EFFECT OF POTASSIUM SORBATE ON SOME ORGANISMS ASSOCIATED WITH COTTAGE CHEESE SPOILAGE[1,2]

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The per capita consumption of creamed cottage cheese increased from 2.9 to 5.5 pounds between 1948 and 1960 *(15)*. Consistent improvement in quality has been largely responsible for this increase, but spoilage by microorganisms continues to be the principal problem in this industry. Several individual manufacturers of cottage cheese have expressed an interest in the use of antimycotic agents. Federal regulations permit the addition of sorbic acid or its salts to several varieties of cheese, but not to cottage cheese. The work reported herein was performed for the purpose of extending the information on the ability of potassium sorbate to inhibit surface spoilage organisms and to determine whether these organisms utilized sorbic acid during normal storage periods.

The antimycotic activity of the fatty acids was first demonstrated by Clark *(2)*. Also, Kiesel *(11)* and Wyss *et al. (23)* contributed to the knowledge concerning the characteristics of fatty acids which make them inhibitory to microorganisms. Most important among these characteristics is the fact that the inhibitory activity of a fatty acid increases with decreasing pH and that unsaturated fatty acids are more inhibitory than the saturated acids. Keeney *(10)* reported fungicidal properties for the 5, 6, 8, 10, and 11 carbon fatty acids; whereas, the shorter chained acids exhibited only fungistatic properties.

Demaree *et al. (5)* and Deuel *et al. (6)* investigated the toxicity of sorbic acid in the diet. They showed that α, β-unsaturated fatty acids were readily metabolized and that they would not interfere with the digestion of foods or cause any histopathological changes to occur in the anatomy of rats fed rations containing as much as 8% sorbic acid. Melnick *et al. (14)* established that mold enzymes concentrated near the surface of cheese metabolized the sorbic acid used in wrappers or coatings. The work of Mukherjee *(16, 17, 18)* proved that the dehydrogenase system in *Aspergillis niger* was inhibited by β-hydroxybutyric acid. The accumulation of β-hydroxy acid inhibits the dehydrogenation which produces the β-unsaturate and the extent of inhibition is a function of the amount of β-hydroxy acid present. The continued production of the dehydrogenase enzyme eventually overcomes the inhibitor which can then be utilized as a source of carbon.

Melnick *et al. (14)* noted that a β-hydroxy acid occurred two times during the degradation of sorbic acid thereby increasing the effectiveness as an inhibitor. Samson *et al. (21)* observed that the inhibitory effect of fatty acids increased with decreasing pH because the cell was permeable only to the undissociated form of the acid. According to O'Neill *(19)* any food product with a pH of 6.5 or lower and a potassium sorbate concentration of 0.1% or less would have all the sorbate hydrolyzed to sorbic acid.

Bell *et al. (1)* reported that sorbic acid was an effective deterrent of growth for many species of bacteria, yeasts and molds in nutrient media ranging in pH from 7.0 to 4.5. Perry and Lawrence *(20)* indicated that sorbic acid did not suppress heavy microbial contaminations but did effectively retard the growth of small contaminations. Geminder *(8)* reported that 0.075% sorbic acid was effective in controlling the growth of contaminants in cottage cheese and that higher concentrations produced a bitter flavor in the cheese.

Experimental Procedure

Cottage Cheese Manufacture.

Skimmilk pasteurized at 145°F for 30 min. 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% milk fat was pasteurized by steaming in an autoclave for 30 minutes, after which 3% salt was added and the mixture was cooled to 40°F.

Propagation of the Organisms.

The following organisms 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.* Active strains were developed by initiating daily transfers into nutrient broth three days before using the organism. Cultures were incubated at 72°F.

[1]Michigan Agricultural Experiment Station Journal Article no. 3008.

[2]From a thesis presented by the senior author in partial fulfillment of the requirements for the Master of Science degree.

The creaming mixtures were inoculated with a broth culture of the bacteria, yeast or mold which had been incubated for 24 hours. The bacteria populations were determined on violet red bile (VRB) agar and the yeasts and molds were enumerated on potato dextrose (PD) agar acidified to pH 3.5 with tartaric acid.

Preparation of Samples.

Twelve lots of cheese were prepared and analyzed, with each of the six spoilage organisms mentioned previously being used to contaminate two lots. Each lot was composed of five groups of samples prepared as follows: *(a)* non-inoculated control; *(b)* inoculated control; *(c)* inoculated + 0.050% potassium sorbate; *(d)* inoculated + 0.075% potassium sorbate; and *(e)* inoculated + 0.100% potassium sorbate.

