SOME FACTORS WHICH INFLUENCE THE BIACETYL CONTENT IN COTTAGE CHEESE

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INTRODUCTION

Biacetyl and acetylmethylcarbinol are recognized as chemical substances which contribute to the desirable flavor and aroma of dairy products. Biacetyl and acetylmethylcarbinol are produced by the decomposition of citric acid and lactose by Leuconostoc citrovorous and Leuconostoc dextranicum. The mechanism of formation of these flavor components by Leuconostoc organisms and the factors affecting their formation have been determined in previous studies primarily involving butter and butter cultures. Fine flavored butter contains from 0.0002 to 0.0004 percent biacetyl.

The increasing consumer demand for cottage cheese has focused attention to the influence of biacetyl and acetylmethylcarbinol on desirable cottage cheese flavor. Moderate increases in amounts of these flavor components could result in a better flavored product which would be beneficial both to the producer and consumer of cottage cheese. One aim of this study was to determine if additions of certain organic acids to wash waters used for cottage cheese would result in increases of biacetyl and acetylmethylcarbinol.

Biacetyl and acetylmethylcarbinol are produced and utilized by Leuconostoc organisms. The decrease in these constituents normally occurs slowly and in most instances does not result in a poor flavored cheese. Some organisms have been reported to destroy biacetyl and acetylmethylcarbinol rapidly and cause the cottage cheese to assume a flat flavor. However, very little information is available concerning destruction of these flavor compounds by organisms which are naturally present in cottage cheese. Knowledge of the effect of organisms isolated from cottage cheese on the biacetyl and acetylmethylcarbinol content of cottage cheese could lead to retention of these flavor components.

REVIEW OF LITERATURE

The recognition of van Niel (1929) that biacetyl either is responsible for the aroma of butter or is the principal flavor component of butter led to studies of biacetyl and acetylmethylcarbinol content of dairy products. Tapernaux (1932) showed that addition of biacetyl improved the flavor of butter and margarine. Davies (1933) found that lactose fermentation yields acetylmethylcarbinol which is oxidized to biacetyl, giving the characteristic flavor and aroma of butter. According to Michaelian et al. (1933), butter cultures with a satisfactory flavor and aroma contained considerable quantities of acetylmethylcarbinol and biacetyl, while cultures lacking in flavor showed little or none. Hammer (1934) reported that fine butter contained from 0.0002 to 0.0004 percent biacetyl. The relationship between acetylmethylcarbinol and biacetyl content, and a satisfactory flavor in butter and butter cultures has been confirmed repeatedly. However, a relatively high biacetyl content is no assurance of a good flavor because definite off-flavors due to a number of causes may be present. According to Wiley et al. (1939) the assumption that the biacetyl content of a butter culture is an accurate guide to its value as a desirable flavor producer is fallacious.

Farmer and Hammer (1931) and Ritter and Christen (1935) found that addition of citric acid greatly increased the amount of biacetyl in butter cultures. Ruehe (1937) obtained maximum biacetyl contents when citric acid was added to a twenty-four hour old culture and incubated for another twenty-four hours. A culture so treated could not be added to butter as a starter but was adapted to the preparation of starter distillate. Prill and Hammer (1939) reported that 0.15 percent citric acid proved to be the most practical amount to add to butter cultures. Klubchandani (1939) added citric acid and sodium citrate to butter cultures as suggested by Prill and Hammer (1939) and reported improved butter flavor. When citric acid was added to butter cultures, a 70 to 90 percent increase in acetylmethylcarbinol and biacetyl content was observed by Gehrke and Weiser (1948). Nelson and Brence (1953) observed that the addition of citric acid to cottage cheese increased the volatile acidity and improved the flavor.

Hammer (1939) proved that biacetyl was the principal flavor component in cottage cheese. The biacetyl content of cottage cheese could be lowered by prolonged washing, or by high cooking temperatures which destroyed flavor producing organisms. Ninety-two specimens of various kinds of cheese examined by Csiszar et al. (1942) contained acetylmethylcarbinol in all samples and biacetyl in

a large majority of the samples. Calvert and Price (1949) found biacetyl in samples of cheddar cheese but could not find a relationship between flavor and biacetyl content. Krishnaswamy and Babel (1951) found 1.06 to 2.25 parts per million of biacetyl in cottage cheese curd obtained by the long set method. Parker and Elliker (1952) demonstrated the importance of biacetyl to cottage cheese flavor by classifying various samples of the product according to aromatic flavor and subjecting the same samples to a chemical analysis for biacetyl. Samples with high biacetyl contents received high flavor scores, with occasional samples criticized for high acid. Low scoring samples were low in biacetyl values.

The Voges-Proskauer reaction (1898) was one of the earliest methods devised for the detection of acetylmethylcarbinol. It was a qualitative-colorimetric determination requiring considerable time. Barritt (1936) found that the Voges-Proskauer reaction (1898) could be intensified and made more delicate by addition of a small amount of alpha naphthol. Eggleston et al. (1943) used a rapid colorimetric method similar to that of Barritt (1936). A rapid qualitative method using creatine and sodium hydroxide was developed by O'Meara (1931). Pien et al. (1936) mentioned a qualitative method reacting biacetyl with phenylhydrazine to yield biacetylphenylhydrazone. Hammer (1935a) applied the rapid method of O'Meara (1931)

to butter cultures. Small amounts of creatine and strong sodium hydroxide solution were added to culture in a test tube. The intensity of the resulting red color depended upon the amount of biacetyl and acetylmethylcarbinol present.

A procedure for measuring 2,3-butylene glycol and acetylmethylcarbinol was developed by Lemoigne (1920). Bromine was used to oxidize 2,3-butylene glycol to acetylmethylcarbinol. The acetylmethylcarbinol was oxidized with ferric chloride to biacetyl and reacted with nickel chloride to form nickel dimethyl glyoximate. The insoluble nickel salt precipitated and could be measured. Van Niel (1927) stated that the method of Lemoigne (1920) did not give quantitative results and described a method, based on the Lemoigne reaction, which permitted a quantitative measurement of the biacetyl and acetylmethylcarbinol. This method required more careful distillation of the biacetyl and a slightly modified procedure for collecting and weighing the precipitated nickel salt. Davies (1933) modified van Niel's method (1927) and obtained better results.

Various factors which influence the determination of acetylmethylcarbinol and biacetyl as nickel dimethyl gloximate were studied by Michaelian et al. (1933). Biacetyl was distilled in a stream of carbon dioxide to prevent atmospheric oxidation. Conditions affecting the completeness of precipitation of acetylmethylcarbinol and biacetyl

as nickel dimethyl glyoximate from butter were studied by Bairncoat (1935). This worker proposed a colorimetric method to determine traces of the nickel salt. The nickel dimethyl glyoximate was dissolved in chloroform and compared with solutions of known amounts of the salt.

Hammer (1935b) steam distilled cultures to which ferric chloride had been added for the purpose of oxidizing the acetylmethylcarbinol to biacetyl. The distillates were treated with hydroxylamine hydrochloride, sodium acetate, and nickel chloride, and the resulting nickel salts determined quantitatively. Results indicated that the diketone produced was biacetyl rather than one of the homologs and if homologs were present, they were limited to relatively insignificant amounts. In the studies of Prill et al. (1939), distillates from ordinary butter cultures gave no evidence of the higher homologs of biacetyl or acetylmethylcarbinol. Dehove and Desirrier (1938) were able to evaluate biacetyl with an accuracy of 0.5 milligrams per kilogram of butter, by purification of the nickel dimethyl glyoximate. Other investigators including Pritzker and Jungkunz (1930), Vizern and Guillot (1932), Mohler and Helberg (1933), Stahly et al. (1935), Mohler and Herzfeld (1935), Mohr and Wellm (1937), Schmalfuss and Werner (1937), Kniphorst and Kruisheer (1937), Parker and Shadwick (1937), Jungkunz (1940), and Wilson (1941)

have reported on the nickel dimethyl glyoximate method and modifications of that method.

A colorimetric method for determining biacetyl and acetylmethylcarbinol was suggested by Testoni and Cuisa (1931). These investigators oxidized nickel dimethyl glyoximate and obtained a soluble red complex in which the nickel had a higher valence number. Kunze (1936) described a micromodification of the gravimetric method and recommended colorimetric methods for amounts less than 0.3 milligrams. Pien et al. (1936) obtained a yellow color when biacetyl was reacted with m-p-toluenediamine and treated with strong sulfuric acid. These workers later (1937) obtained a stronger yellow color using diaminobenzine. This method was accurate for amounts of biacetyl as low as 0.5 milligrams per kilogram of butter. Pien (1948) modified the method of Pien et al. (1937) by using a different procedure for purifying the distillate and obtained more accurate results for small amounts of biacetyl. Ritter and Nussbaumer (1939) used the method of Pien et al. (1936) and suggested use of pure concentrated sulfuric acid and a fresh solution of m-p-toluenediamine. Dehove and Dessirier (1938) noted that the method of Pien et al. (1937) would not permit accuracy of four milligrams per kilogram when fifty grams of butter was used. Cox and Wiley (1939) extended

the method of Pien et al. (1937) by standardizing the sample, the apparatus, and the method of distillation.

