Geminder (10) reported that 0.075 per cent sorbic acid was effective in retarding the growth of yeasts, molds, and slime-forming bacteria on cottage cheese. Higher sorbic concentrations produced a bitter flavor in the finished product.

EXPERIMENTAL PROCEDURE

The purpose of this experiment was to measure the inhibitory effect of potassium sorbate on organisms commonly associated with cottage cheese spoilage and to determine if any of the organisms studied utilized potassium sorbate as a source of carbon.

Cottage Cheese Manufacture

Skimmilk pasteurized at 145° F. for 30 minutes was obtained from the Michigan State University Dairy Plant. Cottage cheese was made from this skimmilk by the short-set method. The creaming mixture containing 12 per cent fat was pasteurized by steaming in an autoclave for 30 minutes. Three per cent salt was added to the pasteurized creaming mixture.

Propagation of Organisms

The following organisms commonly associated with surface spoilage in cottage cheese were used in this study: *Pseudomonas fragi*, *Alcaligenes metalcaligenes*, *Geotrichum candidum*, *Penicillium frequentans*, *Rhodotorula mucilaginosa* and *Torulopsis candida*.

An active strain was developed by initiating daily transfers into nutrient broth three days before the organism was to be used. The cultures were incubated at 72° F.

Four milliliters of the yeast and 4 ml. of the mold cultures were used to inoculate the respective creaming mixtures, whereas, only 2 ml. of the psychrophiles were used to give the desired contamination. The psychrophile populations inoculated into the cottage cheese dressings were

determined on Violet Red Bile agar and the yeasts and molds were enumerated on Potato Dextrose agar acidified to pH 3.5.

Preparation of Samples

A total of 12 lots of cheese were analysed with two lots being contaminated with each of the six spoilage organisms mentioned previously. Each trial (lot) was composed of five groups of cheese samples labelled as follows: group 1) non-inoculated control, group 2) inoculated control, group 3) inoculated + 0.050 per cent potassium sorbate, group 4) inoculated + 0.075 per cent potassium sorbate, and group 5) inoculated + 0.100 per cent potassium sorbate. The diagram (Figure 2) shows how the samples were prepared and indicates the analyses performed on each sample.

Sixty seven grams of the dry curd and 33 grams of the creaming mixture were weighed into each sterilized sample jar. The samples were incubated at 50° F. until surface spoilage was observed.

ANALYTICAL PROCEDURES

Bacteriological Analyses

In the trials involving cheese samples inoculated with psychrophiles, one sample from each of the five groups was analysed daily for sorbic acid content, organism populations, and pH. In the trials with samples inoculated with yeasts or molds, one sample from each of the five groups was analysed every other day. Each sample was mixed for two minutes in a sterilized Waring blendor at slow speed to assure thorough mixing. At high speed, curd particles adhered to the upper surfaces of the jar and were

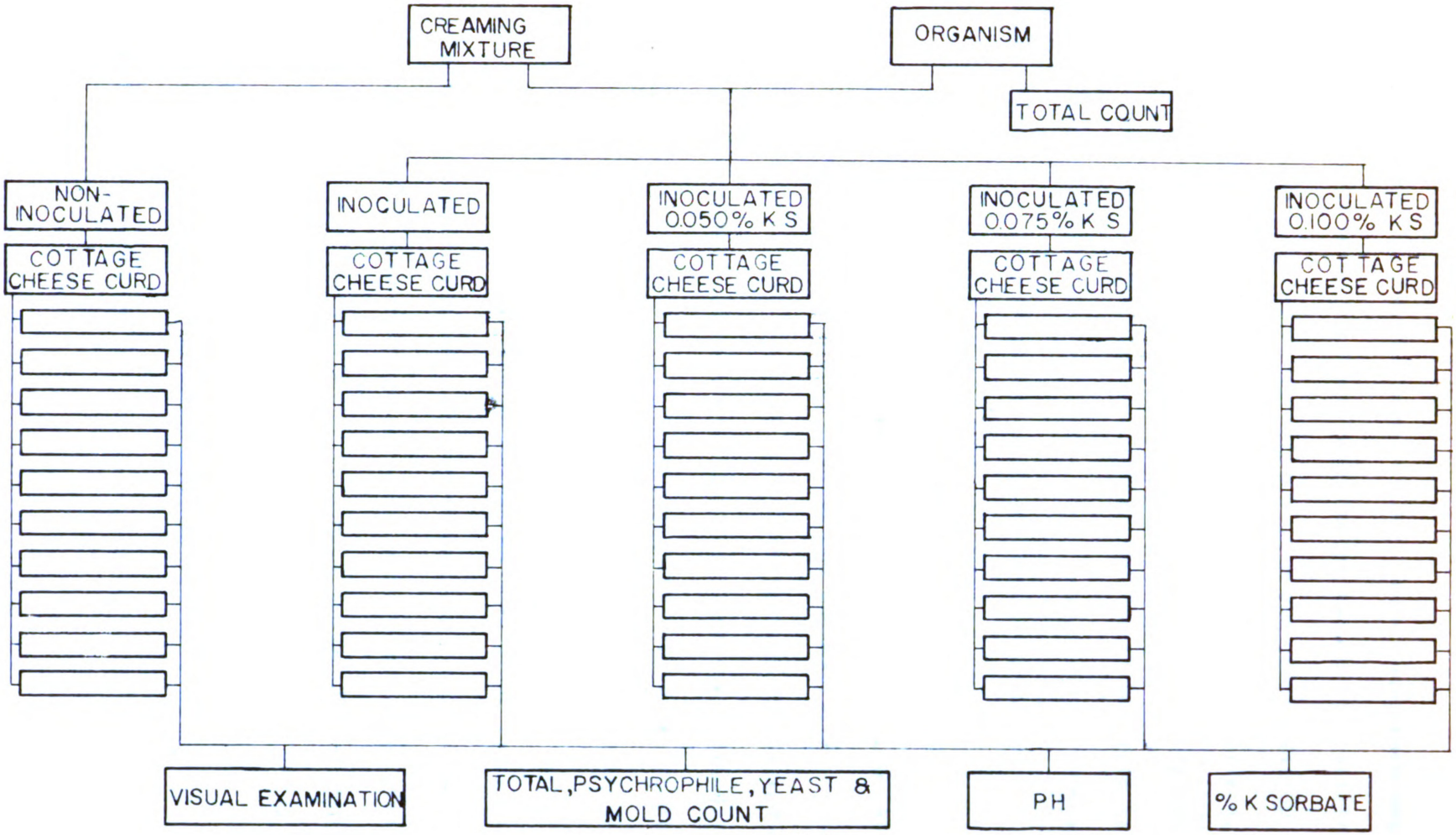

Figure 2. Diagram illustrating procedure for preparing samples of creamed cottage cheese in sterile 8 oz. jars and indicating the analyses performed.

not blended properly. An 11 gram sample of this homogenate was added to 99 ml. of peptone water (0.1 per cent Bacto-peptone in distilled water) and mixed for an additional two minutes in a sterilized Waring blendor jar. Appropriate dilutions were plated with Violet Red Bile agar, Tryptone Glucose Yeast agar, and Potato Dextrose agar acidified to pH 3.5 with tartaric acid. The Violet Red Bile and Potato Dextrose agar plates were incubated at 72° F. for 3 and 4 days, respectively. Tryptone Glucose Yeast agar plates were incubated at 89.6° F. for 48 hours.

Determination of Sorbic Acid

A 2 gram portion of the homogeneous sample of cheese was analyzed for sorbic acid by the method of Melnick and Luckmann (15). The 2 gram sample, 50 grams of $MgSO_4$, and 50 ml. of de-ionized, distilled water were added to a two necked, 500 ml. distillation flask and a Friedrich's condensor was affixed. A Glas-col mantle connected to a powerstat was used for the heat source. When the contents of the flask started to bump, another 50 ml. of de-ionized, distilled water was added through the side inlet. Upon completion of the distillation, the flask was cooled, and the apparatus was dismantled. The condensor was rinsed with hot de-ionized, distilled water to remove any traces of sorbic acid. This rinse water was added to the distillate previously collected in the 500 ml. volumetric flask. The contents of the flask were made up to 500 ml. with de-ionized distilled water and shaken.

A Beckman DK-2 spectrophotometer was used to record the absorbancy of the distillates, in the 255 to 263 mμ range. The controls, having no

potassium sorbate added, were used to determine the irrelevant absorbancy. A previously prepared standard curve was used to convert the absorbancies of the distillates to percentages of potassium sorbate. Appropriate corrections were made for small amounts of irrelevant absorbancy.

pH measurements were made on each sample with a Beckman Zeromatic pH meter using a calomel half cell and a glass electrode.

The analysis of the inoculated samples was terminated when visible spoilage had occurred. No further analyses of the non-inoculated samples were conducted after all the inoculated samples had spoiled.

RESULTS

Each sample of creamed cottage cheese was examined for psychrophile, yeast and mold populations. Graphs were constructed to show organism growth by plotting the logarithm of the count per gram of cheese on the vertical axis and the number of days of incubation on the horizontal axis. The graphs that illustrate the growth of these organisms in the samples of cheese, represent only the specific classification of the organism (psychrophile, yeast, or mold) inoculated into the sample. The counts of the non-inoculated controls which correspond to the particular classification of organism inoculated into the cheese, are also shown on the graphs to illustrate the growth of the inherent contaminants.

A comparison of the populations of the inoculated samples and the non-inoculated controls on the 0 day showed that contamination other than that induced was negligible. Also, the significance of the inherent contaminants was minimized by the dilutions that were performed. Thus, the occurrence of spoilage was assumed to be the result of the organism inoculated into the cheese. The appearance of the surface of the spoiled cheese was typical of the organism inoculated into the samples in all groups except in the samples inoculated with R. mucilaginosa. The samples that contained 0.075 and 0.100 per cent potassium sorbate exhibited no typical growth of the yeast. The lack of growth was attributed to the fungistatic activity of the sorbic acid.

An examination of the counts on 0 day (Tables I through XII) revealed a discrepancy between the populations enumerated in the inoculated control samples of cottage cheese and the populations determined in the inocula. In most cases, the counts of the inoculated control samples were significantly higher within four hours after inoculation than the calculated populations which were added to the cheese. A hypothesis that rapid growth of organisms had occurred could be inferred. However, the method of blending necessary to make the samples homogeneous may have shattered the colonies and caused the higher count in the inoculated control samples on the 0 day.

The difference in time required for the duplicate samples to spoil varied from 0 to 4 days. This variation occurred principally because of three factors: a) variation in the inherent populations present in the non-inoculated cheese, b) variation in populations inoculated into the cheese and activity of the inoculum, and c) variations in the amount of water contained in different lots of cheese. The populations at spoilage in the duplicate samples were also affected by these factors.

Analysis of Samples Inoculated with Pseudomonas fragi.- The data in Tables I and II indicate that the populations of psychrophiles determined on the 0 day in the inoculated control samples, approximately four hours after inoculation, were significantly greater than the calculated number of Ps. fragi organisms introduced into the cheese with the inoculum. Also, the addition of potassium sorbate to the cottage cheese caused a substantial decrease in the psychrophile count on the 0 day which suggested that the sorbic acid was bactericidal to Ps. fragi.

The growth curves of the psychrophiles (Figure 3) exhibited lags at the beginning of storage. The duration of the lag phase increased as the percentages of potassium sorbate in the samples increased. A comparison of Figures 3 and 4 demonstrates the direct relationship of organism population and the length of the lag phase; i.e., the smaller the concentration of organisms, the longer the lag phase. The smaller concentration of organisms in the inoculum added to the samples represented in Figure 3 showed a lag, whereas, the populations added to the samples represented in Figure 4 showed no lag phase. The duration of the lag phases in Figure 3 was 1 day for the samples containing 0.050 per cent potassium sorbate and 2 days for the samples containing 0.100 per cent potassium sorbate. The fact that there was no lag in the sample that contained 0.075 per cent potassium sorbate suggested a possible analytical error in the initial count, which was much lower than the count of the other two samples containing potassium sorbate. When the inhibitory effects of the sorbate ion were no longer apparent, logarithmic growth occurred and the slopes of all the growth curves of the psychrophiles in the inoculated samples were similar.

Standard plate and psychrophile counts (Tables I and II) indicated that growth of Ps. fragi was retarded by the addition of potassium sorbate. Since the strain used in this experiment grew at 89.6°F., the standard plate counts of the inoculated samples were higher than the standard plate counts of the non-inoculated controls. If this strain of Ps. fragi had been unable to grow at 89.6°F., the standard plate counts of the non-inoculated controls and inoculated samples would have been similar.

