obtained at a storage temperature of 32° C.

In general, the moisture content of ingredients with a relative humidity of 72.0% was slightly higher than that reported previously. A maximum difference in moisture content obtained by the two methods was 1.0% each for dehydrated alfalfa leaf and steamed bone meal. From a practical viewpoint, less time is required to obtain the critical moisture level from the relative humidity measurement than by observing the time required for a feed to heat in the heating apparatus. The former procedure required only a few hours; the latter, at least 6 weeks.

Relation of Humidity, Moisture Content, and Spontaneous Heating in Complex Feed Mixtures. It is practically impossible to apply critical moisture levels in predicting moisture conditions that will be safe or unsafe for storage of feed mixtures. Before moisture contents can be used in judging whether a feed mixture will be safe from heating, it is necessary to know the composition, concentration, and critical moisture level of each principal ingredient. On the other hand, the data in Table VI show that all mixtures were safe for a 6-week storage period when the relative humidity was 72.0% or less. The unreliable nature of moisture contents for determining safe storage conditions is illustrated by two mixtures with and without animal feeding fat. In this instance, heating depended upon moisture content of the hygroscopic components of the mixture. When a nonhygroscopic ingredient was added, the total moisture was less but this did not change the ability of the feed mixture to support the growth of molds.

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FOOD ADDITIVES

Preservative Effect of Sorbic Acid on Creamed Cottage Cheese

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Commercially made creamed cottage cheese is a microbiologically unstable product because bacteria, yeast, and mold contaminants cause flavor changes. This deterioration is retarded, but not halted by low temperature storage, or by rigid sanitation. The incorporation of sorbic acid into this product arrests the growth of most contaminants, but not the group of microorganisms associated with flavor formation in dairy products. The efficacy of sorbic acid depended on its concentration, the storage temperature, and the intensity of the initial contamination. Levels of 0.05 and 0.07% sorbic acid in creamed cottage cheese of pH 4.9 increased microbiological and flavor stability.

Many factors affect spoilage in creamed cottage cheese. As a result, opinion varies among investigators in the field as to the type of microflora prevailing. Actually, 17 different organisms capable of causing spoilage were isolated and identified by Bonner and Harmon (*1*). They grew these isolates in laboratory media at three concentrations of sorbic acid and reported that concentrations up to 0.25% were not effective in completely destroying the organisms when the pH was not reduced. They also employed a 0.1% aqueous solution of sorbic acid for curd-washing and reported an adverse effect upon flavor. Geminder (*2*) showed that 0.075% potassium sorbate or sorbic acid in finished cottage cheese retarded the growth of yeasts, molds, and slime-forming bacteria. Stull (*5*) reported that sorbic acid at a level of 0.05% inhibited the growth of psychrophilic (cold-loving) organisms, thereby extending the shelf life of cottage cheese.

Materials and Methods

Portions of curd and cream dressing were taken from regular production in a large, independent dairy plant. The curd was made using the "short-set" method which entails culturing skim milk (of 10% total solids content) at 90° F. for 4 to 4.5 hours. The curd was cut and then cooked until a desirable consistency was attained. After draining, the curd was washed three times using water containing 5 p.p.m. of chlorine, the last wash consisting of 35° F. water. To this curd, salted cream was added as a dressing.

In these studies, sorbic acid (in amounts required to give levels of 0.05 and 0.07% in the finished product) was added to the pasteurized and cooled cream dressing (80° F.) and the treated cream was held at 40° to 45° F. until used.

Table I. Effect of Sorbic Acid on Flavor and pH of Creamed Cottage Cheese

Sorbic Acid	Days at 50° F.			Days at 45° F.			Days at 35° F.	
	1	6	14	8	15	25	30	45
None								
Flavor	Good	Fair	Poor	Fairly good	Fair	[a]	Fair	Poor
pH	4.9	4.75	4.4	4.8	4.7	4.7	4.9	4.9
0.05%								
Flavor	Good	Good	Fairly good	Good	Good	Fairly good[b]	Good	Good
pH	4.9	4.85	4.9	4.8	4.9	4.9	4.9	4.9
0.07%								
Flavor	Good	Good	Fairly good	Good	Good	Good	Good	Good[c]
pH	4.9	4.9	4.85	4.9	4.85	4.9	4.9	4.9

[a] Visible growth, not submitted to panel.
[b] Off-odor noticed after 36 days.
[c] After 120 days judged as palatable.

The solubility of the sorbic acid in cream was enhanced by the 16% butterfat content. In actual plant practice this holding or "aging" period may extend from 1 to 3 days. During this time, psychrophilic bacteria may grow in untreated cream and become a source of contamination in the finished product.