A volumetric method for biacetyl determination, based on the oxidation of one molecule of biacetyl to two molecules of acetic acid with hydrogen peroxide was developed by Ruehe and Corbett (1937).

Prill and Hammer (1938) developed a colorimetric method for the microdetermination of biacetyl based on the formation of the intensely colored ammo-ferrous dimethyl glyoximate. With this method, they were able to detect the difference between 0.001 milligrams of biacetyl and no biacetyl in five milliliters of water. Den Herder (1947) modified the method of Prill and Hammer (1938) using a special apparatus and distilling the biacetyl with a stream of carbon dioxide.

Folke Bange (1943a, 1943b, 1944a, 1944b, 1945) has shown that biacetyl and acetylmethylcarbinol are products of citric acid and sugar fermentation of Streptococcus citrovorous and Streptococcus paracitrovorous. These products are subject to utilization or destruction by the above named flavor organisms during subsequent metabolism. Michaelian et al. (1933) studied destruction of biacetyl and acetylmethylcarbinol using a skimmilk and butter culture medium that had been subjected to high heat treatment. When the medium was held twenty days at 6° C., the biacetyl and acetylmethylcarbinol

content remained constant, but when inoculated with butter culture and similarly held there was a pronounced decrease.

The investigations of Williams and Morrow (1928) revealed that acetylmethylcarbinol is destroyed by certain strains of coli-aerogenes bacteria chiefly Aerobacter aerogenes, by the green fluorescent bacteria and by all the aerobic spore formers tested. Acetylmethylcarbinol was not destroyed by certain representatives of the Salmonella, Eberthella, Proteus and Serratia groups. Virtanen and Kontio (1941) found that Bacillus punctatum and Bacillus vulgatus destroyed both biacetyl and acetylmethylcarbinol. A non-proteolytic coccus and Pseudomonas fluorescens destroyed very little acetylmethylcarbinol but up to half of the biacetyl content. Elliker and Horrall (1943) stated that Pseudomonas putrefaciens destroyed biacetyl in the aqueous phase of butter. A complete lack of aroma followed growth but one-third to one-fourth of the original biacetyl remained in the butter. Elliker (1945) found that Streptococcus lactis had no effect on biacetyl. However, Ps. fluorescens, "Ps. fluroscens var. liquefaciens," Pseudomonas fragi, Ps. putrefaciens, Pseudomonas nigrificans, and some unidentified strains of Pseudodomonas markedly reduced the biacetyl content of butter stored at 60° F. Hietaranta and Gyllenberg (1950) reported that formation of biacetyl, but not of acetylmethylcarbinol, is suppressed

when the oxidation-reduction potential is lowered by the addition of strongly reducing bacteria such as A. aerogenes, Escherichia coli, and Serratia marcesens. The investigations of Parker and Elliker (1952, 1953) showed that Ps. fragi, Pseudomonas viscosa and Alcaligenes metalcaligenes destroyed biacetyl in cottage cheese. Biacetyl was destroyed prior to the appearance of the gelatinous or slimy curd defect associated with these organisms. Ps. fragi converted most of the biacetyl to acetylmethylcarbinol while slightly less was converted by Ps. viscosa. Alc. metalcaligenes reduced biacetyl more slowly to acetylmethylcarbinol and 2,3-butylene glycol than did Ps. fragi and Ps. viscosa.

EXPERIMENTAL PROCEDURE

The cottage cheese curd used in this experiment was obtained from the Michigan State University creamery on the date of manufacture. The cheese was divided into the appropriate number of lots and placed in separate containers which had been rinsed with a two hundred parts per million hypochlorite solution. All creamed cottage cheese was packaged in twelve-ounce cartons and stored for twelve days. One-half of the cartons of each lot were stored at 40° F. and the other half were stored at 50° F.

Biacetyl, acetylmethylcarbinol, pH, and flavor determinations were performed initially and at three-day intervals on each sample of creamed cottage cheese.

Biacetyl and acetylmethylcarbinol were determined by the method of Prill and Hammer (1938). Solutions were prepared containing pure biacetyl in increments of 0.05 milligram between the concentrations of 0 and 10 milligrams. These prepared solutions were developed colorimetrically and the intensity measured on a Cenco-Sheard-Sanford photelometer. Six duplicate trials were performed and the average scale reading for each biacetyl concentration

was calculated. A standard curve was constructed, plotting photelometer scale readings versus milligrams of biacetyl.

Sampling was done in an aseptic manner. Twenty-five grams of cheese were weighed directly into the distillation flasks. For acetylmethylcarbinol determinations, twenty milliliters of 40 percent ferric chloride solution were added to the weighed cheese sample and refluxed strongly for ten minutes and then distilled. All photelometer scale readings of the color intensity of all distilled samples were converted into biacetyl values using the standard curve. When it was impossible to perform biacetyl and acetylmethylcarbinol determinations on the sampling date, samples were frozen promptly and stored at 10° F. The storage time did not exceed two days.

Flavor and pH were determined immediately after sampling. The pH was determined with a Beckman Model G pH meter. A scorecard proposed by the American Dairy Science Association[1] was used in evaluating flavor.

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[1] American Dairy Science Association Committee on Cottage Cheese Scorecard. H. C. Olson, Dairy Department, Oklahoma A. and M. College, Stillwater, chairman.

Addition of Various Organic Acids to Cottage Cheese Wash Water

Citric, lactic, and sorbic acid were added to cottage cheese wash waters to determine if they would increase the biacetyl and acetylmethylcarbinol content of the cottage cheese. The cheese curd, which had been washed once, was divided into ten equal lots. Each lot of cheese was washed separately with wash water which contained lactic, citric, or sorbic acid. Sufficient citric and lactic acids were used to produce pH levels of 6.0, 5.5, and 5.0 in the wash water. pH levels of 6.2, 5.8, and 5.2 were obtained by adding 0.05, 0.1, and 0.25 percent sorbic acid, respectively. The cottage cheese curd was allowed to stand one hour in the treated wash water before draining. One lot of cottage cheese was washed with untreated tap water and used as a control. A creaming mixture containing 14 percent butterfat and 4 percent salt was added to the drained curd to standardize the final product to 4 percent fat and 1.3 percent salt.

Inoculation of the Cheese with Various Microorganisms

Various microorganisms were inoculated into cottage cheese to determine if they reduced the biacetyl and acetylmethylcarbinol

content. Cottage cheese curd was divided into twenty-one equal lots preparatory to creaming. An appropriate amount of creaming mixture of the composition previously described was prepared and also divided into twenty-one equal portions. One lot of cheese was creamed and used as a control. Each of the remaining lots of creaming mixture was inoculated with 0.1 milliliter of a twenty-four hour trypticase soy broth culture of the following organisms: Micrococcus flavus, Micrococcus conglomeratus, Micrococcus candidus, Pseudomonas fragi, Pseudomonas fluorescens, Pseudomonas desmolyticum, Pseudodomonas tralucida, Achromobacter butyri, Achromobacter eurydice, Escherichia coli, Escherichi freundii, Alcaligenes metalcaligenes, Bacillus firmus, Bacterium erythrogenes, Geotrichum candidum, Mucor plumbeus, Penicillium frequentans, Rhodotorula flava, and Torulopsis candida. These organisms were obtained from stock cultures possessed by the Michigan State University Dairy Department. Not all of these organisms are responsible for cottage cheese spoilage, but all have been isolated from spoiled cottage cheese and were included to determine their effect upon biacetyl and acetylmethylcarbinol content. Samples of each creaming mixture were taken before and after inoculation. The total microbiological population of each sample was enumerated according to procedures described by Standard Methods (1953). The counts of organisms

added ranged from 300 to 450,000 per milliliter of cream. Mold and yeast numbers were the lowest, while counts for Micrococci, Achromobacter, and Escherichia tended to be high. After sampling, the inoculated creaming mixture was added to the cottage cheese curd.

Addition of Various Organic Acids and Starter to the Creaming Mixture

Citric, lactic, and sorbic acids and commercial starter were added to creaming mixtures in an effort to increase the biacetyl content of the creamed cottage cheese. A creaming mixture of previously described composition was divided into five equal lots. One-tenth percent lactic acid was added to one portion, 0.1 percent citric acid to a second portion, 0.1 percent sorbic acid to a third portion, and 1.0 percent commercial starter to a fourth portion. The fifth portion had no addition and was used as a control. These five lots of cream were then added to uncreamed cottage cheese.