Analysis of Samples.

In the trials involving the cheese samples inoculated with the psychrophiles, one sample from each of the five groups was analyzed daily for sorbic acid content, organism populations and pH. In the trials in which the samples were inoculated with yeasts or molds, one sample from each of the five groups was analyzed every other day. In preparation for analysis each sample was mixed for two minutes in a sterilized Waring blender jar at slow speed to assure thorough mixing. (At high speed, curd particles adhered to the upper surfaces of the jar and were not blended properly.) An 11-g sample of this homogenate was added to 99 ml of peptone water (0.1% Bactopeptone in distilled water) and mixed for an additional two minutes in a sterilized Waring blender jar. Appropriate dilutions were plated with VRB agar to determine psychrophiles or PD agar acidified to pH 3.5 to determine yeast and/or mold populations. Some data are included showing total counts obtained on tryptone glucose yeast (TGY) agar. The VRB and PD agar plates were incubated at 72°F for 3 and 5 days, respectively. TGY agar plates were incubated at 89.6°F for 48 hours.

Determination of Sorbic Acid.

A 2-g portion of the homogeneous sample of cheese was analyzed for sorbic acid by the method of Melnick and Luckmann *(12)*. A Beckman DK-2 spectrometer was used to record the absorbancies of the distillates. The controls having no potassium sorbate added were used as a basis to determine the irrelevant absorbancy and appropriate corrections were made. A standard curve was prepared to use in converting the absorbancies of the distillates to percentages of potassium sorbate. pH measurements of each sample were made with a Beckman Zeromatic pH meter using a calomel half-cell and a glass electrode.

The analyses of the inoculated samples were terminated when visible spoilage had occurred and no further analyses of the non-inoculated control samples were performed after all the inoculated samples in the same lot had spoiled.

RESULTS AND DISCUSSION

The principal limitation in working with inoculated samples of cottage cheese involves the fact that cheese curd cannot be sterilized without adversely altering its physical structure. However, a comparison of the microbial populations of both the inoculated samples and the non-inoculated controls on the zero day indicate that the inoculum represented the majority of the organisms present in the cottage cheese used in this work. In all of the samples except those inoculated with *R. mucilaginosa*, the spoilage was typical of that caused by the organism used to inoculate the cheese. Among the group of samples inoculated with *R. mucilaginosa* only the inoculated control and the samples containing 0.050% potassium sorbate exhibited spoilage typical of this organism. The lack of growth of *R. mucilaginosa* in the other samples in this group was attributed to the inhibitory activity of the potassium sorbate.

The flavor hazard is a limitation in the use of sorbic acid or sorbate. Occasional organoleptic determinations supported previous work *(8, 9)* which showed that no off-flavor is present when 0.075% sorbic acid is added to cottage cheese, but an off-flavor attributable to sorbic acid frequently occurs when 0.10% is added.

The data in Figures 1 and 2 show the results of analysis of representative lots of cheese inoculated with each spoilage organism. Throughout the entire work the growth curves show a lower initial population with the inoculated samples containing potassium sorbate than with the inoculated control sample. These results indicate that the potassium sorbate was bacteriostatic and mycostatic, particularly since the inhibition against the populations in the inoculated samples seemed to increase as the percentage of potassium sorbate increased. The inhibitory effect was evident in the forms of an initial reduction in organism populations and extended lag periods in the early phase of the growth curve. The latter particularly seemed to be related to the concentration of potassium sorbate added. Following the lag period, the rates of growth and the maximum populations attained in all of the inoculated samples of cheese containing potassum sorbate were approximately the same as the growth rates and maximum populations attained in corresponding inoculated con-