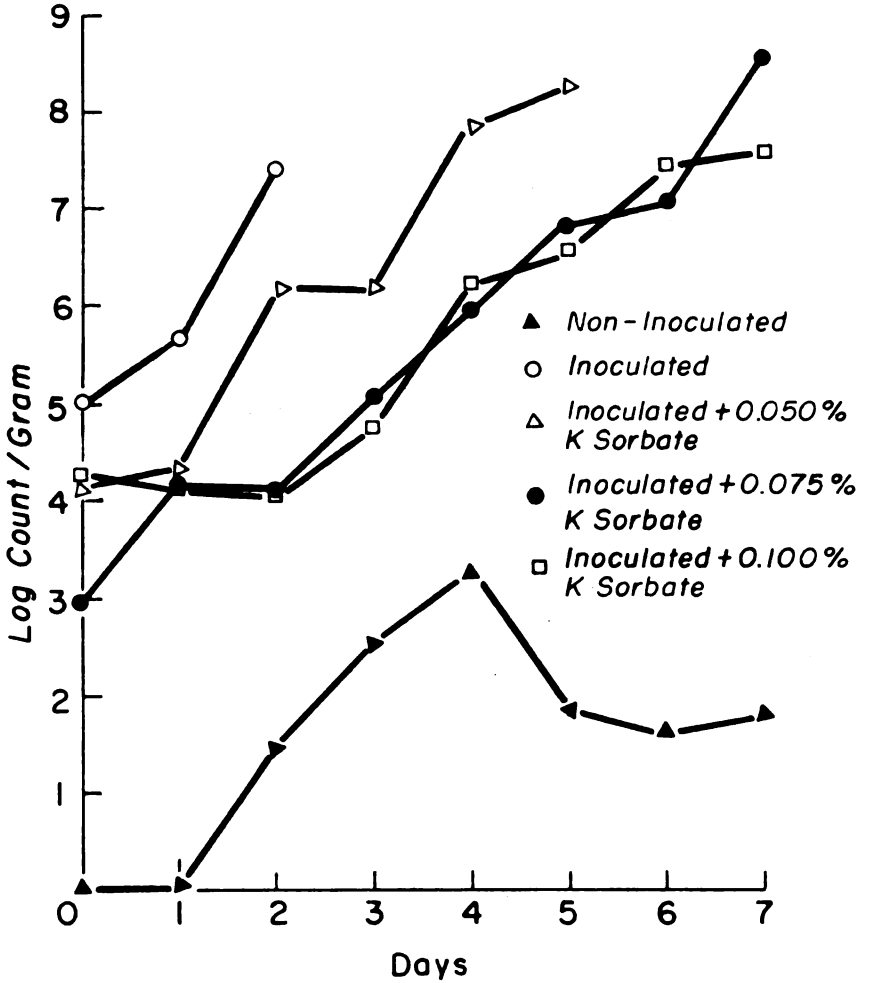

Figure 3. Effect of potassium sorbate on Pseudomonas fragi inoculated into creamed cottage cheese stored at 50°F.

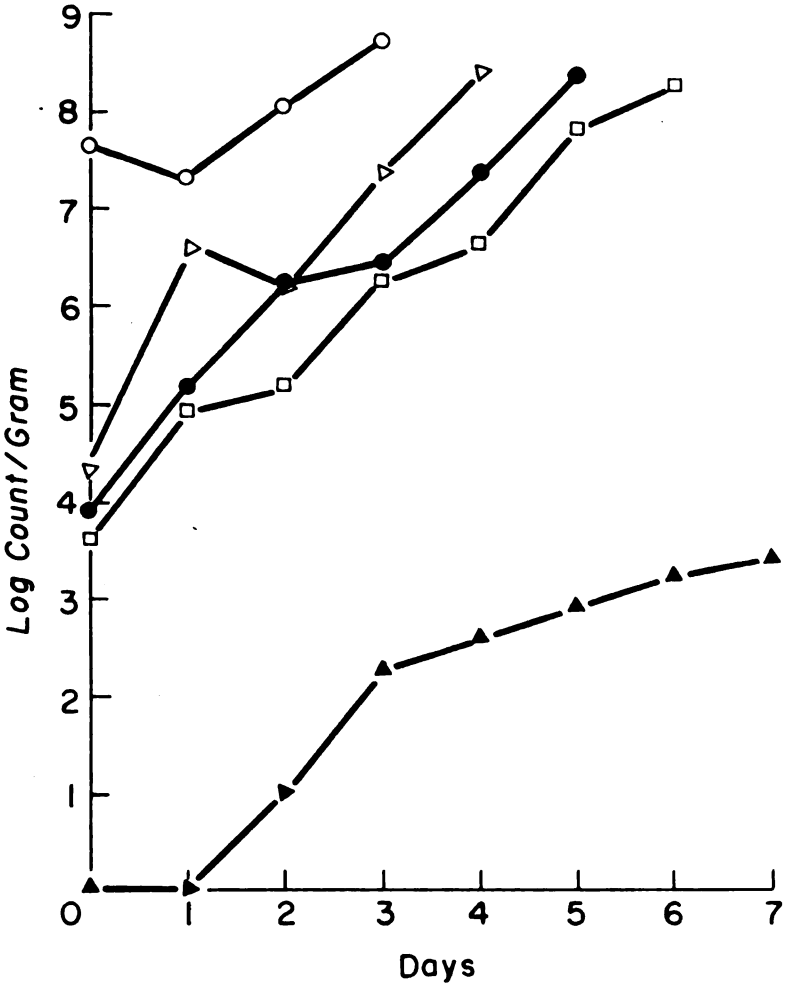

Figure 4. Effect of potassium sorbate on Pseudomonas fragi inoculated into creamed cottage cheese stored at 50°F.

TABLE I

ORGANISM POPULATION, pH, AND PER CENT POTASSIUM SORBATE* IN CREAMED COTTAGE CHEESE INOCULATED WITH PSEUDOMONAS FRAGI (30,000 organisms per gram) AND INCUBATED AT 50°F.

Type of Analysis	Description of Cheese Sample				
	Controls		Inoculated + Potassium Sorbate as Indicated		
	Non-inoculated	Inoculated	0.050%	0.075%	0.100%
		0 Day			
Total Count	720	110,000	50,000	15,000	31,000
Psychrophile	<10	91,000	15,000	9,000	18,000
Yeast and Mold	10	<10	<10	<10	<10
pH	5.30	5.30	5.30	5.35	5.35
% K Sorbate	0.000	0.000	0.048	0.069	0.112
		1 Day			
Total Count	800	840,000	55,000	12,000	11,000
Psychrophile	<10	460,000	23,000	15,000	13,000
Yeast and Mold	<10	<10	<10	<10	<10
pH	5.30	5.30	5.30	5.35	5.35
% K Sorbate	0.000	0.050	0.045	0.077	0.113
		2 Days			
Total Count	17,000	19,000,000	100,000	19,000	13,000
Psychrophile	30	25,000,000	1,400,000	11,000	11,000
Yeast and Mold	20	<10	<10	<10	<10
pH	5.35	5.35	5.30	5.35	5.35
% K Sorbate	0.000	0.000	0.046	0.078	0.111
		3 Days			
Total Count	5,800	30,000,000	3,200,000	140,000	9,500
Psychrophile	340	21,000,000	1,500,000	110,000	60,000
Yeast and Mold	<10	<10	<10	<10	<10
pH	5.25	5.30	5.35	5.35	5.35
% K Sorbate	0.000	0.000	0.045	0.074	0.112

TABLE I (Continued)

Type of Analysis	Description of Cheese Sample: Controls: Non-inoculated	Controls: Inoculated	Inoculated + Potassium Sorbate as Indicated: 0.050%	0.075%	0.100%
		4 Days			
Total Count	66,000	30,000,000	30,000,000	1,300,000	1,800,000
Psychrophile	1,900	320,000,000	78,000,000	900,000	1,700,000
Yeast and Mold	<10	<10	<10	<10	<10
pH	5.35	5.40	5.45	5.30	5.30
% K Sorbate	0.000	0.000	0.042	0.064	0.107
		5 Days			
Total Count	260,000	----	260,000,000	6,700,000	4,700,000
Psychrophile	70	----	180,000,000	6,900,000	3,700,000
Yeast and Mold	<10	----	<10	<10	<10
pH	5.40	----	5.50	5.45	5.45
% K Sorbate	0.000	----	0.043	0.076	0.098
		6 Days			
Total Count	1,100,000	----	----	14,000,000	22,000,000
Psychrophile	40	----	----	12,000,000	27,000,000
Yeast and Mold	30	----	----	<10	<10
pH	5.40	----	----	5.35	5.45
% K Sorbate	0.000	----	----	0.068	0.108
		7 Days			
Total Count	1,200,000	----	----	310,000,000	33,000,000
Psychrophile	60	----	----	380,000,000	35,000,000
Yeast and Mold	20	----	----	90	10
pH	5.40	----	----	5.40	5.45
% K Sorbate	0.000	----	----	0.077	0.111

0.000 corrected for irrelevant absorbancy
* calculated as sorbic acid

TABLE II

ORGANISM POPULATION, pH, AND PER CENT POTASSIUM SORBATE* IN CREAMED COTTAGE CHEESE INOCULATED WITH PSEUDOMONAS FRAGI (460,000 organisms per gram) AND INCUBATED AT 50° F.

Type of Analysis	Description of Cheese Sample				
	Controls		Inoculated + Potassium Sorbate as Indicated		
	Non-inoculated	Inoculated	0.050%	0.075%	0.100%
		0 Day			
Total Count	2,000	43,000,000	68,000	30,000	27,000
Psychrophile	<10	46,000,000	25,000	8,000	4,000
Yeast and Mold	<10	<10	<10	<10	<10
pH	5.35	5.35	5.35	5.30	5.30
% K Sorbate	0.000	0.000	0.046	0.059	0.092
		1 Day			
Total Count	4,700	19,000,000	2,200,000	1,400,000	750,000
Psychrophile	<10	21,000,000	4,900,000	150,000	82,000
Yeast and Mold	<10	<10	<10	<10	<10
pH	5.35	5.30	5.25	5.25	5.20
% K Sorbate	0.000	0.000	0.037	0.052	0.101
		2 Days			
Total Count	4,900	TNTC	8,000,000	2,300,000	230,000
Psychrophile	10	120,000,000	1,800,000	1,700,000	140,000
Yeast and Mold	<10	<10	<10	<10	<10
pH	5.25	5.40	5.25	5.25	5.25
% K Sorbate	0.000	0.000	0.040	0.050	0.092
		3 Days			
Total Count	6,900	790,000,000	90,000,000	5,500,000	3,800,000
Psychrophile	190	540,000,000	27,000,000	2,800,000	1,900,000
Yeast and Mold	10	<10	<10	<10	<10
pH	5.20	5.40	5.25	5.20	5.20
% K Sorbate	0.000	0.000	0.040	0.062	0.100

TABLE II (Continued)

Type of Analysis	Description of Cheese Sample: Controls: Non-inoculated	Controls: Inoculated	Inoculated + Potassium Sorbate as Indicated: 0.050%	0.075%	0.100%
		4 Days			
Total Count	26,000,000	----	530,000,000	35,000,000	11,000,000
Psychrophile	400	----	270,000,000	22,000,000	4,200,000
Yeast and Mold	3,200	----	400	200	210
pH	5.25	----	5.45	5.35	5.30
% K Sorbate	0.000	----	0.052	0.076	0.100
		5 Days			
Total Count	1,400,000	----	----	180,000,000	110,000,000
Psychrophile	800	----	----	22,000,000	1,200,000
Yeast and Mold	3,900	----	----	370	360
pH	5.25	----	----	5.45	5.45
% K Sorbate	0.000	----	----	0.079	0.102
		6 Days			
Total Count	440,000	----	----	210,000,000	100,000,000
Psychrophile	1,700	----	----	230,000,000	65,000,000
Yeast and Mold	4,200	----	----	640	530
pH	5.20	----	----	5.45	5.45
% K Sorbate	0.000	----	----	0.072	0.089
		7 Days			
Total Count	1,600,000	----	----	----	190,000,000
Psychrophile	2,600	----	----	----	190,000,000
Yeast and Mold	7,100	----	----	----	1,100
pH	5.30	----	----	----	5.45
% K Sorbate	0.000	----	----	----	0.094

0.000 corrected for irrelevant absorbancy
*calculated as sorbic acid

Ps. fragi showed no evidence of metabolizing potassium sorbate during the storage period since no decrease in sorbate concentration was noted. In one of the duplicate trials the pH values of the samples containing sorbate showed a gradual increase from 5.30 to 5.45 (Table I); whereas, in the other trial (Table II) the pH values showed a slight decrease from 5.30 to 5.20 followed by an increase to 5.45.