RESULTS

Effect of Various Organic Acids Used to Acidulate Wash Waters on the Biacetyl Content of Cottage Cheese

The initial biacetyl values of fresh cottage cheese analyzed in this experiment usually ranged from 0.3 to 0.8 milligrams per 100-gram sample (3 to 8 parts per million), and most samples were within the range of 0.4 to 0.6 milligrams (4 to 6 parts per million). Variations were noted in the biacetyl content of individual samples. Generally, however, biacetyl increased to a maximum on the sixth or ninth day of storage and decreased on the twelfth day.

(a) Influence of organic acids on the biacetyl content of cottage cheese stored at 40° F. The effect of washing curd with wash waters acidified to pH 6.0, 5.5, and 5.0 with lactic or citric acid, and to pH 6.2, 5.8, and 5.2 with sorbic acid on the biacetyl content of creamed cottage cheese stored twelve days at 40° F. is shown in Figures 1 to 6, inclusive. A comparison of data in Figures 1, 2, and 3 shows that the samples washed with waters acidulated with lactic acid contained more biacetyl than the other samples. The data indicate that lactic acid was the most effective in permitting the biacetyl content to increase above that of the control. The

higher amount of biacetyl is evident especially in the data of Figures 2 and 3.

The effect of various levels of lactic acid in the wash water on the biacetyl content of the cheese is shown in Figure 5. The biacetyl values of each sample are significantly higher than those of the control. Unlike the control in which a decrease in biacetyl was noted at three days, the biacetyl content of all samples washed with waters containing lactic acid increased similarly, reaching a maximum at nine days. Beyond the nine-day storage period, the biacetyl value varied according to the pH of the wash water. The biacetyl content of the cheese washed with waters acidified to pH 5.5 with lactic acid increased on the twelfth day, whereas the biacetyl of the cheese washed with waters acidified to pH 5.0 and 6.0 with lactic acid decreased on the twelfth day.

No significant differences between the biacetyl contents of the control and the cheese washed with waters containing citric and sorbic acids were noted (Figures 4 and 6). However, slight deviations were evident. The biacetyl of the control decreased at three days, while that in the samples washed with waters adjusted to pH 5.0 with citric acid and to pH 5.2 with sorbic acid increased the third day. Also, the biacetyl content of the control increased on the twelfth day, whereas the biacetyl content of samples washed with

waters acidified to pH 6.2 with sorbic acid, as well as that of all samples washed with citric acid, decreased on the twelfth day.

The pH values of all samples stored at 40° F. varied from pH 5.48 to 4.88 regardless of sampling interval (Table 1). The pH of most of the samples decreased slightly to a minimum on the ninth day and were perceptibly higher the twelfth day.

(b) Influence of organic acids on the biacetyl content of cottage cheese stored at 50° F. The changes occurring in the biacetyl content of cottage cheese washed with acidulated waters and stored twelve days at 50° F. are shown in Figures 7 to 12, inclusive, and in Table 1. The higher storage temperature had a great effect upon the biacetyl content of the control. The biacetyl content of the control sample stored at 40° F. (Figure 1) decreased slightly the third day before increasing continuously and uniformly to a maximum of 1.28 milligrams per 100-gram sample on the twelfth day. The biacetyl content of the control stored at 50° F. (Figure 7) increased gradually to a maximum of 1.0 milligrams per 100-gram sample on the sixth day and uniformly decreased to a minimum of 0.5 milligrams on the twelfth day. With the exception of the cheese sample washed with water acidified to pH 5.2 with sorbic acid (Figure 9), similar differences due to temperature were noted between

the biacetyl content of cheese stored at 40° and that of the corresponding sample stored at 50° F.

The biacetyl contents resulting from acidifying wash waters to pH 6.0, 5.5, and 5.0 are shown in Figures 7, 8, and 9, respectively. The biacetyl contents of the cheese washed with water acidulated with citric acid (Figure 7) and lactic acid (Figure 8) were higher on the third day than the control, but decreased and remained below the control on the sixth, ninth, and twelfth day. The remaining cheese samples in Figures 7 and 8 and those washed with citric and lactic acid in Figure 9 had biacetyl contents that were lower than the control for the duration of the storage period.

The biacetyl content of the cheese washed with water containing sorbic acid (Figure 9) increased markedly and continuously above the biacetyl level of the control. This increase may be due to the continued production of biacetyl and inhibition of the fermentation which destroys biacetyl. Leuconostoc citrovorous and/or Leuconostoc dextranicum which produce biacetyl, were quite tolerant to sorbic acid. These organisms grew well in broth containing all combinations of sorbic acid and pH used in this experiment (Table 2). Good growth was observed in trypticase soy broth containing 0.05 to 0.25 percent sorbic acid and adjusted to a pH range of 4.0

to 6.6. Growth was slightly heavier in broth of pH 5.0 and below, than in broth above pH 5.0.

Data representing the biacetyl values of the cheese washed with the three pH levels of each acid have been grouped (Figures 10, 11, and 12). Analysis of these graphs indicates that the sample washed with water acidified to pH 5.2 with sorbic acid (Figure 12) was the only sample with a biacetyl content significantly higher than the control. This sample contained 1.1 milligrams per 100-gram sample more biacetyl than the control on the twelfth day.

The pH values of all cheese samples stored at 50° F. are shown in Table 1. The pH of all samples decreased to a minimum on the sixth and ninth day and increased slightly on the twelfth day. At the same sampling interval, the pH of the cheese stored at 50° F. was lower in all cases than the corresponding sample stored at 40° F. Attention should be focused on the pH values of the cheese washed with waters containing sorbic acid. These pH values did not decrease as rapidly or extend as low as the other samples. A correlation is evident between the amount of sorbic acid and the decrease in pH. The larger the amount of sorbic acid, the higher the pH of the sample, which indicates that acid production by organisms is inhibited by sorbic acid. The sample washed with water adjusted

Table . pH measurements of creamed cottage cheese which were washed with waters containing citric, lactic, or sorbic acids and stored for twelve days at 40° and 50° F.

Wash Waters Used	0 da
Control	5.45
Acidified with:	
citric acid (pH 6.0)	5.35
citric acid (pH 5.5)	5.25
citric acid (pH 5.0)	5.22
lactic acid (pH 6.0)	5.25
lactic acid (pH 5.5)	5.22
lactic acid (pH 5.0)	5.15
sorbic acid (pH 6.2)	5.28
sorbic acid (pH 5.8)	5.22
sorbic acid (pH 5.2)	5.02

Table 1 (Continued)

pH of Cottage Cheese at Various Storage Conditions When Washed with Acidified Water							
3 da.		6 da.		9 da.		12 da.	
40° F.	50° F.	40° F.	50° F.	40° F.	50° F.	40° F.	50° F.
5.25	4.65	5.18	4.30	5.00	4.32	5.08	4.40
5.00	4.68	5.08	4.40	4.98	4.30	5.05	4.40
5.00	4.65	4.95	4.35	4.88	4.35	5.00	4.45
5.05	4.78	4.90	4.35	4.90	4.38	5.00	4.43
5.12	4.70	5.00	4.35	4.95	4.35	5.02	4.45
5.10	4.65	5.00	4.32	4.98	4.32	5.00	4.40
5.05	4.60	4.95	4.28	4.98	4.28	5.00	4.35
5.00	4.90	4.88	4.45	4.80	4.35	5.00	4.48
5.00	4.95	4.90	4.65	4.82	4.50	5.00	4.60
4.90	4.95	4.90	4.75	4.85	4.75	4.90	4.80

Table 2. Sorbic acid tolerance of Leuconostoc citrovorous and/or Leuconostoc dextranicum in trypticase soy broth at various levels of pH (avg. of 4 trials).

Sorbic Acid Added to Broth Before Adjusting pH with Lactic Acid (%)	Final Reaction of Broth (pH)	Turbidity of Culture Indicating Growth
0.05	6.65	+
0.05	6.00	+
0.05	5.00	+
0.05	4.50	+
0.05	4.00	+
0.10	6.60	+
0.10	6.00	+
0.10	5.00	+
0.10	4.50	+
0.10	4.00	+
0.25	6.00	+
0.25	5.90	+
0.25	5.00	+
0.25	4.50	+
0.25	4.00	+

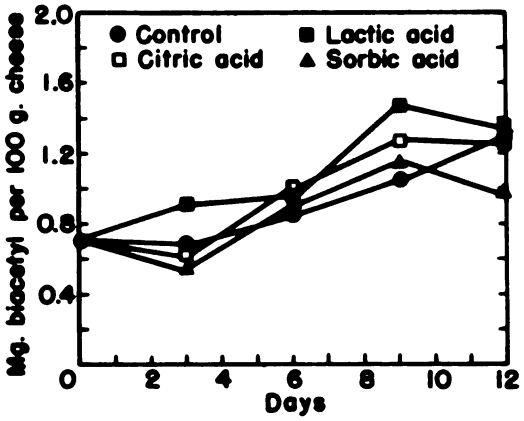

Fig. 1 Wash waters acidulated to pH 6.0 with citric and lactic acids and to pH 6.2 with sorbic acid.