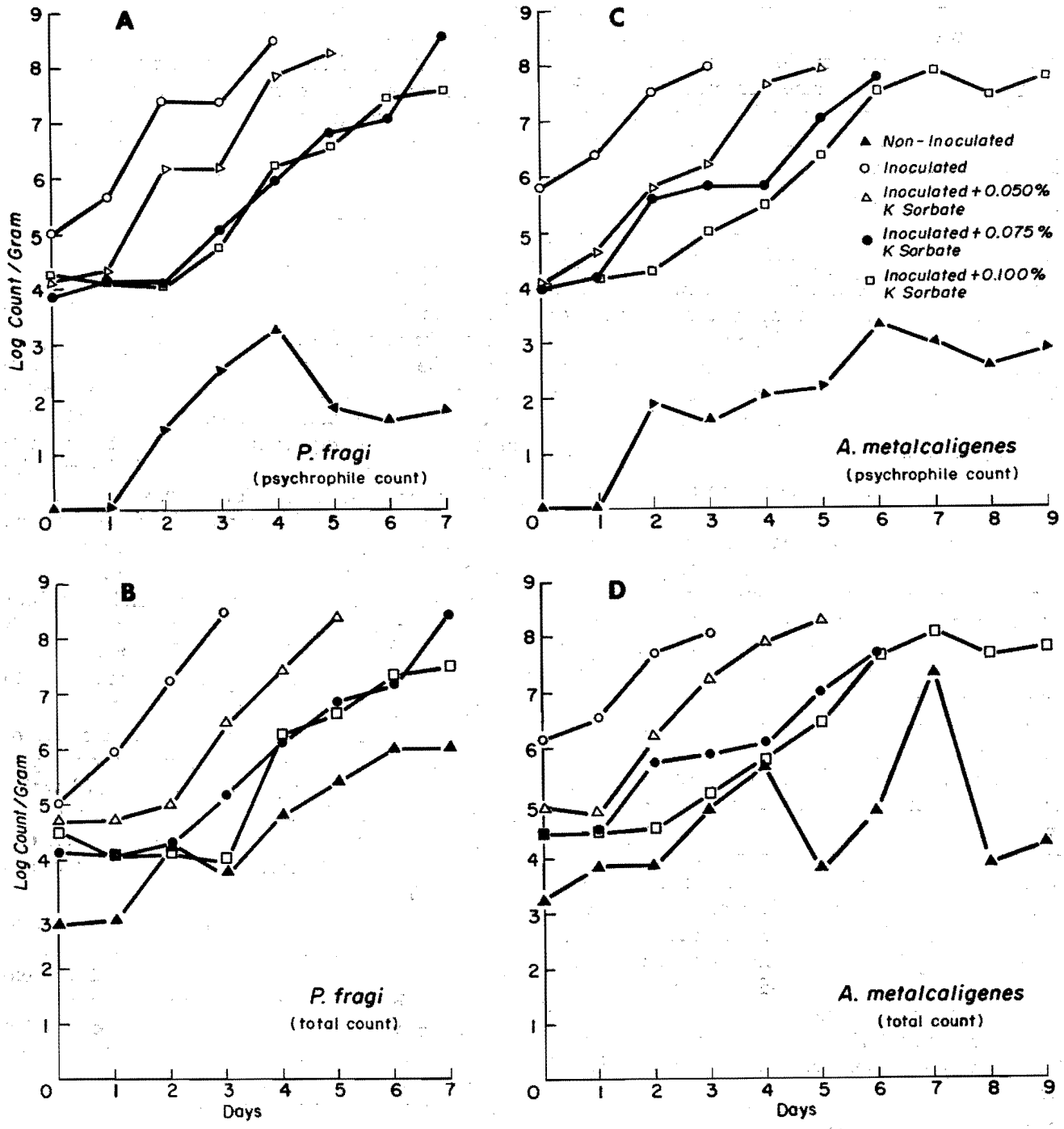

Figure 1. The effect of potassium sorbate on the rate of increase of *P. fragi* or *A. metalcaligenes* inoculated into creamed cottage cheese and stored at 50°F.

trols which contained no sorbate. These results substantiate the opinion of Mukherjee *(16-18)* that continued production of dehydrogenase enzyme by microorganisms would eventually overcome the inhibitory effect of sorbic acid.

Cottage cheese inoculated with *P. fragi* and *A. metalcaligenes* spoiled after 2 or 3 days, respectively, (Figure 1-A, C) when no potassium sorbate was added. Spoilage was associated with populations of approximately 10^8 per g. In samples containing potassium sorbate the populations at the time of spoilage also were approximately 10^8 per g but the time required for spoilage was 5 to 7 days, depending on the amount of potassium sorbate present. The total counts on both of the above organisms enumerated on TGY agar (Figure 1-B, D) represent the sum of the inherent and the induced contamination.

In the cheese inoculated with *G. candidum* and *P. frequentans* (Figure 2-A, B) spoilage was associated with populations on acidified PD agar of ap-

proximately 10^5 to 10^6 per g, regardless of the presence or absence of potassium sorbate. However, the length of time required to attain these populations varied from 4 days in the inoculated cheese containing no potassium sorbate to 8 days in the cheese inoculated with *P. frequentans* and containing 0.1% sorbate.