A fruity odor was present in the inoculated control samples on the first day; however, surface deterioration was not observed until the second or third days.

Analysis of Samples Inoculated with Alcaligenes metalcaligenes.- The growth curves of Alc. metalcaligenes (Figures 5 and 6) showed a lag in the growth of the organism which increased as the percentage of potassium sorbate increased. The population of psychrophiles in the inoculated control sample on the 0 day was 1,500,000 (Table IV). This count was 480, 790, and 2300 per cent greater than the counts in the inoculated samples containing 0.050, 0.075, and 0.100 per cent potassium sorbate, respectively, which suggested a bactericidal activity attributable to the undissociated sorbic acid. Moreover, the lag of the growth curve extended in length to as much as 2 days in the samples that contained 0.100 per cent potassium sorbate. A logarithmic growth rate occurred in the samples inoculated with Alc. metalcaligenes after the inhibitory property of the sorbate became ineffective.

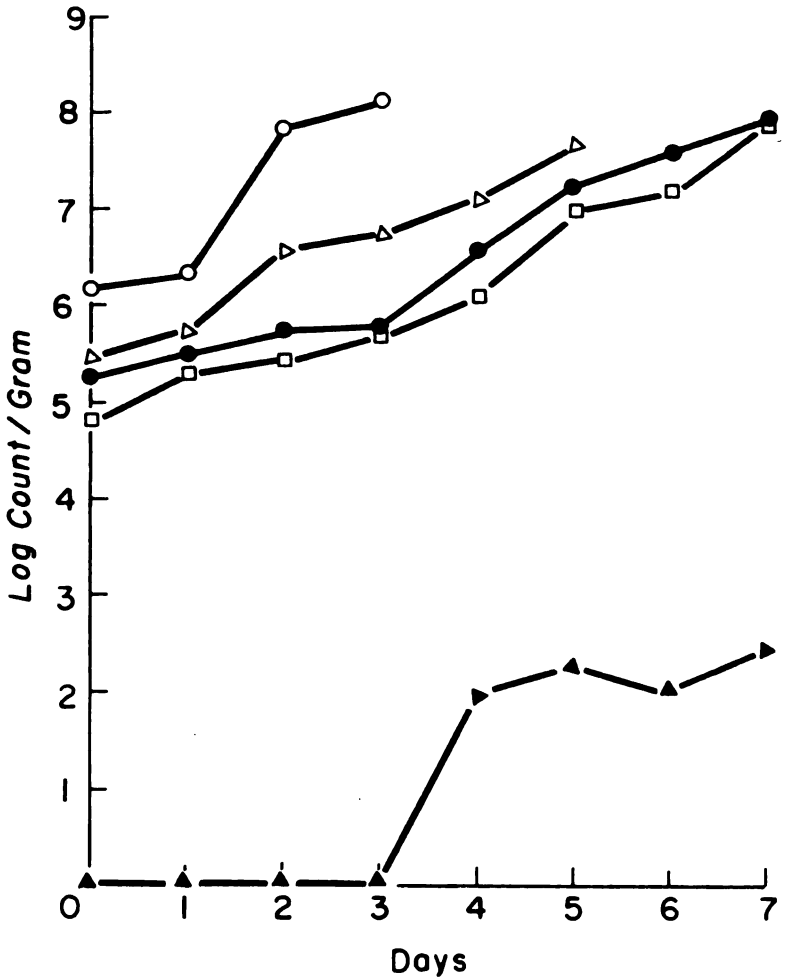

Figure 5. Effect of potassium sorbate on Alcaligenes metalcaligenes inoculated into creamed cottage cheese stored at 50°F.

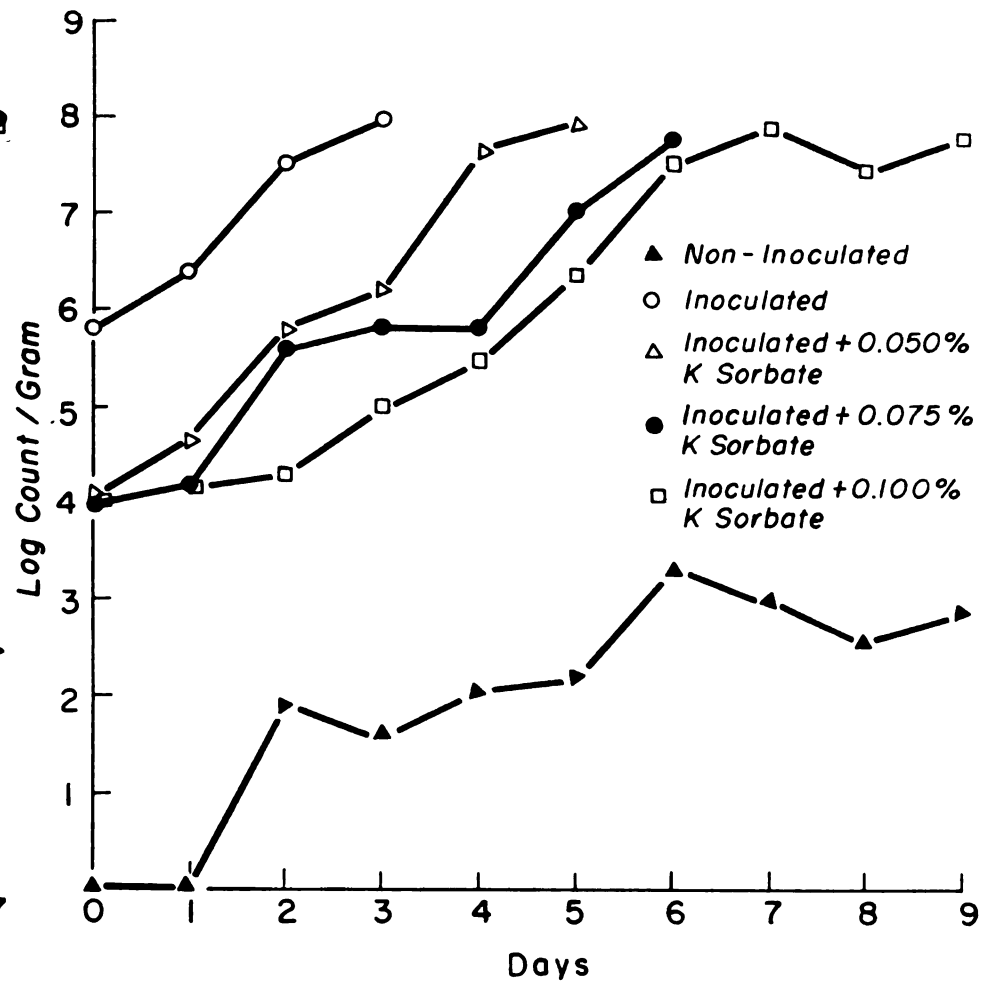

Figure 6. Effect of potassium sorbate on Alcaligenes metalcaligenes inoculated into creamed cottage cheese stored at 50°F.

TABLE III

ORGANISM POPULATION, pH, AND PER CENT POTASSIUM SORBATE* IN CREAMED COTTAGE CHEESE INOCULATED WITH ALCALIGENES METALCALIGENES (840,000 organisms per gram) AND INCUBATED AT 50°F.

Type of Analysis	Description of Cheese Sample				
	Controls		Inoculated + Potassium Sorbate as Indicated		
	Non-inoculated	Inoculated	0.050%	0.075%	0.100%
		0 Day			
Total Count	3,300	2,100,000	480,000	360,000	220,000
Psychrophile	<10	1,500,000	310,000	190,000	64,000
Yeast and Mold	<10	<10	10	10	<10
pH	5.30	5.30	5.30	5.30	5.35
% K Sorbate	0.000	0.000	0.043	0.060	0.098
		1 Day			
Total Count	11,000	2,800,000	840,000	390,000	270,000
Psychrophile	<10	2,100,000	520,000	290,000	180,000
Yeast and Mold	<10	<10	10	<10	<10
pH	5.40	5.45	5.45	5.40	5.40
% K Sorbate	0.000	0.000	LA	0.074	0.106
		2 Days			
Total Count	120,000	89,000,000	7,400,000	890,000	360,000
Psychrophile	<10	71,000,000	4,200,000	530,000	260,000
Yeast and Mold	<10	<10	<10	<10	10
pH	5.40	5.40	5.40	5.45	5.40
% K Sorbate	0.000	0.000	0.045	0.065	0.096
		3 Days			
Total Count	160,000	220,000,000	11,000,000	1,400,000	540,000
Psychrophile	<10	130,000,000	5,600,000	680,000	460,000
Yeast and Mold	<10	<10	<10	<10	<10
pH	5.40	5.40	5.45	5.40	5.40
% K Sorbate	0.000	0.000	0.051	0.062	0.098

TABLE III (Continued)

Type of Analysis	Description of Cheese Sample: Controls: Non-inoculated	Controls: Inoculated	Inoculated + Potassium Sorbate as Indicated: 0.050%	0.075%	0.100%
		5 Days			
Total Count	9,200,000	----	23,000,000	7,100,000	2,200,000
Psychrophile	100	----	13,000,000	3,700,000	1,300,000
Yeast and Mold	30	----	<10	<10	<10
pH	5.40	----	5.40	5.40	5.40
% K Sorbate	0.000	----	0.046	0.069	0.076
		6 Days			
Total Count	40,000,000	----	76,000,000	19,000,000	10,000,000
Psychrophile	1,900	----	52,000,000	17,000,000	10,000,000
Yeast and Mold	620	----	10	<10	<10
pH	5.40	----	5.40	5.40	5.40
% K Sorbate	0.000	----	0.034	0.075	0.086
		7 Days			
Total Count	59,000,000	----	----	100,000,000	74,000,000
Psychrophile	1,100	----	----	38,000,000	15,000,000
Yeast and Mold	1,200	----	----	<10	<10
pH	5.35	----	----	5.45	5.40
% K Sorbate	0.000	----	----	0.065	0.085
		8 Days			
Total Count	71,000,000	----	----	130,000,000	110,000,000
Psychrophile	2,700	----	----	90,000,000	81,000,000
Yeast and Mold	1,300	----	----	<10	<10
pH	5.40	----	----	5.40	5.40
% K Sorbate	0.000	----	----	0.072	0.086

0.000 corrected for irrelevant absorbancy
*calculated as sirbic acid

TABLE IV

ORGANISM POPULATION, pH, AND PER CENT POTASSIUM SORBATE* IN CREAMED COTTAGE CHEESE INOCULATED WITH ALCALIGENES METALCALIGENES (700,000 organisms per gram) AND INCUBATED AT 50°F.