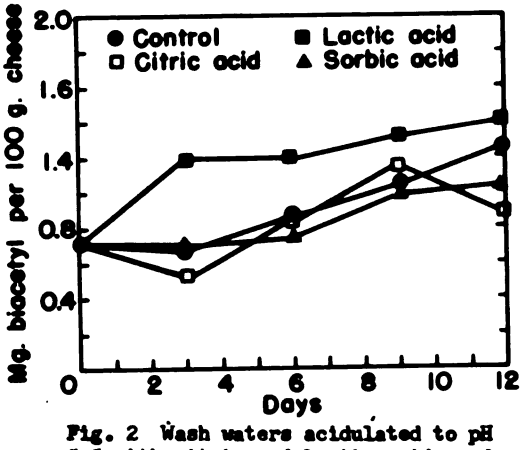

Fig. 2 Wash waters acidulated to pH 5.5 with citric and lactic acids and to pH 5.8 with sorbic acid.

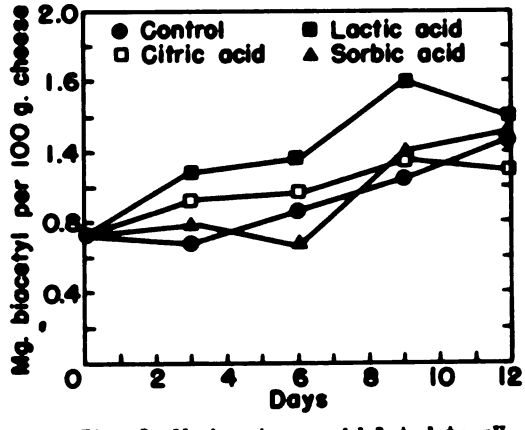

Fig. 3 Wash waters acidulated to pH 5.0 with citric and lactic acids and to pH 5.2 with sorbic acid.

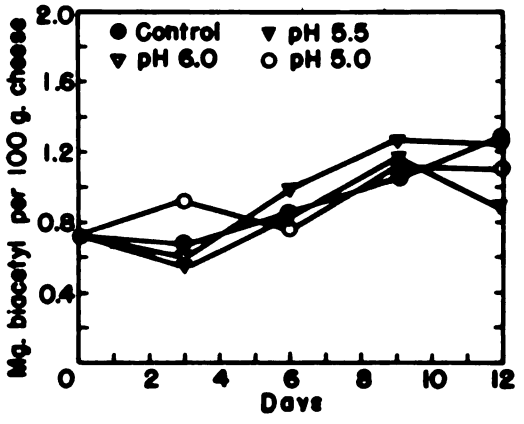

Fig. 4 Wash waters acidulated to pH 6.0, 5.5 and 5.0 with citric acid.

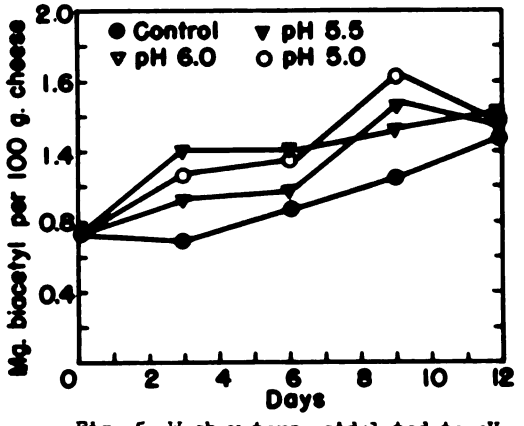

Fig. 5 Wash waters acidulated to pH 6.0, 5.5 and 5.0 with lactic acid.

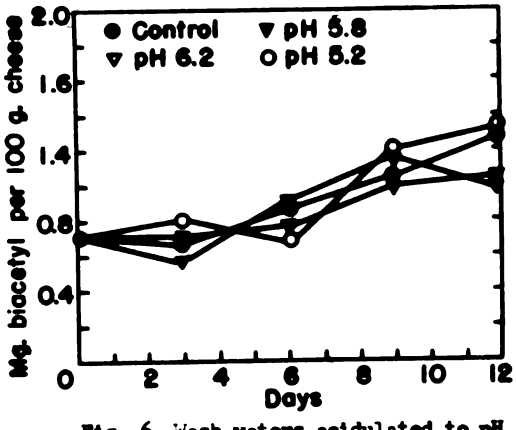

Fig. 6 Wash waters acidulated to pH 6.2, 5.8 and 5.2 with sorbic acid.

Effect of using wash water containing citric, lactic or sorbic acid on the biacetyl content of creamed cottage cheese stored 12 days at 40°F.

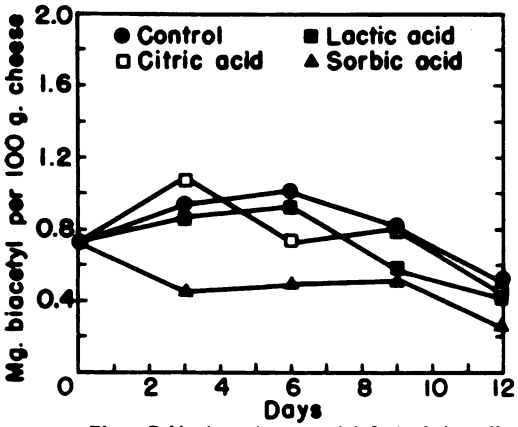

Fig. 7 Wash waters acidulated to pH 5.0 with citric and lactic acids and to pH 6.2 with sorbic acid.

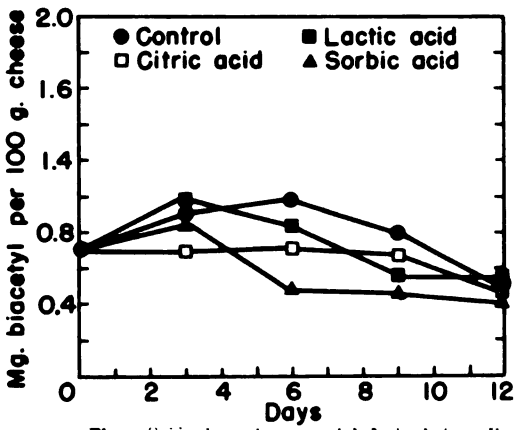

Fig. 8 Wash waters acidulated to pH 5.5 with citric and lactic acids and to pH 5.8 with sorbic acid.

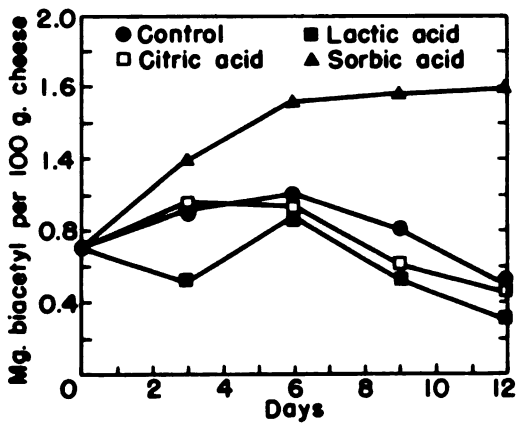

Fig. 9 Wash waters acidulated to pH 5.0 with citric and lactic acids and to pH 5.2 with sorbic acid.

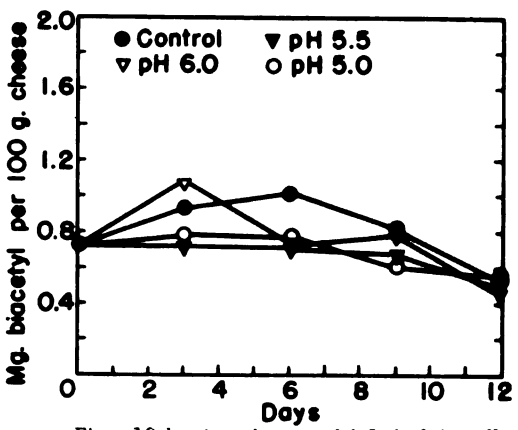

Fig. 10 Wash waters acidulated to pH 6.0, 5.5 and 5.0 with citric acid.