The data in Figure 2-C show that potassium sorbate had little effect on the growth rate, maximum population attained or keeping quality of cheese inoculated with *T. candida*. At the time of spoilage the organism populations on acidified PD agar varied from approximately 10^7 to 10^8 per g.

R. mucilaginosa seemed to be more sensitive to potassium sorbate than the other organisms used in the experiment (Figure 2-D). The spoilage time varied from 8 days in the samples containing 0.05% potassium sorbate, to 18 days in the samples containing 0.1% sorbate. The organism populations on acidified PD agar varied from approximately 10^6 to

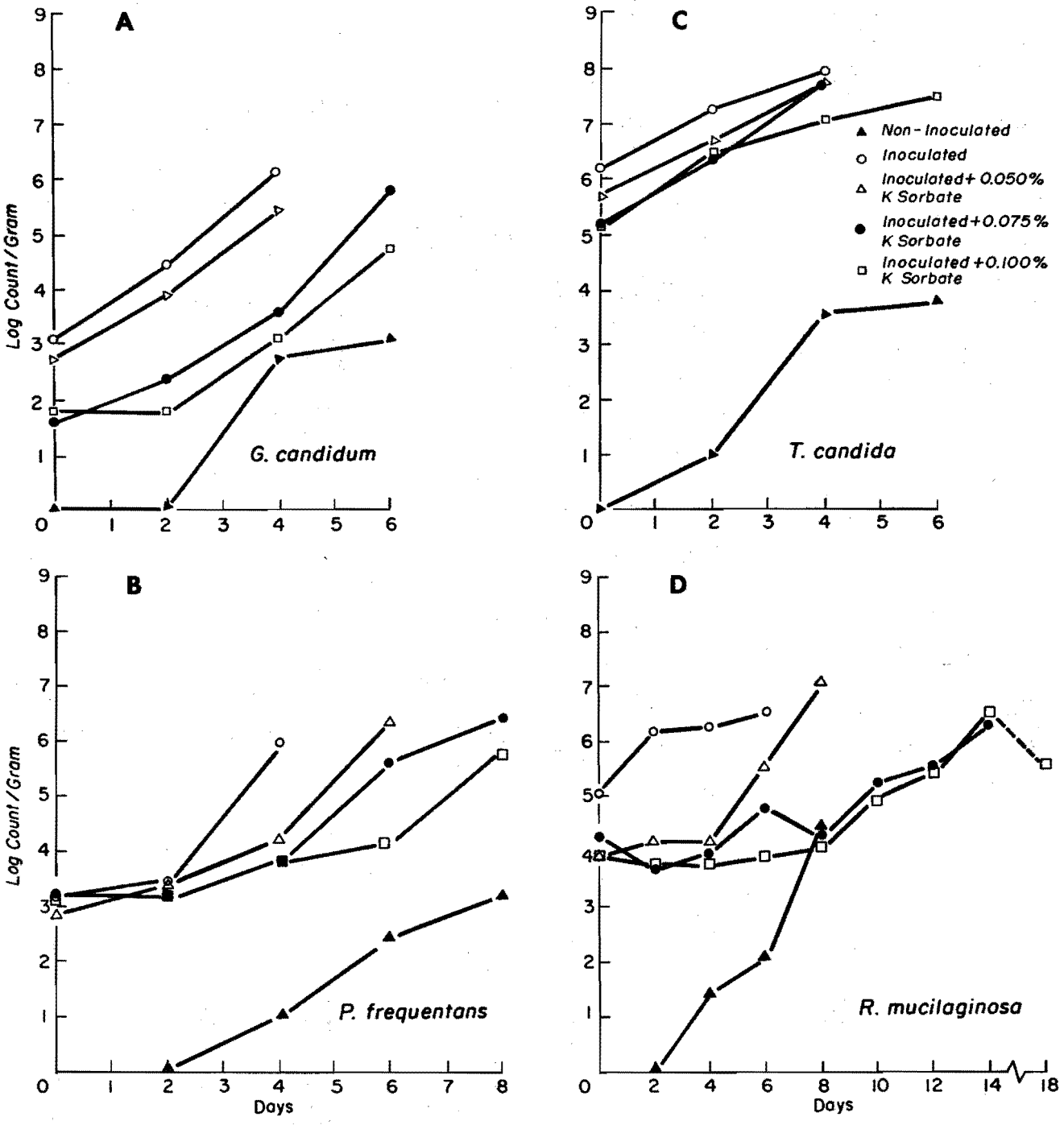

Figure 2. The effect of potassium sorbate on the rate of increase of selected yeasts or molds inoculated into creamed cottage cheese and stored at 50°F.