Type of Analysis	Description of Cheese Sample: Controls: Non-inoculated	Controls: Inoculated	Inoculated + Potassium Sorbate as Indicated: 0.050%	0.075%	0.100%
		0 Day			
Total Count	1,900	1,500,000	84,000	26,000	26,000
Psychrophile	<10	590,000	13,000	15,000	10,000
Yeast and Mold	<10	20	<10	10	30
pH	5.20	5.15	5.20	5.15	5.20
% K Sorbate	0.000	0.000	0.056	0.066	0.122
		1 Day			
Total Count	7,400	3,500,000	73,000	31,000	28,000
Psychrophile	<10	2,300,000	46,000	16,000	15,000
Yeast and Mold	<10	<10	<10	<10	<10
pH	5.10	5.20	5.15	5.20	5.20
% K Sorbate	0.000	0.000	0.045	0.085	0.094
		2 Days			
Total Count	7,600	52,000,000	1,700,000	560,000	33,000
Psychrophile	90	32,000,000	650,000	400,000	18,000
Yeast and Mold	<10	<10	<10	<10	<10
pH	5.15	5.30	5.20	5.15	5.15
% K Sorbate	0.000	0.000	0.059	0.084	0.108
		3 Days			
Total Count	77,000	120,000,000	19,000,000	790,000	130,000
Psychrophile	40	87,000,000	1,700,000	630,000	91,000
Yeast and Mold	<10	<10	<10	<10	<10
pH	5.25	5.40	5.35	5.25	5.25
% K Sorbate	0.000	0.000	0.044	0.079	0.081
		4 Days			
Total Count	460,000	----	87,000,000	1,300,000	610,000
Psychrophile	110	----	51,000,000	640,000	290,000
Yeast and Mold	10	----	<10	<10	<10
pH	5.30	----	5.30	5.25	5.30
% K Sorbate	0.000	----	0.046	0.074	0.083

TABLE IV (Continued)

Type of Analysis	Description of Cheese Sample: Controls: Non-inoculated	Controls: Inoculated	Inoculated + Potassium Sorbate as Indicated: 0.050%	0.075%	0.100%
		5 Days			
Total Count	6,100	----	210,000,000	9,900,000	2,900,000
Psychrophile	160	----	90,000,000	11,000,000	2,300,000
Yeast and Mold	2,200	----	40	20	<10
pH	5.15	----	5.20	5.15	5.15
% K Sorbate	0.000	----	0.058	0.079	0.104
		6 Days			
Total Count	69,000	----	----	61,000,000	43,000,000
Psychrophile	2,200	----	----	58,000,000	33,000,000
Yeast and Mold	300	----	----	<10	<10
pH	5.15	----	----	5.20	5.20
% K Sorbate	0.000	----	----	0.066	0.083
		7 Days			
Total Count	27,000,000	----	----	----	120,000,000
Psychrophile	1,000	----	----	----	80,000,000
Yeast and Mold	700	----	----	----	10
pH	5.25	----	----	----	5.35
% K Sorbate	0.000	----	----	----	0.085
		8 Days			
Total Count	7,600	----	----	----	41,000,000
Psychrophile	400	----	----	----	27,000,000
Yeast and Mold	1,800	----	----	----	<10
pH	5.30	----	----	----	5.40
% K Sorbate	0.000	----	----	----	0.081
		9 Days			
Total Count	19,000	----	----	----	62,000,000
Psychrophile	800	----	----	----	59,000,000
Yeast and Mold	4,400	----	----	----	<10
pH	5.35	----	----	----	5.40
% K Sorbate	0.000	----	----	----	0.082

0.000 corrected for irrelevant absorbancy
*calculated as sorbic acid

The growth curve of the psychrophiles in the samples (Figures 5 and 6) containing 0.050 per cent potassium sorbate showed no lag. In the inoculated samples containing 0.075 and 0.100 per cent potassium sorbate, the lags in the growth phase were apparent after the first day in the samples described in Figure 5 and during the first 1 or 2 days in the corresponding samples in Figure 6. As a result of the addition of potassium sorbate to the cheese, the standard plate counts of the inoculated samples containing sorbate (Tables III and IV) were significantly less on the 0 day than the corresponding counts of the inoculated control sample. The populations assumed a logarithmic growth rate when the inhibitory property of the sorbate ion was no longer effective.

Since Alc. metalcaligenes grew at 89.6°F., the standard plate counts for the inoculated control samples were higher than the counts of the non-inoculated controls. Alc. metalcaligenes demonstrated no evidence of sorbate metabolism, and pH values of the inoculated samples showed no significant change as decomposition progressed.

Analysis of Samples Inoculated with Geotrichum candidum.- The populations of G. candidum (Figures 7 and 8) in the samples on the 0 day indicated that there was a greater destruction of the mold in the samples containing the higher percentages of potassium sorbate. A 2 to 4 day lag was evident in the growth curves of the mold in the inoculated samples containing 0.075 and 0.100 per cent potassium sorbate, whereas, the growth curve of the mold in the inoculated samples containing 0.050 per cent potassium sorbate showed relatively little lag. After the inhibitory effect of the sorbic acid was no longer apparent, the slope of the growth curves

of the mold in the sample containing potassium sorbate was similar to the slope of the growth curve of the mold in the inoculated control.

In Table V, the inoculated samples that contained potassium sorbate in the following percentages: 0.050, 0.075, and 0.100 per cent, showed decreases in the population of the yeasts and molds on the 0 day of 68, 99, and 98 per cent, respectively. The corresponding samples (Table VI) showed reductions in population on the 0 day of 54, 97, and 95 per cent, respectively. These decreases, calculated from the count of the inoculated control sample on the 0 day, exemplified the fungicidal property of the sorbic acid against G. candidum.

In most inoculated samples the total count decreased as the percentages of potassium sorbate increased; however, in one trial inoculated with G. candidum, the standard plate counts (Table VI) on the 0 day showed greater populations in the samples containing potassium sorbate than in the inoculated control samples. Large increases in the standard plate counts were observed after the second day of incubation. The numbers of psychrophiles were negligible throughout the sampling period.

G. candidum showed no utilization of potassium sorbate as a source of carbon. During the storage period there were slight, but inconsistent fluctuations in pH with the terminal pH being slightly higher than the initial pH.

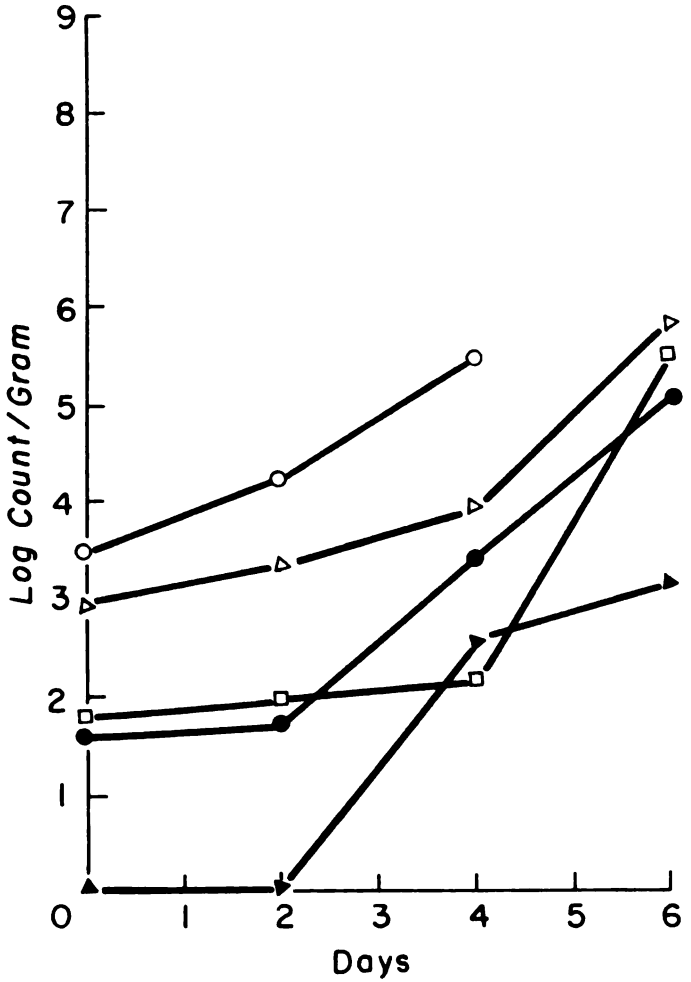

Figure 7. Effect of potassium sorbate on Geotrichum candidum inoculated into creamed cottage cheese stored at 50°F.

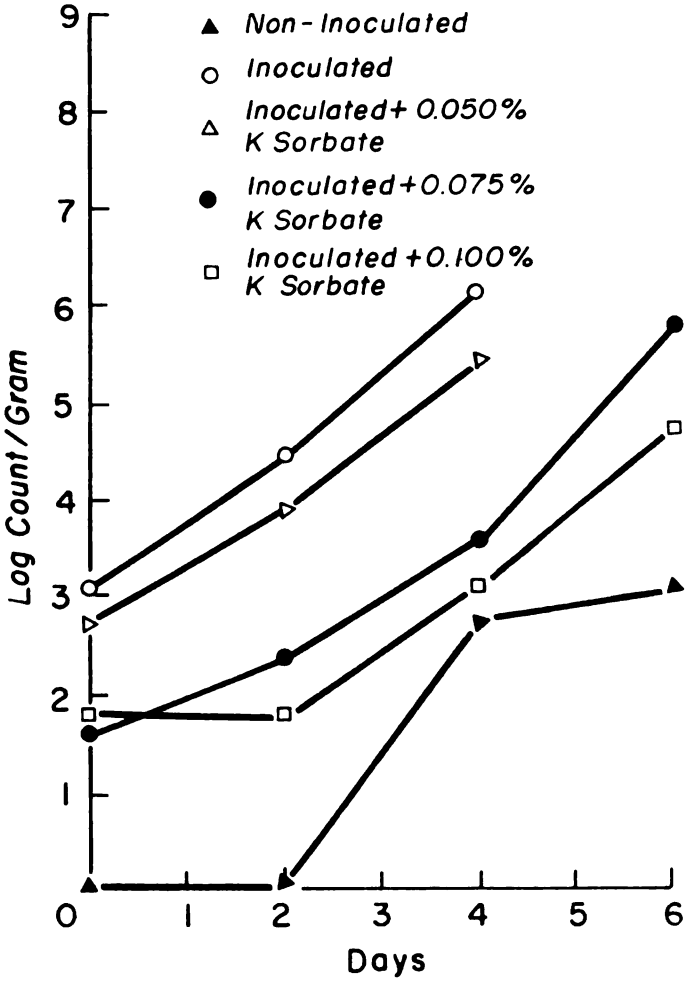

Figure 8. Effect of potassium sorbate on Geotrichum candidum inoculated into creamed cottage cheese stored at 50°F.

TABLE V

ORGANISM POPULATION, pH, AND PER CENT POTASSIUM SORBATE* IN CREAMED COTTAGE CHEESE INOCULATED WITH GEOTRICHUM CANDIDUM (560 organisms per gram) AND INCUBATED AT 50° F.

Type of Analysis	Description of Cheese Sample: Controls: Non-inoculated	Controls: Inoculated	Inoculated + Potassium Sorbate as Indicated: 0.050%	0.075%	0.100%
		0 Day			
Total Count	1,900	5,600	3,000	2,700	2,600
Psychrophile	30	<10	<10	20	10
Yeast and Mold	<10	3,100	1,000	40	60
pH	5.35	5.35	5.30	5.35	5.40
% K Sorbate	0.000	0.000	0.050	0.079	0.092
		2 Days			
Total Count	4,900	13,000	7,200	5,700	4,300
Psychrophile	<10	<10	<10	20	10
Yeast and Mold	<10	17,000	2,400	50	90
pH	5.25	5.20	5.25	5.20	5.20
% K Sorbate	0.000	0.000	0.041	0.076	0.087
		4 Days			
Total Count	260,000	250,000	430,000	530,000	540,000
Psychrophile	<10	<10	10	60	30
Yeast and Mold	400	300,000	9,000	2,600	140
pH	5.25	5.45	5.50	5.45	5.45
% K Sorbate	0.000	0.000	0.047	0.084	0.103
		6 Days			
Total Count	440,000	----	700,000	670,000	620,000
Psychrophile	40	----	70	100	60
Yeast and Mold	1,700	----	730,000	120,000	350,000
pH	5.20	----	5.45	5.50	5.45
% K Sorbate	0.000	----	0.039	0.078	0.098

0.000 corrected for irrelevant absorbancy
*calculated as sorbic acid

TABLE VI

ORGANISM POPULATION, pH, AND PER CENT POTASSIUM SORBATE* IN CREAMED COTTAGE CHEESE INOCULATED WITH GEOTRICHUM CANDIDUM (150 organisms per gram) AND INCUBATED AT 50° F.

Type of Analysis	Description of Cheese Sample: Controls: Non-inoculated	Controls: Inoculated	Inoculated + Potassium Sorbate as Indicated: 0.050%	0.075%	0.100%
		0 Day			
Total Count	11,000	7,900	100,000	8,700	14,000
Psychrophile	<10	<10	10	20	<10
Yeast and Mold	<10	1,300	600	40	60
pH	5.40	5.40	5.40	5.40	5.40
% K Sorbate	LA	0.000	0.046	0.077	0.098
		2 Days			
Total Count	160,000	65,000	23,000	7,600	9,200
Psychrophile	<10	<10	<10	<10	<10
Yeast and Mold	<10	30,000	9,000	240	60
pH	5.40	5.45	5.40	5.40	5.35
% K Sorbate	0.000	0.000	0.043	0.067	0.092
		4 Days			
Total Count	40,000,000	7,800,000	310,000	43,000	810,000
Psychrophile	1,900	20	<10	10	<10
Yeast and Mold	620	1,400,000	300,000	39,000	1,300
pH	5.40	5.35	5.40	5.35	5.40
% K Sorbate	0.000	0.000	0.035	0.082	0.095
		6 Days			
Total Count	59,000,000	----	----	850,000	1,200,000
Psychrophile	6,700	----	----	90	40
Yeast and Mold	1,300	----	----	710,000	60,000
pH	5.40	----	----	5.40	5.45
% K Sorbate	0.000	----	----	0.059	0.095

0.000 corrected for irrelevant absorbancy
calculated as sorbic acid

Analysis of Samples Inoculated with Penicillium frequentans.- The populations of P. frequentans in the samples on the 0 day showed no decrease when 0.050, 0.075, and 0.100 per cent potassium sorbate were added to the samples. In fact the three samples containing sorbate (Figure 9) attained slightly higher populations than the inoculated controls, but these differences in population did not influence on the rate of spoilage of the samples.