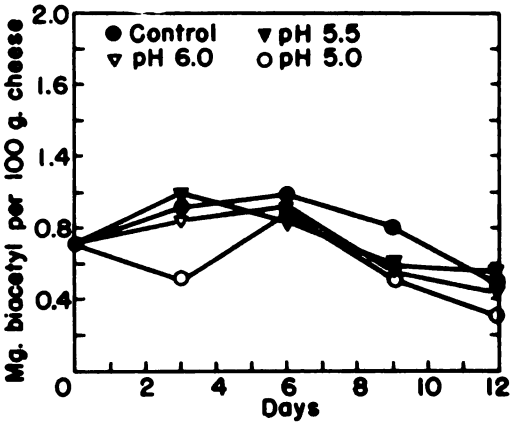

Fig. 11 Wash waters acidulated to pH 6.0, 5.5 and 5.0 with lactic acid.

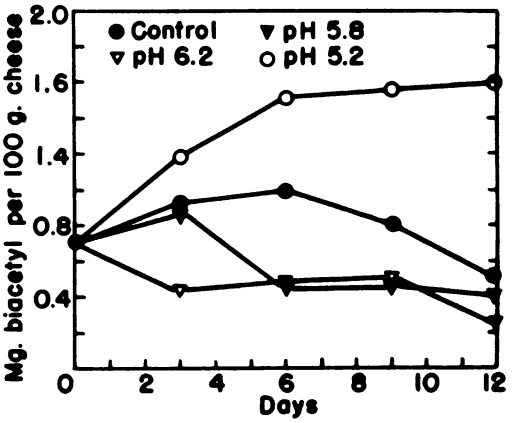

Fig. 12 Wash waters acidulated to pH 6.2, 5.8 and 5.2 with sorbic acid.

Effect of using wash water containing citric, lactic or sorbic acid on the biacetyl content of creamed cottage cheese stored 12 days at 50°F.

to pH 5.2 with sorbic acid had a pH value of 4.80 on the twelfth day (Table 1) as compared to 4.40 for the control on the twelfth day.

Effect of Various Organic Acids Used to Acidulate Wash Waters on the Acetylmethylcarbinol Content of Cottage Cheese

The initial acetylmethylcarbinol values for fresh cottage cheese ranged from 1.5 to 5.0 milligrams per 100-gram sample with the average ranging from 3.2 to 3.8 milligrams. Acetylmethylcarbinol values tended to increase considerably during the storage period and usually attained a maximum on the sixth or ninth days. The values tended to be lower on the twelfth day.

(a) Influence of organic acids on the acetylmethylcarbinol content of cottage cheese stored at 40° F. The acetylmethylcarbinol contents of cottage cheese, washed with waters acidified to pH 6.0, 5.5, and 5.0 with citric or lactic acid and to pH 6.2, 5.8, and 5.2 with sorbic acid and stored twelve days at 40° F., are shown in Figures 13 through 18. The acetylmethylcarbinol content of the control increased from the initial value of 3.6 milligrams to a maximum of 5.95 milligrams per 100-gram sample on the sixth day and on the twelfth day decreased to approximately the original value.

The acetylmethylcarbinol contents of the cheese washed with waters adjusted to pH 6.0 are illustrated in Figure 18. The

acetylmethylcarbinol was highest on the sixth day in the cheese washed with water containing lactic acid, but decreased more abruptly on the ninth day than did the control. When washed with waters acidulated with citric acid, the cheese reached a maximum acetylmethylcarbinol content on the ninth day and remained higher than the control on the twelfth day.

The use of lactic acid in the wash waters, as shown in Figures 14 and 15, enormously increased the acetylmethylcarbinol content of the cheese. Maximum values of 8.6 and 7.7 milligrams per 100-gram sample, respectively, were attained on the third day and the acetylmethylcarbinol content remained above the control until the twelfth day, at which time it was slightly lower. Cheese washed with waters containing citric acid reached a maximum acetylmethylcarbinol content later than the corresponding control but these acetylmethylcarbinol values did not decrease as rapidly as the other samples, including the control.

The acetylmethylcarbinol content of the samples which are grouped according to the acid used in the wash waters (Figures 16, 17, and 18) show a conformity between the acetylmethylcarbinol contents at the three pH values produced by each acid. The data in Figure 16 show that the acetylmethylcarbinol content of all samples washed with waters containing citric acid reached a

maximum on the ninth day and decreased slightly on the twelfth day. The acetylmethylcarbinol content of cheese washed with waters containing lactic acid (Figure 17) increased significantly above that of the control. The acetylmethylcarbinol contents of cheese washed with waters acidified with sorbic acid (Figure 18) were below the level of the corresponding control.

A comparison of data in Figures 4, 5, and 6 with data in Figures 16, 17, and 18, respectively, indicates a general relationship between biacetyl and acetylmethylcarbinol in the cheese samples. There was a tendency for high biacetyl values to be associated with high acetylmethylcarbinol values and for low biacetyl values to be associated with low acetylmethylcarbinol values.

(b) Influence of organic acids on the acetylmethylcarbinol content of cottage cheese stored at 50° F. The data for the acetylmethylcarbinol content in cottage cheese samples stored at 50° F. are presented in Figures 19 to 24, inclusive. These graphs show that the sample washed with water acidified to pH 5.2 with sorbic acid (Figure 21) had a much higher acetylmethylcarbinol content than the control. This is the only sample in which the acetylmethylcarbinol content exceeded the control. Generally, the acetylmethylcarbinol content of cheese samples stored at 50° tended to be lower than that of the corresponding sample stored at 40° F.

An examination of data in Figures 22, 23, and 24 shows a consistent relationship between the acetylmethylcarbinol content of the cheese samples washed with waters containing three different amounts of each acid. The acetylmethylcarbinol contents of all samples washed with waters containing citric acid (Figure 22) were lower than the control; these values were maximum the sixth day and decreased thereafter. An extremely close relationship is apparent among the acetylmethylcarbinol contents of cheese washed with waters acidulated with lactic acid (Figure 23). These acetylmethylcarbinol values decreased on the third day, increased sharply on the sixth day, decreased abruptly on the ninth day, and increased on the twelfth day. The acetylmethylcarbinol contents of the cheese washed with waters acidified to pH 6.2 and 5.8 with sorbic acid (Figure 24) decreased on the third day and remained well below the control. The acetylmethylcarbinol content of the sample washed with water adjusted to pH 5.2 with sorbic acid remained above the control for the duration of the storage period and was 4.3 milligrams per 100-gram sample higher than the control on the twelfth day.

A comparison of data in Figures 10, 11, and 12 with data in Figures 22, 23, and 24, respectively, shows a general relationship between biacetyl and acetylmethylcarbinol contents of corresponding samples stored at 50° F. High acetylmethylcarbinol values seemed

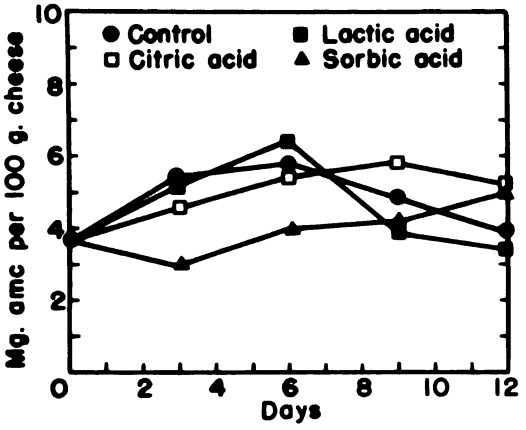

Fig. 13 Wash waters acidulated to pH 6.0 with citric and lactic acid and to pH 6.2 with sorbic acid.

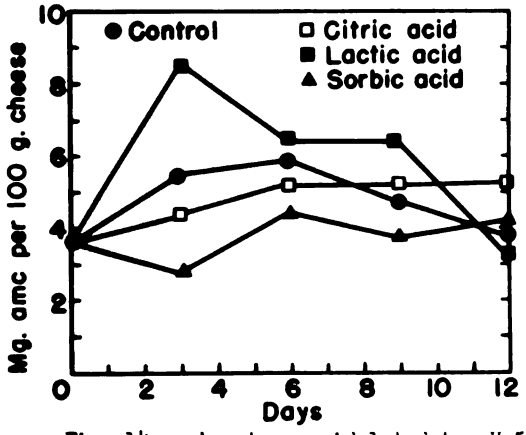

Fig. 14 Wash waters acidulated to pH 5.5 with citric and lactic acids and to pH 5.8 with sorbic acid.