10^7 per g. Visible surface mold appeared on the non-inoculated control sample after 8 days of storage, and analysis of this sample was terminated.

Several authors *(13, 14, 22)* have reported that molds metabolize sorbic acid as a source of carbon; however, Costilow, *et al. (3)* found no evidence of utilization of sorbic acid in pickle brines containing a large inoculum of yeast *(Torulopsis holmii)*. Emard and Vaughn *(7)* reported that sorbic acid could be used as a nutritional component in selective enrichment media for catalase-negative bacteria.

In the work reported herein, fluctuations in the amounts of sorbic acid recovered at examination intervals during storage were probably within the range of analytical error (Table 1). There was no evidence that sorbic acid was utilized by any of the organisms. If the organisms use sorbic acid, spoilage of the cheese was accomplished by lower populations than required to cause measurable diminution of the sorbate.

Some evidence of bactericidal action by potassium sorbate is suggested by the decreases in the populations enumerated on TGY and VRB on the zero day in the inoculated samples containing sorbate (Figure 1, 2). This property of sorbic acid was also observed by Cowles *(4)* who stated that fatty acids have bactericidal properties at pH 4.7 and lower. Because of the two conjugated double bonds sorbic acid should be active at a higher pH and should possess greater bactericidal ability than caproic acid, the saturated fatty acid of the same chain length.

There was no evidence that the keeping quality of any particular group of samples was influenced by pH which varied from 5.0 to 5.3, the normal range for creamed cottage cheese.

SUMMARY

Samples of creamed cottage cheese containing 0.050, 0.075, and 0.100 per cent potassium sorbate were contaminated with *Pseudomonas fragi, Alcaligenes metalcaligenes, Geotrichum candidum, Penicillium frequentans, Rhodotorula mucilaginosa* or *Torulopsis candida* and stored at 50° F. Samples inoculated with the psychrophiles were analyzed daily and those inoculated with yeasts and molds were analyzed on alternate days for total, psychrophile, yeast and mold counts, pH, and percentage of potassium sorbate. The initial counts indicated that some destruction of the organisms occurred during the first few hours after the cheese was inoculated, and the reduction in population usually increased as the amount of potassium sorbate increased.

The presence of potassium sorbate usually caused a definite lag in the growth curve of the organisms. The lag extended for a period of 1 to 8 days depending upon the concentration of the sorbate and the sensitivity of the organism. The extension in the shelf-life of the cheese was approximately equivalent to the length of the lag period. Upon expiration of the lag phase, the rate of growth and the maximum population attained in the cheese were approximately the same as in the inoculated control samples which contained no potassium sorbate. An off-flavor was

TABLE 1. AMOUNT OF POTASSIUM SORBATE RECOVERED FROM CREAMED COTTAGE CHEESE INOCULATED WITH VARIOUS SPOILAGE ORGANISMS AND STORED AT 50°F. (VALUES CORRECTED FOR IRRELEVANT ADSORPTION).

Age of the cheese when analyzed (in days)	Percentage potassium sorbate added to the cheese	Percentage potassium sorbate present in samples of creamed cottage cheese contaminated with:					
		P. fragi	*A. metalcaligenes*	*G. candidum*	*P. frequentans*	*R. mucilaginosa*	*T. candida*
0	0.050	0.048	0.043	0.046	0.039	0.046	0.046
	0.075	0.069	0.060	0.077	0.070	0.065	0.078
	0.100	0.112	0.098	0.098	0.117	0.085	0.092
2	0.050	0.046	0.045	0.043	0.037	0.039	0.046
	0.075	0.078	0.065	0.067	0.080	0.079	0.076
	0.100	0.111	0.092	0.102	0.085	0.085	0.086
4	0.050	0.042	0.046	0.035	0.049	0.040	0.034
	0.075	0.064	0.069	0.082	0.073	0.061	0.064
	0.100	0.107	0.076	0.095	0.079	0.074	0.094
6	0.050	*	*	*	0.046	0.044	*
	0.075	0.068	0.065	0.059	0.080	0.081	*
	0.100	0.108	0.085	0.098	0.093	0.099	0.072
8	0.050	*	*	*	*	0.052	*
	0.075	*	*	*	0.052	0.088	*
	0.100	*	*	*	0.080	0.094	*

*Samples spoiled; no analyses performed.

frequently present in samples of cheese containing 0.1% sorbate but not in cheese containing 0.75%.

No evidence was found indicating that any of the organisms utilized potassium sorbate. However, the populations may have been insufficient to cause measurable diminution of the sorbate.

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