After mixing the curd with cream at a 3 to 1 ratio, the creamed cottage cheese was packaged in waxed, 12-ounce cups, capped with plastic lids using a commercial filling and packing machine. Except for the presence of sorbic acid, all standards of identity for creamed cottage cheese were met. Fourteen cups of each series were held at 35°, 45°, and 50° F.

Sensory Evaluation and Diacetyl. Freshly made and incubated samples were scored by a flavor panel of five members. In a study to determine the effect of sorbic acid on flavor, sets of cheeses were made with and without diacetyl. Analysis for diacetyl was carried out using a modified nickel dimethylglyoxime test (*3*).

Microbiological Procedure. Both the surface and interior portions of the cheese were sampled at periodic intervals. For the surface examinations, 1-gram samples were taken (about 1/16-inch surface layer) and for the interior examination, 11-gram samples were used, each representing a composite of two or three containers. Sampling and preparation for plating were conducted under aseptic conditions. The first dilutions for plating were made in 2% aqueous sodium citrate solution by macerating the cheese, using a mortar and pestle and then disintegrating in an Osterizer blender (John Oster Mfg. Co.) using a pint jar in place of the regular container. Preliminary macerating of the cheese with 2% sodium citrate solution followed by 2.5 minutes at high speed in an Osterizer was necessary to obtain complete solubilization of this particular type of cheese. Serial dilutions were plated on nutrient agar (1.5% agar, 0.5% peptone, 0.3% beef extract) and acidified potato dextrose agar (Difco Laboratories). All plates were incubated at 72° F. for 6 days and pH determinations were made on composite samples using a Beckman Model G pH meter.

Microorganisms growing on nutrient agar in the form of pinpoint colonies following the plating of samples containing sorbic acid were subjected to identification studies.

The effect of sorbic acid on the microbiological stability of cream dressing was also studied; and a special experiment was conducted to determine the effect of 0.05% sorbic acid toward large numbers of contaminants in cottage cheese. Surface-growing isolates from spoiled, creamed cottage cheese were used as inoculum.

Results and Discussion

At the three temperatures studied, all control samples were microbiologically unstable. At the very low temperature of 35° F., the growth of contaminants in the controls was slow, but was not arrested completely. The encountered microorganisms grew most intensely on the surface portions of the examined samples. Molds were the slowest to grow; therefore the counts on potato dextrose agar were mainly those of yeast. Surface mold growth could be observed visually over the period of incubation—the samples with visible growth were not plated. Off-flavors (bitter) developed in the controls and after 45 days, total counts reached 9,000,000 per gram *vs.* less than 10,000 per gram in the 0.05% sorbic acid samples. The cheeses with 0.07% sorbic acid were still palatable after 120 days at this temperature and were free of visible growth.

At 50° F., off-flavors were noticed in the controls in 6 days with total counts of 5,000,000 per gram. After 14 days, sourness was pronounced and pH decreased from an initial of 4.9 to 4.4. Cheeses made with 0.05% sorbic acid were still acceptable flavorwise after 14 days of incubation (total counts were less than 10,000 per gram).

There was no change in pH in any of the other cheeses, regardless of the temperature or time of storage, as indicated in Table I.

Microbial growth retardation was accompanied with longer retention of fresh flavor. The least retarded, if any, were the microorganisms of noncontaminant origin which grew as pinpoint colonies on nutrient agar. The growth of these was noted in the cheeses containing sorbic acid after prolonged incubation, but never in the control samples.

The results of microbiological studies of cheeses held at 45° F. are seen in Figures 1 and 2. These are reported in detail, as 45° F. is considered more representative of actual retail storage conditions than are 50° and 35° F. Figure 1 shows the microbiological population in the 1/16-inch surface sample and Figure 2 that of the interior of creamed cottage cheese. The control was off in flavor in 15 days, while neither the 0.05 nor 0.07% sorbic acid samples had detectable off-flavors after 25 days. The slight rise in the curves indicating an increase in yeast count in the 0.05% sorbic acid samples is too insignificant to confer any defect in the cheese.

In both figures it will be noticed that the bacterial population increased during prolonged storage in the cheeses containing sorbic acid. The increase was due to the natural microbial flora associated with cultured dairy products, whereas in the control, the bacterial population was predominantly of the spoilage type—pinpoint *vs.* large colonies. Yeast and mold inhibition by sorbic acid is shown. Pinpoint colonies on nutrient agar were isolated and found to be cocci of the genus Leuconostoc. These were gram-positive and possessed the rather distinctive characteristics of being catalase-negative and producing slime from sucrose. In milk containing the pH indicator, bromocresol purple, they grew with a slight acid reaction.