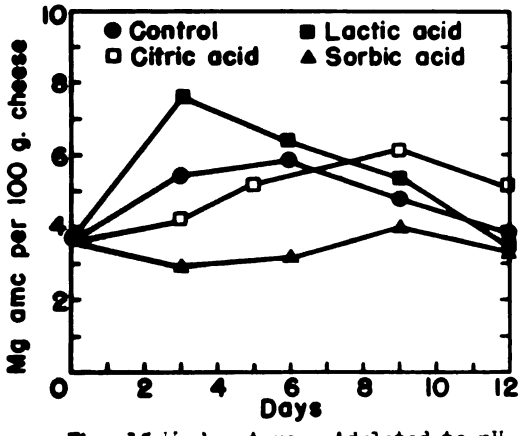

Fig. 15 Wash waters acidulated to pH 5.0 with citric and lactic acids and to pH 5.2 with sorbic acid.

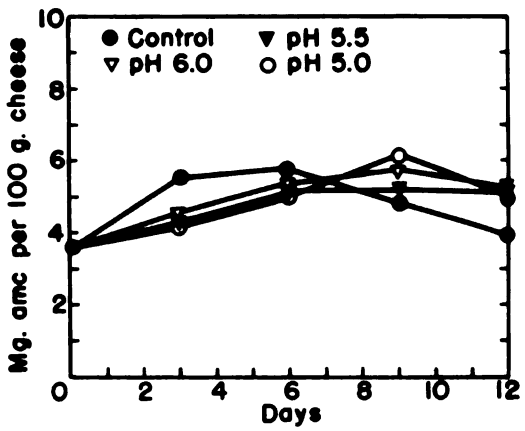

Fig. 16 Wash waters acidulated to pH 6.0, 5.5 and 5.0 with citric acid.

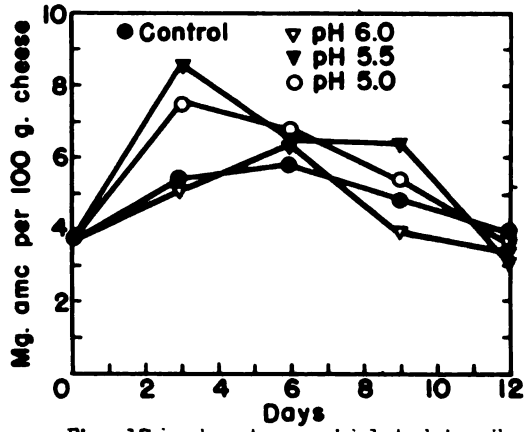

Fig. 17 Wash waters acidulated to pH 6.0, 5.5 and 5.0 with lactic acid.

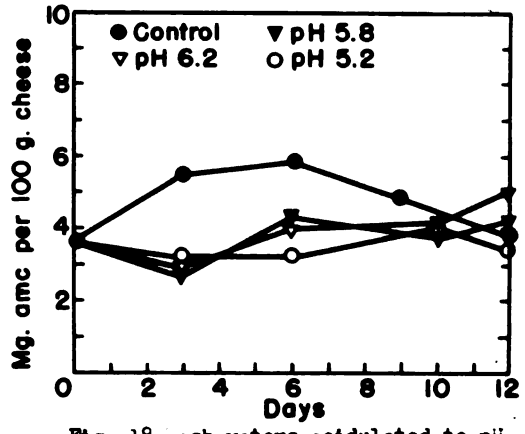

Fig. 18 Wash waters acidulated to pH 6.2, 5.8 and 5.2 with sorbic acid.

Effect of using wash water containing citric, lactic or sorbic acid on the acetylmethylcarbinol content of creamed cottage cheese stored 12 days at 40°F.

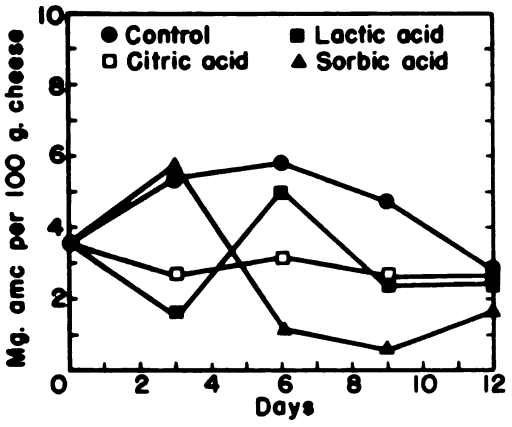

Fig. 19 Wash waters acidulated to pH 6.0 with citric and lactic acids and to pH 6.2 with sorbic acid.

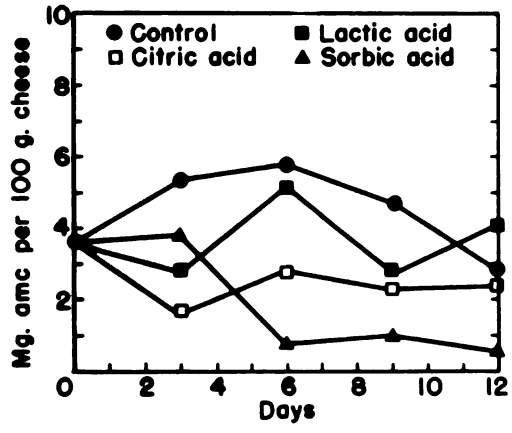

Fig. 20 Wash waters acidulated to pH 5.5 with citric and lactic acids and to pH 5.8 with sorbic acid.

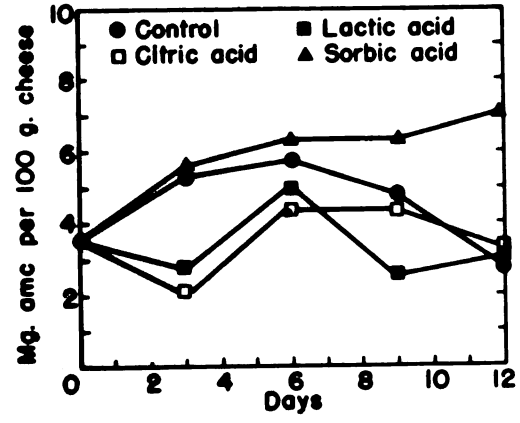

Fig. 21 Wash waters acidulated to pH 5.0 with citric and lactic acids and to pH 5.2 with sorbic acid.

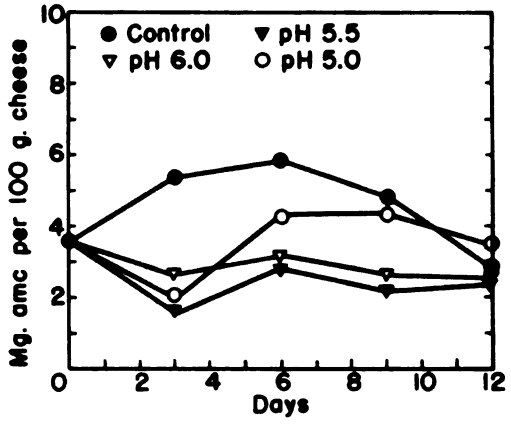

Fig. 22 Wash waters acidulated to pH 6.0, 5.5 and 5.0 with citric acid.

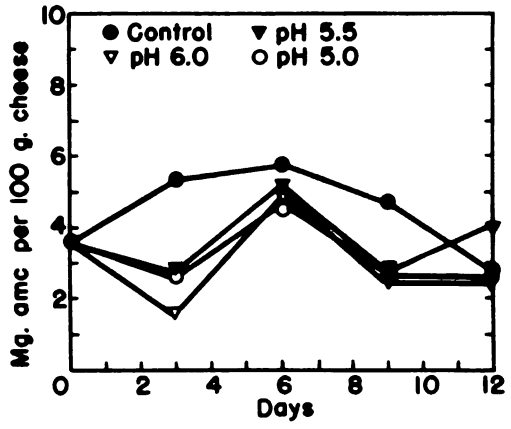

Fig. 23 Wash waters acidulated to pH 6.0, 5.5 and 5.0 with lactic acid.

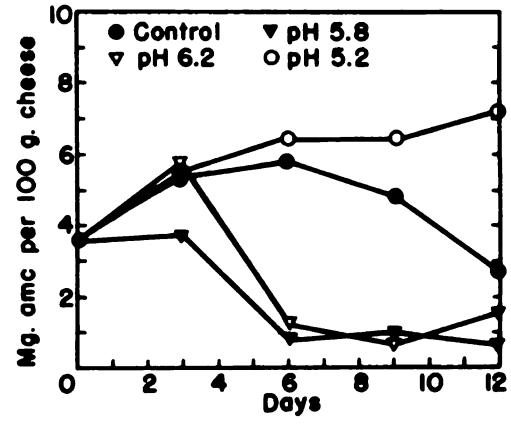

Fig. 24 Wash waters acidulated to pH 6.2, 5.8 and 5.2 with sorbic acid.

Effect of using wash water containing citric, lactic or sorbic acid on the acetylmethylcarbinol content of creamed cottage cheese stored 12 days at 50°F.

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to occur in samples with high biacetyl contents and low acetylmethylcarbinol values seemed to occur in samples with low biacetyl contents.