The lag phase of the growth curves of P. frequentans lengthened as the percentages of potassium sorbate in the samples of cheese increased from 0.050 to 0.100 per cent (Figures 9 and 10). In the samples illustrated in Figure 9 the growth curves of the mold in the samples containing 0.075 and 0.100 per cent potassium sorbate showed a tendency towards a secondary lag between the sixth and eighth days, after which, logarithmic growth was resumed.

Inconsistencies, such as the variations between the estimated population added to the samples of cheese and the actual count of the organism in the 0 day samples (Tables VII and VIII), are difficult to explain. After the second day, the populations increased less rapidly in the samples containing the higher percentages of potassium sorbate. This was the expected pattern of growth which was also exhibited by the other organisms used in this experiment.

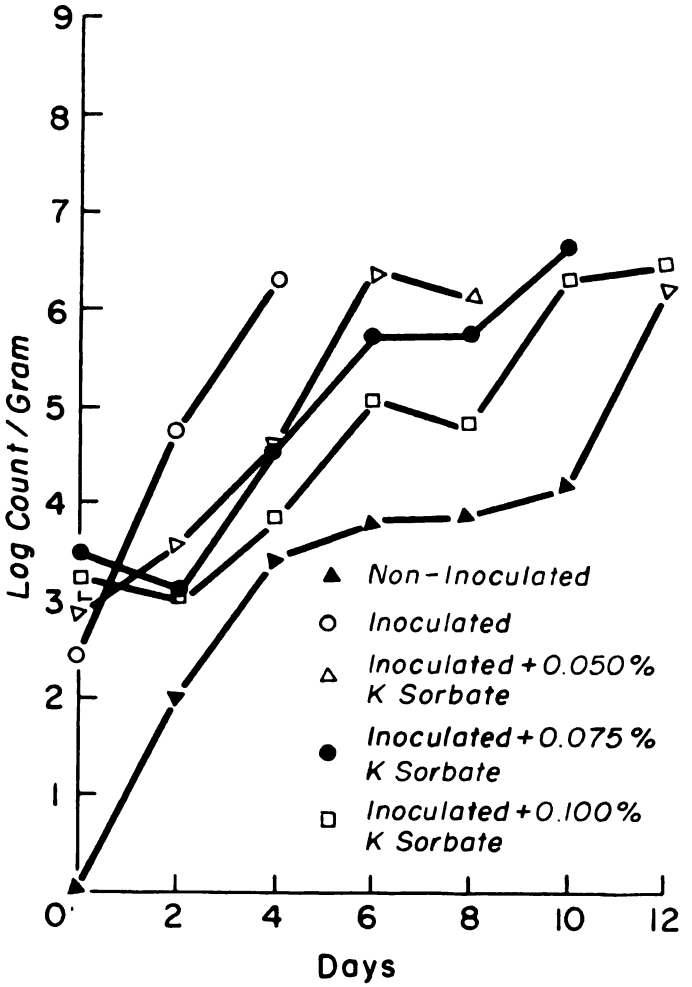

Figure 9. Effect of potassium sorbate on Penicillium frequentans inoculated into creamed cottage cheese stored at 50°F.

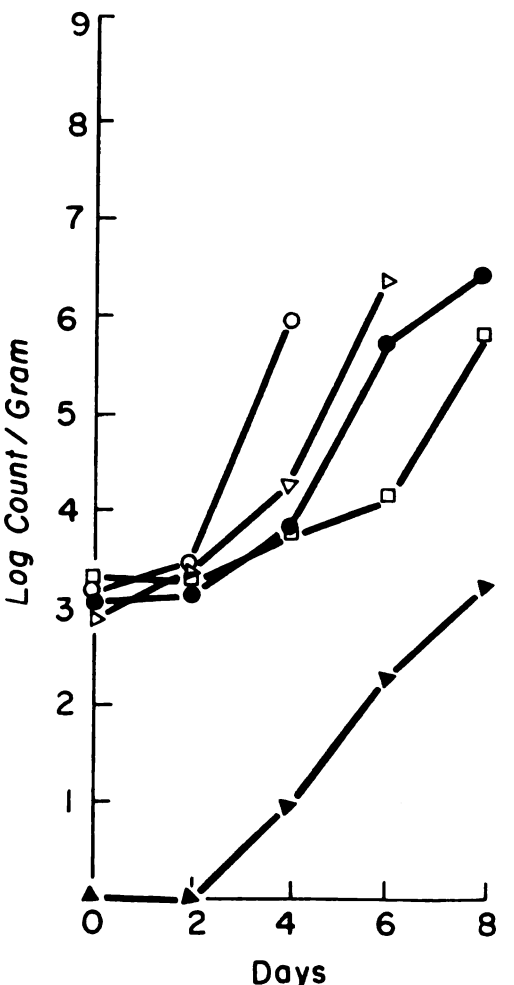

Figure 10. Effect of potassium sorbate on Penicillium frequentans inoculated into creamed cottage cheese stored at 50°F.

TABLE VII

ORGANISM POPULATION, pH, AND PER CENT POTASSIUM SORBATE* IN CREAMED COTTAGE CHEESE INOCULATED WITH PENICILLIUM FREQUENTANS (19,000 organisms per gram) AND INCUBATED AT 50° F.

Type of Analysis	Description of Cheese Sample: Controls: Non-inoculated	Controls: Inoculated	Inoculated + Potassium Sorbate as Indicated: 0.050%	0.075%	0.100%
		0 Day			
Total Count	2,000	41,000	9,900	8,300	2,200
Psychrophile	120	180	20	30	10
Yeast and Mold	<10	270	780	3,000	1,700
pH	5.40	5.45	5.50	5.45	5.45
% K Sorbate	0.000	0.000	0 .050	0.081	0.101
		2 Days			
Total Count	2,300	56,000	39,000	8,700	2,000
Psychrophile	100	130,000	16,000	2,700	11,000
Yeast and Mold	100	51,000	4,000	1,300	1,200
pH	5.30	5.30	5.30	5.25	5.30
% K Sorbate	0.000	0.000	0.052	0.070	0.097
		4 Days			
Total Count	8,400	2,200,000	55,000	34,000	12,000
Psychrophile	20	LA	100	500	300
Yeast and Mold	2,700	2,000,000	49,000	35,000	6,800
pH	5.25	5.40	5.35	5.30	5.25
% K Sorbate	0.000	0.000	0.039	0.075	0.101
		6 Days			
Total Count	190,000	----	2,800,000	630,000	80,000
Psychrophile	120	----	LA	1,000	300
Yeasts and Mold	6,800	----	3,000,000	570,000	120,000
pH	5.25	----	5.35	5.30	5.30
% K Sorbate	0.000	----	0.047	0.071	0.088

TABLE VII (Continued)

Type of Analysis	Description of Cheese Sample: Controls: Non-inoculated	Controls: Inoculated	Inoculated + Potassium Sorbate as Indicated: 0.050%	0.075%	0.100%
		8 Days			
Total Count	1,000,000	----	2,100,000	560,000	60,000
Psychrophile	710	----	LA	3,000	1,000
Yeast and Mold	7,400	----	1,600,000	560,000	66,000
pH	5.25	----	5.35	5.35	5.35
% K Sorbate	0.000	----	0.037	0.068	0.102
		10 Days			
Total Count	2,400,000	----	----	4,000,000	2,700,000
Psychrophile	2,300	----	----	7,500	3,000
Yeast and Mold	17,000	----	----	4,600,000	2,300,000
pH	5.35	----	----	5.35	5.30
% K Sorbate	0.000	----	----	0.068	0.095
		12 Days			
Total Count	2,800,000	----	----	----	3,700,000
Psychrophile	3,000	----	----	----	10,000
Yeast and Mold	600,000	----	----	----	2,700,000
pH	5.30	----	----	----	5.30
% K Sorbate	0.000	----	----	----	0.090

0.000 corrected for irrelevant absorbancy
* calculated as sorbic acid

TABLE VIII

ORGANISM POPULATION, pH, AND PER CENT POTASSIUM SORBATE* IN CREAMED COTTAGE CHEESE INOCULATED WITH PENICILLIUM FREQUENTANS (10,400 organisms per gram) AND INCUBATED AT 50° F.

Type of Analysis	Description of Cheese Sample: Controls: Non-inoculated	Controls: Inoculated	Inoculated + Potassium Sorbate as Indicated: 0.050%	0.075%	0.100%
		0 Day			
Total Count	1,900	5,500	4,900	4,800	4,100
Psychrophile	<10	<10	<10	<10	<10
Yeast and Mold	<10	1,500	900	1,200	1,700
pH	5.20	5.15	5.20	5.20	5.20
% K Sorbate	0.000	0.000	0.039	0.070	0.117
		2 Days			
Total Count	7,600	4,500	3,800	4,300	4,800
Psychrophile	90	<10	<10	<10	<10
Yeast and Mold	<10	2,700	2,600	1,400	1,600
pH	5.15	5.15	5.20	5.20	5.15
% K Sorbate	0.000	0.000	0.037	0.080	0.102
		4 Days			
Total Count	460,000	1,700,000	34,000	17,000	9,100
Psychrophile	110	20	<10	<10	<10
Yeast and Mold	10	940,000	21,000	7,000	6,200
pH	5.30	5.35	5.25	5.20	5.20
% K Sorbate	0.000	0.000	0.049	0.073	0.079
		6 Days			
Total Count	69,000	----	3,400,000	610,000	29,000
Psychrophile	2,200	----	40	70	10
Yeast and Mold	300	----	2,900,000	580,000	14,000
pH	5.15	----	5.30	5.20	5.15
% K Sorbate	0.000	----	0.046	0.080	0.093
		8 Days			
Total Count	7,600	----	----	3,600,000	1,000,000
Psychrophile	400	----	----	70	60
Yeast and Mold	1,800	----	----	3,000,000	700,000
pH	5.30	----	----	5.35	5.35
% K Sorbate	0.000	----	----	0.052	0.080

0.000 corrected for irrelevant absorbancy

* calculated as sorbic acid

Analysis of Samples Inoculated with Rhodotorula mucilaginosa.- On the 0 day, no decreases in population attributable to the presence of potassium sorbate were observed in the samples containing 0.050 and 0.075 per cent potassium sorbate and inoculated with R. mucilaginosa (Figure 11), but the sample containing 0.100 per cent potassium sorbate showed a 42 per cent decrease in count. However, in the duplicate trial (Figure 12), the populations of the samples containing 0.050, 0.075, and 0.100 per cent sorbate showed decreases in count of 82, 58, and 73 per cent, respectively.

This experiment suggested that R. mucilaginosa was very sensitive to the effects of potassium sorbate. Some fungicidal action was exhibited in addition to an extensive fungistatic activity which attenuated the growth of the populations inoculated into the samples containing potassium sorbate. The growth curves of the yeast in the samples containing 0.050 and 0.075 per cent sorbate (Figure 11) showed lag phses of 6 and 4 days, respectively. An increase in the populations occurred after the initial lag phase. However, the slope of the growth curves of the yeast in the inoculated samples containing sorbate showed less increase in elevation than the slope of the corresponding curve representing the inoculated control sample. The growth curve of the yeast in the sample containing 0.100 per cent potassium sorbate indicated only a slight variation in population from the initial count, until the fourteenth day of storage when the only colonies that appeared on potato dextrose agar plates were mold contaminants; therefore, a <10 population of yeast was plotted on Figure II.

The growth of the yeast in the inoculated samples containing 0.050, 0.075, and 0.100 per cent potassium sorbate (Figure 12) showed 4, 4, and

8 day lags, respectively. During the phase of logarithmic growth that followed the lag phase, the growth curves of the yeast in the inoculated samples containing potassium sorbate approximated the growth curve of the yeast in the inoculated control sample. The lag phases shown in Figures 11 and 12 suggested that the potassium sorbate was functioning as a fungistat against the populations of R. mucilaginosa.

The pH of the inoculated control (Tables IX and X) decreased significantly after the second day of storage. However, as the sorbate concentration increased from 0.050 to 0.100 per cent in the inoculated samples, a longer time elapsed before a decrease in pH occurred.