Nutrient agar proved to be an adequate differentiating medium. Leuconostocs—i.e., noncontaminants—grew as readily recognizable pinpoint colonies,

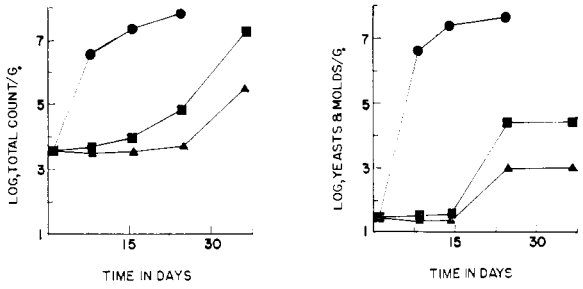

Figure 1. Microbiological population in 1/16-inch surface sample of creamed cottage cheese held at 45°F.

● Control ■ 0.05% sorbic acid ▲ 0.07% sorbic acid

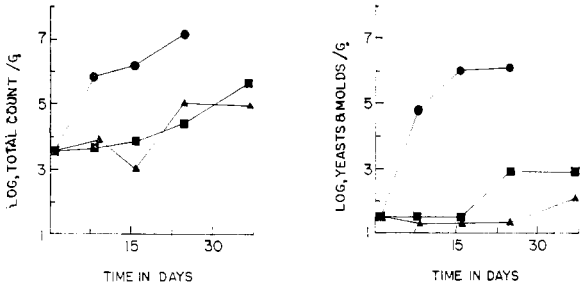

Figure 2. Microbiological population of interior sample of creamed cottage cheese held at 45°F.

● Control ■ 0.05% sorbic acid ▲ 0.07% sorbic acid

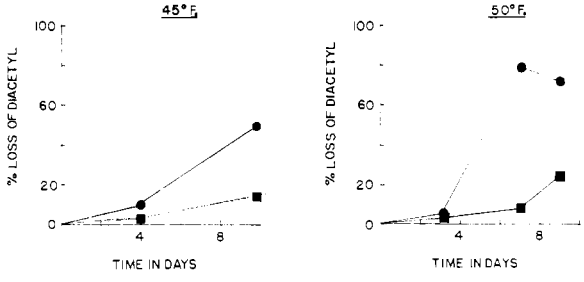

Figure 3. Per cent loss of diacetyl in creamed cottage cheese

● Control ■ 0.05% sorbic acid

while contaminants which were less fastidious were characterized by distinctly larger size colonies, among which bacteria as well as yeasts were encountered. Nutrient agar is a poor but not inhibitory medium for the growth of the latter contaminants. Thus, the bacterial population could be evaluated by subtracting the number of yeast colonies as counted on potato dextrose agar from the numbers representing the total count on nutrient agar. This applied to the counts as obtained on similar dilutions.

The changes of microbial population in the samples incubated at 50° and 35° F. were similar to those at 45° F. The difference was in the growth rate, being faster at 50° F. and slower at 35° F. The growth-retarding effect of 0.07% sorbic acid as compared with 0.05% was not significantly greater within the time limits indicated in the figures. However, a pungent odor developed in the cheeses containing 0.05% sorbic acid at 45° F. after 36 days which is beyond shelf life expectancy. The origin of the odor was not identified. The cheeses containing 0.07% sorbic acid were free of this defect.

Parker and Elliker (*4*) have reported that the loss of diacetyl—a natural flavor component in cultured milk products—in creamed cottage cheese is related to the growth of spoilage microorganisms.

The per cent loss of diacetyl in creamed cottage cheese is shown in Figure 3. Using 150 p.p.m. as the initial concentration, the loss of diacetyl at 50° F. in the control is rapid, with about 80% decrease in 7 days. The sample with sorbic acid shows less than a 10% loss after 7 days at 50° F. At 45° F. after 10 days, the control had decreased 50% *vs.* 10% in the sorbic acid sample.

The viscosity of cream dressing was not affected by sorbic acid added at a 0.2% level after aging at 45° F. and the cream dressing was microbiologically stable during the 4-day holding period. Controls were overgrown with rod-shaped bacteria, when held under these conditions. Experimentation with potassium and calcium sorbate suggested that these compounds are adequate preservers for creamed cottage cheese of pH 4.9 as administered at concentrations of 0.05% equivalent to sorbic acid. However, these compounds at 0.2% concentrations (calculated as sorbic acid) exerted no preserving effect on cream dressing during aging at 45° F. This, presumably, was due to the failure of the salts to lower the pH of this medium.

In earlier studies a deliberately inoculated series was included to determine the effect of 0.05% sorbic acid toward large numbers of initially present contaminants. Examinations had to be limited to visual observations and fairly rapid growth of spoilage organisms in all samples at three temperatures was obvious. No preservative should be a substitute for poor quality milk or unsanitary conditions, and for this reason a preservative should not be effective where initial contamination is large.

Acknowledgment

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