Destruction of Biacetyl by Microorganisms in Creamed Cottage Cheese

(a) Destruction of biacetyl in cottage cheese stored at 40° F.

The population of microorganisms inoculated into cottage cheese creaming mixtures is shown in Table 3. Counts ranged from 300 per milliliter in cream inoculated with Mucor plumbeus to 450,000 per milliliter in cream inoculated with E. coli.

The effect of microorganisms on the biacetyl content of cottage cheese stored twelve days at 40° F. is shown by data in Figures 25 through 30. The cheese sample inoculated with Ach. butyri (Figure 29) is the only one in which the maximum biacetyl content of an inoculated sample exceeds that of the control. An examination of all data presented in Figures 25 through 30 indicates that the cheese samples inoculated with G. candidum and Pen. frequentans (Figure 25), Ps. desmolyticum, Ps. fluorescens, and Ps. tralucida (Figure 26), T. candida (Figure 28), E. freundii (Figure 29), and Alc. metalcaligenes (Figure 30) achieved a maximum biacetyl content on the sixth day, were much lower on the ninth day and increased on the twelfth day.

The biacetyl content of cheese inoculated with Ps. fragi (Figure 26) and R. flava (Figure 28) reached a maximum on the third day and decreased continuously thereafter. The biacetyl content of cheese inoculated with M. flavus, M. conglomeratus, and M. candidus (Figure 26), Mucor plumbeus (Figure 25), Bact. erythrogenes, C. filamentosum, and B. firmus (Figure 30) did not vary significantly from the biacetyl content of the control.

Increases or decreases in pH values (Table 4) could not be correlated with increases or decreases in biacetyl content. pH values of all samples stored at 40° F. varied between 5.4 and 4.5 during the sampling period.

(b) Destruction of biacetyl in cottage cheese stored at 50° F. The samples illustrated in Figures 31 to 35, inclusive, were stored at 50° F. Biacetyl determinations were performed on all inoculated samples up to and including the sixth day but were discontinued thereafter as spoilage was evident. Studies of the graphs (Figures 31 to 35) indicate that biacetyl destruction is more rapid and complete at 50° than at 40° F. The biacetyl contents of samples inoculated with G. candidum and Pen. frequentans (Figure 31), Ps. desmolyticum, Ps. fluorescens, and Ps. tralucida (Figure 32), T. candida (Figure 34), E. freundii (Figure 35), and Alc. metalcaligenes

(Figure 36) and stored at 50° F. were substantially below the control throughout the storage period. Attention should be focused on the cheese samples inoculated with the organisms which appeared to reduce the biacetyl content at 40° F. These organisms also appeared to reduce the biacetyl content in samples stored at 50° F. Comparison with the control shows a significantly lower biacetyl content at three and six days in samples inoculated with Mucor plumbeus (Figure 31), Ps. fragi (Figure 32), M. conglomeratus (Figure 33), R. flava (Figure 34), Ach. eurydice, and E. coli (Figure 35). The biacetyl content of cheese inoculated with M. flavus and M. candidus (Figure 33), C. filamentosum, Bact. erythrogenes, and B. firmus (Figure 36) did not vary significantly from that of the control. All organisms which did not appear to reduce biacetyl in samples stored at 50° F., did not appear to reduce biacetyl in samples stored at 40° F.

The pH values of all samples stored at 50° F. varied between 5.2 and 4.5 regardless of sampling interval (Table 4). High or low pH values could not be correlated with high or low biacetyl contents.

Destruction of Acetylmethylcarbinol by Microorganisms in Cottage Cheese

(a) Destruction of acetylmethylcarbinol in cottage cheese stored at 40° F. Data showing the acetylmethylcarbinol content of

Table 3. Microorganism population inoculated into the cottage cheese creaming mixture.

Organism Inoculated into the Creaming Mixture	Plate Count Per Ml. When Added to Cottage Cheese
Bacteria:	
Ach. butyri	50,000
Ach. eurydice	33,000
Alc. metalcaligenes	150,000
B. firmus	77,000
Bact. erythrogenes	78,000
C. filamentosum	107,000
E. coli	450,000
E. freundii	400,000
M. candidus	200,000
M. conglomeratus	140,000
M. flavus	340,000
Ps. desmolyticum	186,000
Ps. fragi	270,000
Ps. fluorescens	246,000
Ps. tralucida	400,000
Molds:	
G. candidum	2,500
Mucor plumbeus	300
Pen. frequentans	6,000
Yeasts:	
R. flava	400
T. candida	2,000

Table 4. pH of cottage cheese inoculated with twenty microorganisms associated with cottage cheese spoilage under different storage conditions.

Organisms Inoculated into Creaming Mixture	The pH of Inoculated Cottage Cheese at Various Storage Times and Temperatures						
	0 da.	3 da.		6 da.		9 da.	12 da.
		40° F.	50° F.	40° F.	50° F.	40° F.	40° F.
Control	4.85	5.00	5.10	4.70	4.68	4.70	4.65
Bacteria:							
Ach. butyri	4.85	4.85	4.75	4.90	4.70	5.30	5.02
Ach. eurydice	4.85	5.10	5.20	4.73	4.75	4.62	4.65
Alc. metalcaligenes	4.85	4.82	4.73	5.05	5.00	5.35	4.95
B. firmus	4.85	5.00	5.05	4.78	4.70	4.55	4.60
Bact. erythrogenes	4.85	4.75	4.85	5.04	4.70	5.25	4.85
C. filamentosum	4.85	4.80	4.82	5.08	4.73	5.28	4.90
E. coli	4.85	5.10	5.10	4.70	4.80	4.75	4.60
E. freundii	4.85	4.78	4.80	5.02	4.75	5.24	4.88
M. candidus	4.85	4.85	4.80	5.05	4.75	5.30	5.00
M. conglomeratus	4.85	5.05	5.00	4.75	4.50	4.72	4.65
M. flavus	4.85	5.00	5.05	4.70	4.72	4.60	4.58
Ps. desmolyticum	4.85	4.74	4.80	5.02	4.80	5.42	5.02
Ps. fragi	4.85	5.00	5.05	4.70	4.80	4.65	4.60
Ps. fluorescens	4.85	4.98	5.15	4.85	4.80	4.72	4.65
Ps. tralucida	4.85	4.80	4.78	5.08	4.85	5.28	4.90
Molds:							
G. candidum	4.85	5.05	5.00	4.80	4.52	4.50	4.60
Mucor plumbeus	4.85	5.00	5.10	4.75	4.68	4.60	4.80
Pen. frequentans	4.85	4.80	4.68	5.10	4.68	5.25	4.78
Yeasts:							
R. flava	4.85	5.10	5.10	4.80	4.80	4.60	4.58
T. candida	4.85	4.75	4.82	5.05	4.70	5.05	4.85

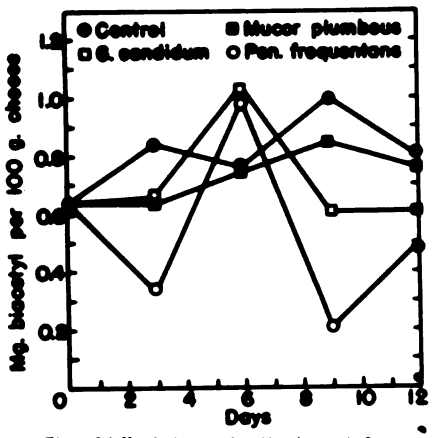

Fig. 25 Variations in the biacetyl content of creamed cottage cheese inoculated with G. candidum, Mucor plumbeus or Pen. frequentans.

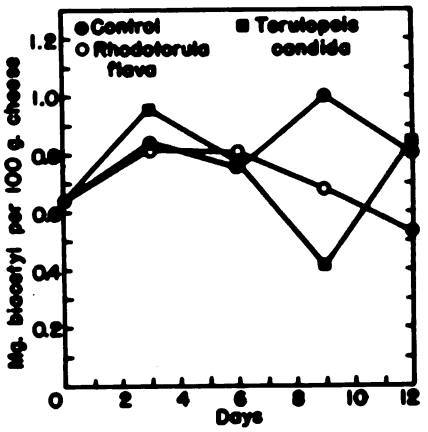

Fig. 28 Variations in the biacetyl content of creamed cottage cheese inoculated with R. flava or T. candida.

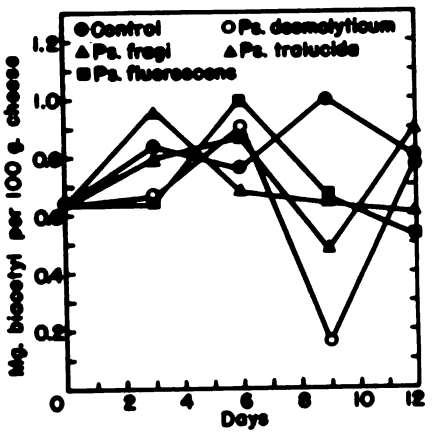

Fig. 26 Variations in the biacetyl content of creamed cottage cheese inoculated with Ps. fragi, Ps. fluorescens, Ps. desmolyticum or Ps. tralucida.