The populations of the psychrophiles increased slowly in the samples inoculated with R. mucilaginosa, and containing the concentrations of potassium sorbate used in this experiment. The total counts (Table IX) showed no decrease as a result of the inhibitory activity of the potassium sorbate until the second day of storage. Throughout the storage period the standard plate counts were substantially lower in the cheese samples containing potassium sorbate.

The non-inoculated control samples (Tables IX and X) showed mold growth on the surface after the twelfth and eighth days, respectively. The last yeast and mold count of the non-inoculated control sample (Table X, 8th day) could not be plotted accurately since insufficient dilutions made the plates impossible to count; therefore, a greater than 30,000 population was recorded.

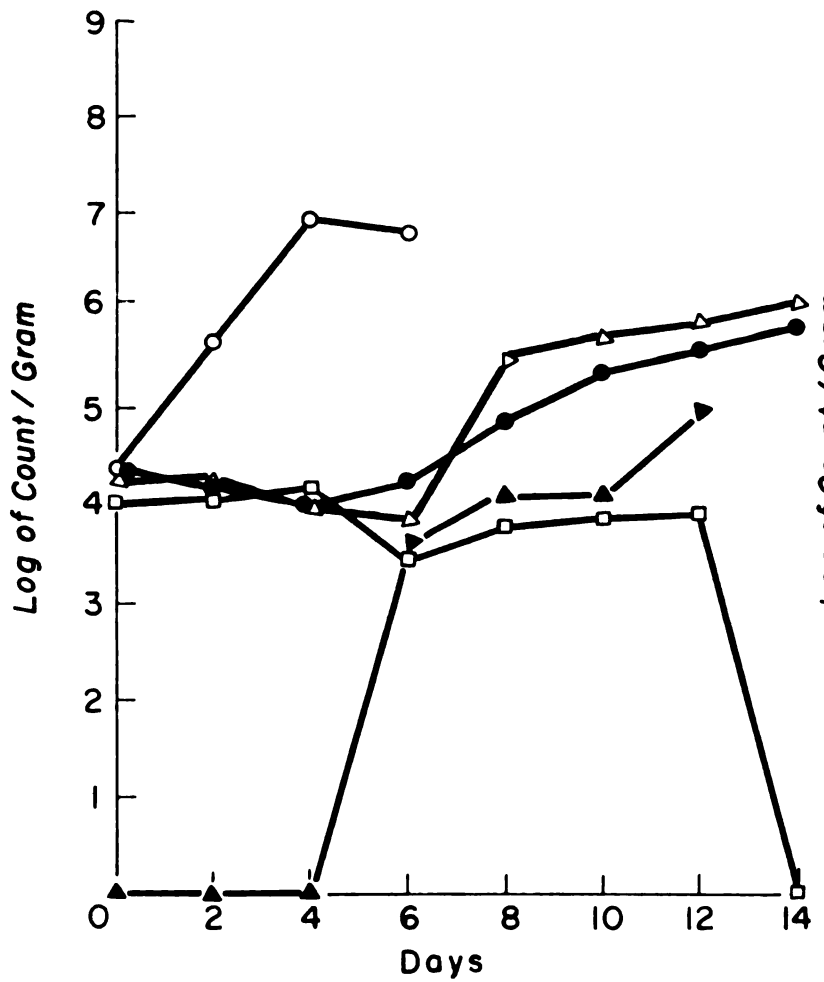

Figure 11. Effect of potassium sorbate on Rhodotorula mucilaginosa inoculated into creamed cottage cheese stored at 50°F.

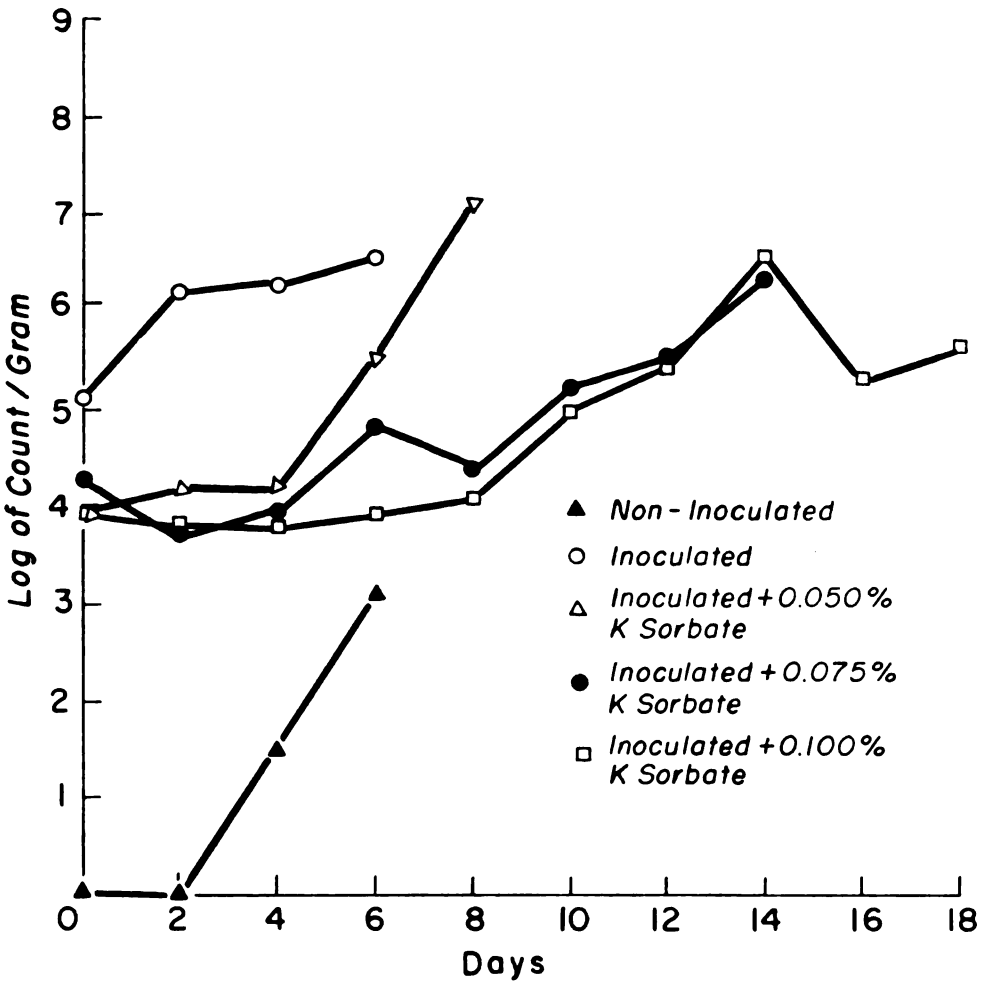

Figure 12. Effect of potassium sorbate on Rhodotorula mucilaginosa inoculated into creamed cottage cheese stored at 50°F.

TABLE IX

ORGANISM POPULATION, pH and PER CENT POTASSIUM SORBATE* IN CREAMED COTTAGE CHEESE INOCULATED WITH *RHODOTORULA MUCILAGINOSA* (270,000 organisms per gram) AND INCUBATED AT 50°F.

Type of Analysis	Description of Cheese Sample: Control — Non-inoculated	Control — Inoculated	Inoculated + Potassium Sorbate as Indicated — 0.050%	0.075%	0.100%
		0 Day			
Total Count	3,300	39,000	31,000	35,000	32,000
Psychrophile	5,100	3,000	5,800	3,200	3,100
Yeast and Mold	<10	24,000	22,000	23,000	10,000
pH	5.25	5.20	5.30	5.20	5.30
% K Sorbate	0.000	0.000	0.050	0.073	0.096
		2 Days			
Total Count	2,000	1,100,000	15,000	86,000	26,000
Psychrophile	90	300	120	60	430
Yeast and Mold	<10	490,000	19,000	16,000	12,000
pH	5.30	5.15	5.15	5.20	5.25
% K Sorbate	0.000	0.000	0.046	0.076	0.106
		4 Days			
Total Count	2,800	6,000,000	15,000	20,000	21,000
Psychrophile	30	<10	160	230	650
Yeast and Mold	<10	8,300,000	10,000	10,000	14,000
pH	5.25	4.80	5.15	5.20	5.20
% K Sorbate	0.000	0.000	0.047	0.068	0.101
		6 Days			
Total Count	30,000	6,800,000	52,000	20,000	18,000
Psychrophile	90	360	740	300	190
Yeast and Mold	4,200	6,400,000	7,000	22,000	2,600
pH	5.25	4.75	5.05	5.15	5.15
% K Sorbate	0.000	0.000	0.046	0.073	0.105

TABLE IX (Continued)

Type of Analysis	Description of Cheese Sample: Control — Non-inoculated	Control — Inoculated	Inoculated + Potassium Sorbate as Indicated — 0.050%	0.075%	0.100%
		8 Days			
Total Count	780,000	----	480,000	60,000	9,100
Psychrophile	d 340	----	120	170	150
Yeast and Mold	13,000	----	340,000	73,000	5,400
pH	5.25	----	4.75	5.00	5.10
% K Sorbate	0.000	----	0.051	0.073	0.103
		10 Days			
Total Count	1,700,000	----	320,000	320,000	61,000
Psychrophile	1,600	----	80	30	<10
Yeast and Mold	13,000	----	620,000	230,000	7,100
pH	5.25	----	4.65	4.80	4.95
% K Sorbate	0.000	----	0.053	0.078	0.095
		12 Days			
Total Count	2,100,000	----	730,000	770,000	42,000
Psychrophile	4,000	----	140	40	150
Yeast and Mold	93,000	----	80,000	38,000	9,000
pH	5.25	----	4.65	4.75	4.85
% K Sorbate	0.000	----	0.050	0.080	0.093
		14 Days			
Total Count	----	----	1,200,000	690,000	200,000
Psychrophile	----	----	280	100	50
Yeast and Mold	----	----	1,300,000	67,000	20
pH	----	----	4.65	4.75	4.90
% K Sorbate	----	----	0.045	0.082	0.124

0.000 corrected for irrelevant absorbancy
* calculated as sorbic acid

TABLE X

ORGANISM POPULATION, pH, AND PER CENT POTASSIUM SORBATE* IN CREAMED COTTAGE CHEESE INOCULATED WITH *RHODOTORULA MUCILAGINOSA* (23,000 organisms per gram) AND INCUBATED AT 50° F.

Type of Analysis	Description of Cheese Sample				
	Control		Inoculated + Potassium Sorbate as Indicated		
	Non-inoculated	Inoculated	0.050%	0.075%	0.100%
		0 Day			
Total Count	3,300	110,000	26,000	20,000	22,000
Psychrophile	<10	60	100	70	10
Yeast and Mold	<10	110,000	9,000	19,000	8,000
pH	5.30	5.35	5.40	5.35	5.40
% K Sorbate	0.000	0.000	0.046	0.065	0.085
		2 Days			
Total Count	120,000	2,700,000	23,000	12,000	8,700
Psychrophile	<10	<10	<10	<10	<10
Yeast and Mold	10	1,600,000	16,000	5,000	6,000
pH	5.40	5.35	5.35	5.35	5.35
% K Sorbate	0.000	0.000	0.039	0.079	0.085
		4 Days			
Total Count	9,200,000	2,900,000	67,000	30,000	12,000
Psychrophile	<10	<10	<10	<10	<10
Yeast and Mold	30	1,900,000	16,000	9,000	6,000
pH	5.45	5.10	5.35	5.40	5.40
% K Sorbate	0.000	0.000	0.040	0.061	0.074
		6 Days			
Total Count	59,000,000	5,000,000	530,000	86,000	19,000
Psychrophile	1,100	<10	30	10	<10
Yeast and Mold	1,200	3,600,000	380,000	67,000	8,000
pH	5.35	4.70	4.90	5.00	5.10
% K Sorbate	0.000	0.000	0.044	0.081	0.099
		8 Days			
Total Count	83,000,000	----	16,000,000	120,000	70,000
Psychrophile	10,000	----	120	290	60
Yeast and Mold	>30,000	----	13,000,000	24,000	12,000
pH	5.40	----	4.80	4.95	5.05
% K Sorbate	0.000	----	0.052	0.088	0.094

TABLE X (Continued)

Type of Analysis	Description of Cheese Sample: Control: Non-inoculated	Control: Inoculated	Inoculated + Potassium Sorbate as Indicated: 0.050%	0.075%	0.100%
		10 Days			
Total Count	----	----	----	180,000	70,000
Psychrophile	----	----	----	630	190
Yeast and Mold	----	----	----	170,000	93,000
pH	----	----	----	4.80	4.95
% K Sorbate	----	----	----	0.083	0.088
		12 Days			
Total Count	----	----	----	260,000	140,000
Psychrophile	----	----	----	900	400
Yeast and Mold	----	----	----	350,000	270,000
pH	----	----	----	4.70	4.75
% K Sorbate	----	----	----	0.082	0.100
		14 Days			
Total Count	----	----	----	2,600,000	5,300,000
Psychrophile	----	----	----	1,100	700
Yeast and Mold	----	----	----	2,200,000	3,700,000
pH	----	----	----	4.65	4.75
% K Sorbate	----	----	----	0.078	0.099
		16 Days			
Total Count	----	----	----	----	430,000
Psychrophile	----	----	----	----	1,100
Yeast and Mold	----	----	----	----	200,000
pH	----	----	----	----	4.60
% K Sorbate	----	----	----	----	0.102
		18 Days			
Total Count	----	----	----	----	510,000
Psychrophile	----	----	----	----	1,500
Yeast and Mold	----	----	----	----	410,000
pH	----	----	----	----	4.60
% K Sorbate	----	----	----	----	0.108

0.000 corrected for irrelevant absorbancy
* calculated as sorbic acid

Analysis of Samples Inoculated with Torulopsis candida.-

The populations of T. candida on the 0 day (Figures 13 and 14) were only slightly affected by the addition of potassium sorbate to the creamed cottage cheese. A progressive decrease in the population of the yeast was noted as the percentages of potassium sorbate were increased; however, the actual count of the yeast on the 0 day was many times higher than the estimated population added to the cheese. The growth curves of the yeast in the inoculated samples containing sorbate approximately paralleled the curve of the yeast in the inoculated control sample. This parallelism, which occurred from the beginning of storage, suggested that the sorbic acid had little fungistatic activity against this yeast. The counts of the yeasts and molds in the non-inoculated controls showed rapid increases after the second day of storage.

Standard plate and psychrophile counts (Tables XI and XII) indicated a slight inhibition of the growth of the organisms particularly at the higher concentration of potassium sorbate in the inoculated samples. The pH values of the inoculated samples showed a slight increase as spoilage progressed. An apparent decrease in the percentage of potassium sorbate during storage was attributed to discrepancies in the recovery of the sorbate from the creamed cottage cheese.

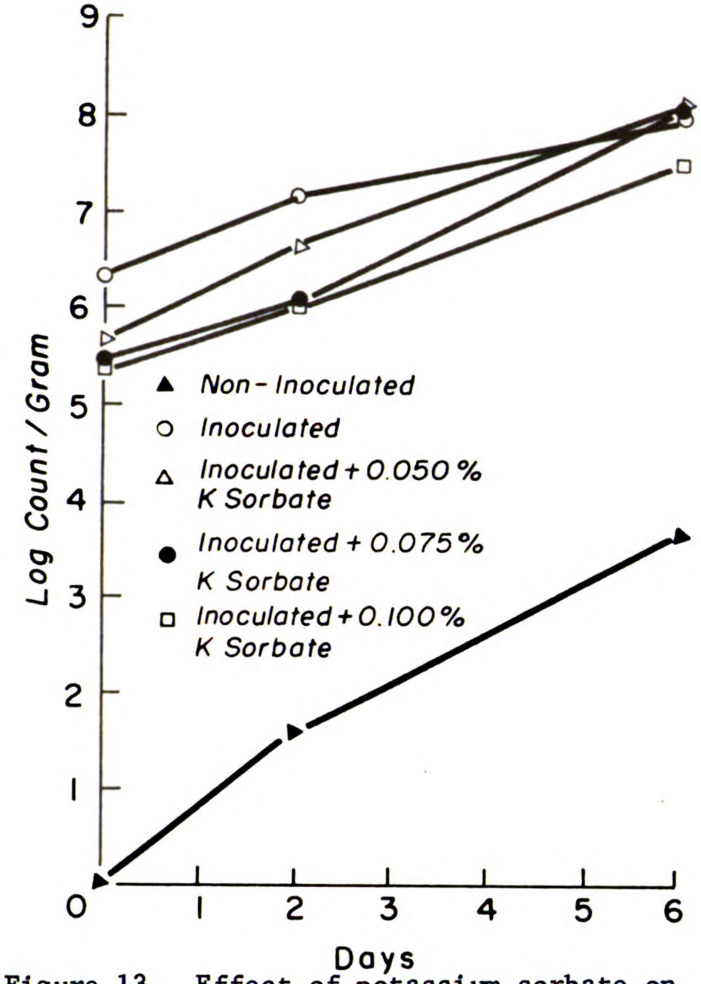

Figure 13. Effect of potassium sorbate on Torulopsis candida inoculated into creamed cottage cheese stored at 50°F.

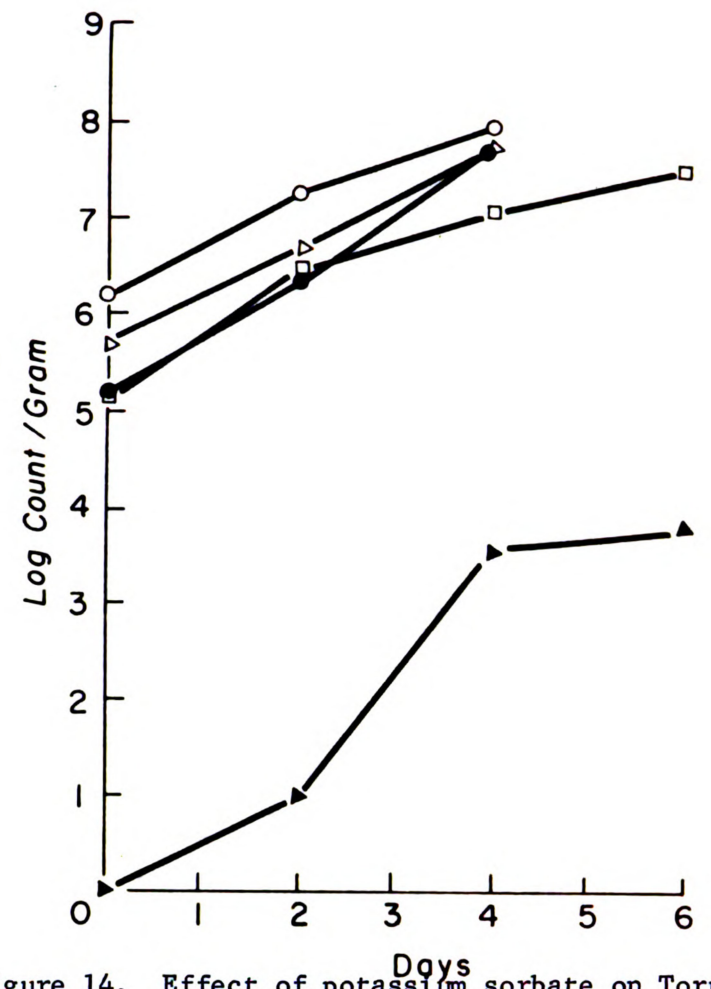

Figure 14. Effect of potassium sorbate on Torulopsis candida inoculated into creamed cottage cheese stored at 50°F.

TABLE XI

ORGANISM POPULATION, pH, AND PER CENT POTASSIUM SORBATE* IN CREAMED COTTAGE CHEESE INOCULATED WITH TORULOPSIS CANDIDA (130,000 orgamisms per gram) AND INCUBATED AT 50° F.

Type of Analysis	Description of Cheese Sample: Control — Non-inoculated	Control — Inoculated	Inoculated + Potassium Sorbate as Indicated — 0.050%	0.075%	0.100%
		0 Day			
Total Count	12,000	940,000	430,000	250,000	150,000
Psychrophile	740	210	190	150	30
Yeast and Mold	10	2,200,000	480,000	310,000	240,000
pH	5.35	5.35	5.40	5.35	5.40
% K Sorbate	0.000	0.000	0.044	0.072	0.107
		2 Days			
Total Count	3,100,000	11,000,000	4,400,000	1,700,000	1,400,000
Psychrophile	3,500	2,700	300	70	10
Yeast and Mold	40	14,000,000	4,900,000	1,200,000	1,100,000
pH	5.40	5.45	5.50	5.50	5.40
% K Sorbate	0.000	0.000	0.033	0.065	0.099
		6 Days			
Total Count	150,000,000	250,000,000	130,000,000	94,000,000	21,000,000
Psychrophile	470,000,000	590,000,000	29,000,000	4,100,000	30,000
Yeast and Mold	4,900	100,000,000	170,000,000	130,000,000	34,000,000
pH	5.40	5.40	5.45	5.45	5.40
% K Sorbate	0.000	0.000	0.027	0.053	0.086

0.000 corrected for irrelevant absorbancy
* calculated as sorbic acid

TABLE XII

ORGANISM POPULATION, pH, AND PER CENT POTASSIUM SORBATE* IN CREAMED COTTAGE CHEESE INOCULATED WITH TORULOPSIS CANDIDA (3,200 organisms per gram) AND INCUBATED AT 50° F.

Type of Analysis	Description of Cheese Sample				
	Control		Inoculated + Potassium Sorbate as Indicated		
	Non-inoculated	Inoculated	0.050%	0.075%	0.100%
		0 Day			
Total Count	4,700	1,100,000	620,000	320,000	150,000
Psychrophile	<10	<10	<10	<10	<10
Yeast and Mold	<10	1,500,000	560,000	170,000	140,000
pH	5.35	5.25	5.20	5.25	5.20
% K Sorbate	0.000	0.000	0.046	0.078	0.092
		2 Days			
Total Count	6,900	39,000,000	5,800,000	4,700,000	3,000,000
Psychrophile	190	100	60	70	<10
Yeast and Mold	10	19,000,000	5,800,000	2,400,000	3,100,000
pH	5.25	5.20	5.20	5.25	5.20
% K Sorbate	0.000	0.000	0.046	0.076	0.086
		4 Days			
Total Count	1,400,000	54,000,000	29,000,000	34,000,000	17,000,000
Psychrophile	800	490	400	520	700
Yeast and Mold	3,900	95,000,000	67,000,000	58,000,000	13,000,000
pH	5.25	5.40	5.35	5.35	5.35
% K Sorbate	0.000	0.000	0.034	0.064	0.094
		6 Days			
Total Count	1,600,000	----	----	----	42,000,000
Psychrophile	2,600	----	----	----	1,500
Yeast and Mold	7,100	----	----	----	35,000,000
pH	5.35	----	----	----	5.35
% K Sorbate	0.000	----	----	----	0.072

0.000 corrected for irrelevant absorbancy
* calculated as sorbic acid

DISCUSSION

Differences in the rate of spoilage of the samples of creamed cottage cheese were observed in many instances in the duplicate trials. The ratio of two parts of curd to one part of creaming mixture was used in all trials. However, in some instances where the moisture content of the curd was low, the curd was not completely covered by the creaming mixture. R. mucilaginosa was sensitive to all concentrations of potassium sorbate used in this experiment and in the samples inoculated with this organism, surface spoilage occurred much more rapidly when the surface of the curd was not covered by the creaming mixture. Surface spoilage, observed in the inoculated samples of cottage cheese, started on the particles of curd protruding from the creaming mixture. This suggested that the sorbic acid concentration was less on the exposed surface of the curd and greater in the creamed cottage cheese. Melnick and Luckmann (16) reported that the migration of sorbic acid was directly related to the moisture content of various variaties of cheese. Although cottage cheese was not studied by these authors, the high moisture content and the permeability of the particles of curd suggest that migration of sorbic acid should have permitted the concentration of sorbic acid to become equalized rapidly; but the spoilage exhibited by R. mucilaginosa indicated that equalization did not occur.

Several authors (16, 17, 27) have reported that molds metabolized sorbic acid as a source of carbon. Yeasts also have the necessary dehydrogenase enzyme system; however, Costilow et al. (4) demonstrated that a large inoculum of T. holmii would not decrease the concentration of the sorbate in pickle brines. In the work reported herein, there was no positive proof that any of the organisms used sorbic acid as a source of carbon. Apparently, spoilage of cottage cheese is accomplished by lower populations than required to cause measurable diminution of the sorbate.

No objectionable flavor was noted with the samples containing 0.100 per cent sorbate. When hydrolized, a 0.100 per cent solution of potassium sorbate is only 0.075 per cent sorbic acid. These results were in agreement with the observations of Geminder (10) and Perry and Lawrence (23) who reported no perceptible typical "bitter" flavor in creamed cottage cheese containing 0.075 per cent sorbic acid or less.

Since the creamed cottage cheese contained one part of the creaming mixture to two parts of curd, the individual creaming mixtures were prepared by adding three times the quantity of potassium sorbate desired in the finished product. On the 0 day, reductions in the populations attributable to the activity of the sorbate ion in the inoculated samples containing sorbate were evident in all instances except the samples inoculated with P. frequentans. Together with the three per cent salt, the tripled concentration of potassium sorbate could have caused some destruction of the organisms during the interval after the organisms were added to the creaming mixture and before the mixture was blended with the cheese.

In the 0 day analysis of the samples containing no sorbate, the populations of the inoculated controls were the same or higher than the calculated populations inoculated into the cottage cheese. In most determinations, the populations of organisms actually enumerated in the inoculated samples containing sorbate were lower than the calculated counts inoculated into the creamed cottage cheese. Exceptions were noted in the samples inoculated with T. candida. These samples showed that some destruction had occurred within 4 hours after inoculation into the creamed cottage cheese and the degree of destruction was greater as the percentages of potassium sorbate were increased. Nevertheless, all the initial counts of T. candida in the samples of creamed cottage cheese were greater than the calculated population of the inoculum added to the cheese. The samples in both of the trials inoculated with P. frequentans and the samples in one trial inoculated with R. mucilaginosa showed no decrease in the initial count when potassium sorbate was added to the cottage cheese.

When inoculated into creamed cottage cheese, R. mucilaginosa was the only organism used in this experiment in which the growth was retarded significantly, and some destruction continued from the 0 through the fourth day. Costilow et al. (4) reported that sorbic acid was a fungistat and slightly fungicidal. Keeney (13) stated that a 6 carbon fatty acid has fungicidal properties. These findings with R. mucilaginosa confirmed the statements of these workers.

There was some decrease of potassium sorbate in samples inoculated with P. frequentans and T. candida. The decrease was attributed to inherent

weaknesses in the analytical recovery of the sorbate rather than to metabolism of sorbate by the organisms.

Variations experienced in analyzing the samples suggested that decreases in sorbate of less than 10 per cent should not be construed as an indication of utilization of sorbate by the organism. If this assumed standard is valid, there is no evidence that sorbate was metabolized by any of the organisms used in this work. It is possible that higher population concentrations of the organisms might have been able to effect some decrease in the sorbate, but under the conditions of this study in which the populations inoculated simulated extreme contamination, the approximate initial concentration of sorbic acid was maintained throughout the period the cheese was in storage.

Yeasts and molds have a dehydrogenase system, but none has been reported for bacteria. Emard and Vaughn (9) demonstrated the inhibitory nature of sorbic acid on catalase positive organisms. Such catalase activity for the Pseudomonas and Alcaligenes used in this experiment is not listed in Bergey's Manual (2). There is evidently a mechanism of inhibition still undiscovered. The bacteriostatic nature of the sorbate ion was exhibited in the experiment with both of the psychrophiles.

Some evidence of bactericidal tendencies is suggested by the decrease in the total count and the psychrophile count on the 0 day in the inoculated samples containing potassium sorbate. This property of sorbic acid was confirmed by Cowles (6) who stated that fatty acids had bactericidal properties at pH 4.7 and lower. However, the only samples of cottage cheese in which the pH decreased below 5.00 were those inoculated with R. mucilaginosa.

Also, a retarded growth of the psychrophiles was observed in these samples at pH 4.7. Sorbic acid has a greater bactericidal power than the 6-carbon fatty acid because the two conjugated double bonds in the structure of the sorbic acid add bactericidal activity. Therefore, since sorbic acid has a greater bactericidal power than a 6-carbon fatty acid, this greater power should exert an influence at a higher pH.

Smith and Rollin (27) noted that the initial contamination of mold must be relatively light in order to obtain the high sorbic acid-to-mold dehydrogenase ratio necessary for inhibition. Melnick et al. (18) stated similar findings with their work on bakery products. These findings explained the reason for an increased initial lag phase when the population in the inocula decreased. All samples containing yeasts, molds, and psychrophiles except those samples inoculated with T. candida, exhibited this property. Perhaps a smaller population of T. candida would also demonstrate a lengthened lag phase in the growth curves, similar to those of R. mucilaginosa.

Bell et al. (1) reported that six species representing three genera of lactic bacteria were inhibited by 0.1 per cent sorbic acid at pH 3.5. pH 3.5 is a little low for most lactics to grow regardless of whether sorbic acid is present. No mention was made in their report as to whether growth tests were performed at pH 3.5 without sorbic acid added. Such a comparison would be necessary to determine the validity of the effect of sorbic acid on the lactics tested.

SUMMARY AND CONCLUSIONS

Samples of cottage cheese containing 0.050, 0.075, and 0.100 per cent potassium sorbate were contaminated with six organisms commonly associated with surface spoilage in cottage cheese. The organisms used were Pseudomonas fragi, Alcaligenes metalcaligenes, Geotrichum candidum, Penicillium frequentans, Torulopsis candida, and Rhodotorula mucilaginosa. Each sample of cheese was analyzed for total count, psychrophile population, yeast and mold population, pH, and percentage of potassium sorbate. Analyses were performed daily on samples contaminated with psychrophiles, and samples contaminated with yeasts or molds were examined on alternate days.

None of the organisms tested metabolized potassium sorbate as a source of carbon before the sample was spoiled. In general, increases in population were inversely related to the percentage of potassium sorbate in the samples.

Some destruction in the populations inoculated into the creamed cottage cheese was shown in the initial analysis, but usually this destruction did not continue after the second day. In most instances, the amount of reduction in population was directly related to the percentage of potassium sorbate.

Some of the organisms showed a definite lag in the growth curve which extended as the percentage of potassium sorbate in the sample increased.

Another factor influencing the lag was the population of the inoculum. A higher population in the initial samples resulted in a shorter lag phase. The observation that a low initial population would give a longer lag phase exemplifies the requirement for a ratio of high sorbic acid to low dehydrogenase enzyme to increase cottage cheese shelf-life.

A bactericidal property was evidenced by the decrease in the populations of the psychrophiles inoculated into the creamed cottage cheese.

Potassium sorbate at a 0.100 per cent concentration imparted no objectionable flavor to the creamed cottage cheese.

LITERATURE CITED

(1) Bell, T. A., Etchells, J. L., and Borg, A. F. Influence of Sorbic Acid on the Growth of Certain Species of Filamentous Fungi. J. Bact., 77: 573. 1959.

(2) Breed, R. S., Murray, E. G. D., and Smith, N. R. Bergey's Manual of Determinative Bacteriology. 7th ed. The Williams and Wilkins Co., Baltimore, Md. 1957. 1094 pp., plus xviii.

(3) Clark, J. F. On the Toxic Effect of Deleterious Agents on the Germination and Development of Certain Filamentous Fungi. Bot. Gaz., 28: 289. 1899.

(4) Costilow, R. N., Ferguson, W. E., and Ray, S. Sorbic Acid as a Selective Agent in Cucumber Fermentations. I. Effect of Sorbic Acid on Microorganisms Associated with Cucumber Fermentations. Appl. Microbiol., 3: 341. 1955.

(5) Costilow, R. N., Coughlin, F. M., Robbins, E. K., and Wen-tah, Hsu. Sorbic Acid as a Selective Agent in Cucumber Fermentations. II. Effect of Sorbic Acid on the Yeast and Lactic Fermentations in Brined Cucumbers. Appl. Microbiol., 5: 373. 1957.

(6) Cowles, P. B. The Germicidal Action of the Hydrogen Ion and of the Lower Fatty Acids. Yale J. Biol. and Med., 13: 571. 1941.

(7) Demaree, G. E., Sjogren, D. W., McCashland, B. W., and Cosgrove, F. P. Preliminary Studies on Feeding Sorbic Acid upon the Growth, Reproduction, and Cellular Metabolism of Albino Rats. J. Am. Pharm. Assoc., Sci. Ed., 44: 619. 1955.

(8) Deuel, H. J., Jr., Afin-Slater, R., Weil, C. S., and Smyth, H. F., Jr. Sorbic Acid as a Fungistatic Agent for Foods. I. Harmlessness of Sorbic Acid as a Dietary Constituent. Food Res., 19: 1. 1954.

(9) Emard, L. O. and Vaughn, R. H. Selectivity of Sorbic Acid Media for the Catalase Negative Lactic Acid Bacteria and Clostridia. J. Bact., 63: 487. 1952.

(10) Geminder, J. Extending the Shelf-life of Creamed Cottage Cheese with Sorbic Acid. Milk Dealer, 48: 44. 1959.

(11) Harmon, L. G., Trout, G. M., and Bonner, M. D. A Market Survey of Cottage Cheese. I. Some Characteristics Influencing Consumer Acceptance and Shelf-life. Mich. Agr. Expt. Sta. Quart. Bul., 38: 146. 1955.

(12) Hartman, R. J. Colloidal Chemistry. 2nd ed. Houghton Mifflin Co., New York. 1947. 537 pp. plus xxxii.

(13) Keeney, E. L. New Preparations for the Treatment of Fungous Infections In Vitro and In Vivo Experiments with Fatty Acid Salts, Penicillin, and Sodium Sulfathiazole. J. Clin. Invest., 23: 929. 1944.

(14) Kiesel, A. The Action of Different Acids and Acid Salts upon the Development of Aspergillus niger. Ann. inst. Pasteur, 27: 391. 1913. cited in Chem Abs., 7: 3350. 1913.

(15) Melnick, D. and Luckmann, F. H. Sorbic Acid as a Fungistatic Agent for Foods. III. Spectrophotometric Determination of Sorbic Acid in Cheese and Cheese Wrappers. Food Res., 19: 20. 1954.

(16) Melnick, D. and Luckmann, F. H. Sorbic Acid as a Fungistatic Agent for Foods. IV. Migration of Sorbic Acid from the Wrapper into Cheese. Food Rds., 19: 28. 1954.

(17) Melnick, D., Luckmann, R. H., and Gooding, C. M. Sorbic Acid as a Fungistatic Agent in Foods. VI. Metabolic Degradation of Sorbic Acid in Cheese by Molds and the Mechanism of Mold Inhibition. Food Res., 19: 44. 1954.

(18) Melnick, D., Gooding, C. M., and Vahlteich, H. W. Sorbic Acid as a Fungistat in Bakery Production with Special Emphasis on a Novel Fungistatic Shortening. Bakers' Digest, 30: 1956.

(19) Mukherjee, S. Studies of the Degradation of Fats by Microorganisms. I. Preliminary Investigations on Enzyme Systems Involved in the Spoilage of Fats. Arch. Biochem. and Biophys., 33: 364. 1951.

(20) Mukherjee, S. Studies of the Degradation of Fats by Microorganisms. II. The Mechanism of Formation of Ketones from Fatty Acids. Arch. Biochem. and Biophys., 35: 23. 1952.

(21) Mukherjee, S. Studies on the Degradation of Fats by Microorganisms. III. The Metabolism of Butyric Acid by Aspergillus niger. Arch. Biochem. and Biophys., 35: 34. 1952.

(22) O'Neill, E. F., Chas. Pfizer and Co. Personal Communication. Letter Nov. 12, 1959.

(23) Perry, G. and Lawrence, R. L. Extending the Shelf-life of Creamed Cottage Cheese by means of Sorbic Acid. Food Tech., 13: 25. 1959.

(24) Pfizer and Co., Inc. Sorbic Acid. Data Sheet no. 510. 5 pp. Chas. Pfizer and Co., Inc., 630 Flushing Ave., Brooklyn 6, New York.

(25) Pfizer and Co., Inc. Sorbistat-K. Data Sheet no. 543. 5 pp. Chas. Pfizer and Co., Inc., 630 Flushing Ave., Brooklyn 6, New York.

(26) Samson, F. E., Katz, A. M., and Harris, D. L. Effects of Acetate and Other Short-Chained Fatty Acids on Yeast Metabolism. Arch. Biochem. and Biophys., 54: 406. 1955.

(27) Smith, D. P. and Rollin, N. J. Sorbic Acid as a Fungistatic Agent for Foods. VIII. Need and Efficacy in Protecting Packaged Cheese. Food Tech., 8: 133. 1954.

(28) U. S. Dept. Health, Education, and Welfare. Food and Drug Administration. Cheeses and Cheese Products. Definitions and Standards. Part 19, Title 21. Reprinted Nov. 1959.

(29) Wyss, O., Ludwig, B. J. and Joiner, R. R. The Fungistatic and Fungicidal Action of Fatty Acids and Related Compounds. Arch. Biochem., 7: 415. 1945.

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