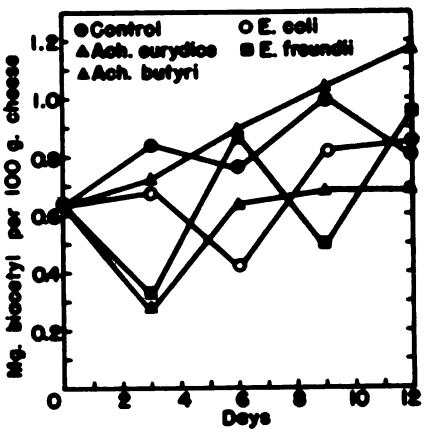

Fig. 29 Variations in the biacetyl content of creamed cottage cheese inoculated with Ach. eurydice, Ach. butyri, E. coli or E. freundii.

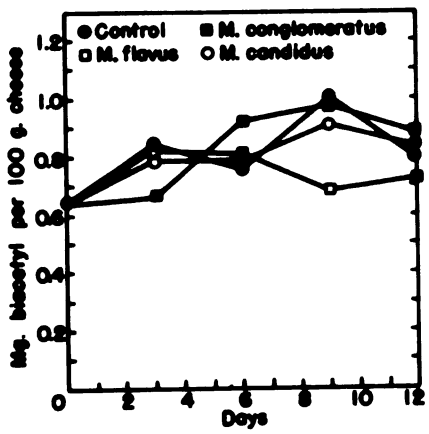

Fig. 27 Variations in the biacetyl content of creamed cottage cheese inoculated with M. flavus, M. conglomeratus or M. candidus.

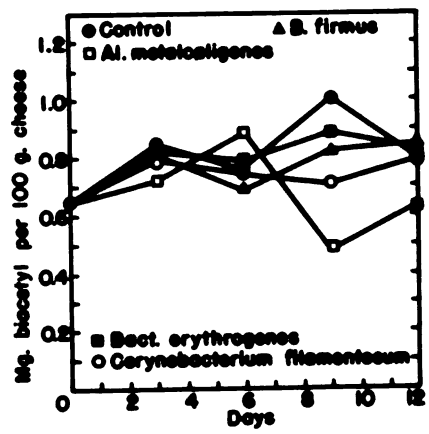

Fig. 30 Variations in the biacetyl content of creamed cottage cheese inoculated with Alc. metalcaligenes, C. filamentosum Bact. erythrogenes or B. firmus.

Effect of microorganisms on the biacetyl content of creamed cottage cheese stored 12 days at 40°F.

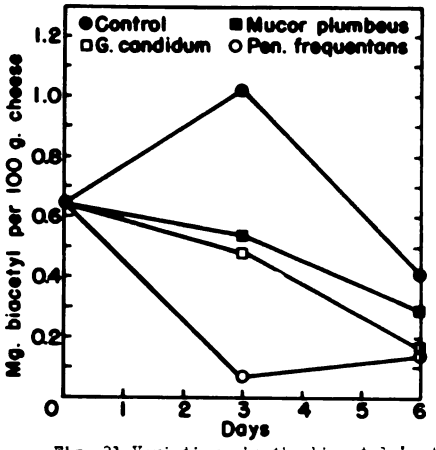

Fig. 31 Variations in the biacetyl content of creamed cottage cheese inoculated with G. candidum, Mucor plumbeus or Pen. frequentans.

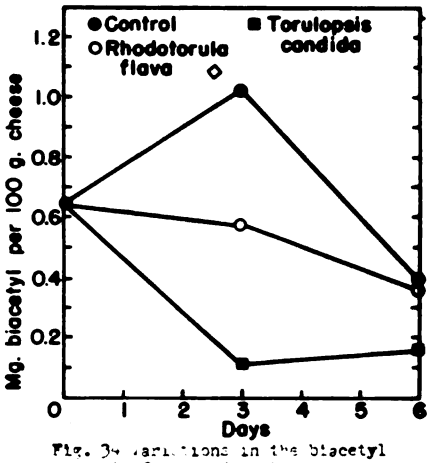

Fig. 34 Variations in the biacetyl content of creamed cottage cheese inoculated with R. flava or T. candida.

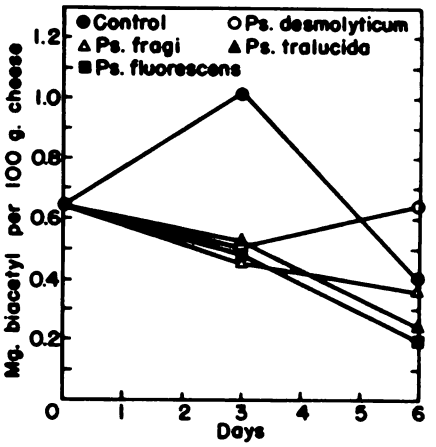

Fig. 32 Variations in the biacetyl content of creamed cottage cheese inoculated with Ps. fragi Ps. fluorescens, Ps. fraucida or Ps. desmolyticum.

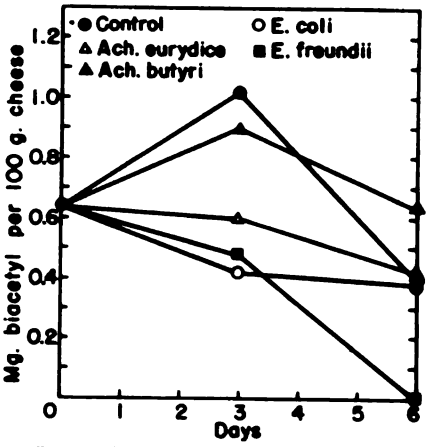

Fig. 35 Variations in the biacetyl content of creamed cottage cheese inoculated with Ach. eurydice, Ach. butyri, E. coli or E. freundii.

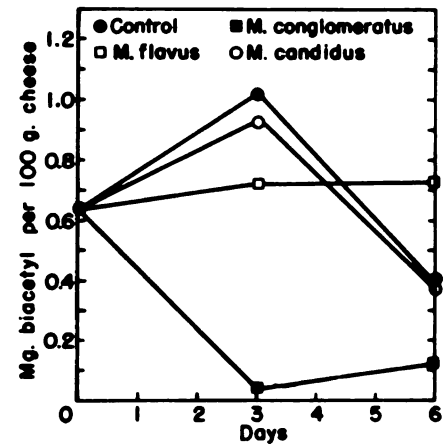

Fig. 33 Variations in the biacetyl content of creamed cottage cheese inoculated with M. flavus, M. conglomeratus or M. candidus.

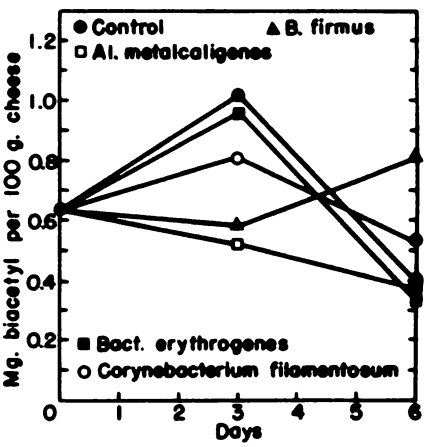

Fig. 36 Variations in the biacetyl content of creamed cottage cheese inoculated with Alc. metalcaligenes, C. filamentosum Bact. erythrogenes or B. firmus.

Effect of microorganisms on biacetyl content of creamed cottage cheese stored 6 days at 50°F.

cottage cheese inoculated with various organisms and stored at 40° F. are presented in Figures 37 to 42, inclusive. The acetylmethylcarbinol content of the control fluctuated slightly with the final value being only slightly lower than the initial value. Cheese inoculated with Pen. frequentans (Figure 37), Ps. desmolyticum (Figure 38), and Alc. metalcaligenes (Figure 42) were the only samples in which the acetylmethylcarbinol content decreased uniformly. The acetylmethylcarbinol content of cheese samples inoculated with the majority of the remaining organisms was approximately the same as the control. Reduced acetylmethylcarbinol contents were noted at three days in cheese inoculated with Mucor plumbeus (Figure 37) and at nine days in cheese inoculated with B. firmus (Figure 42) and Ach. eurydice (Figure 41). An increased acetylmethylcarbinol content was evident at nine days in the sample inoculated with T. candida (Figure 40).

(b) Destruction of acetylmethylcarbinol in cottage cheese stored at 50° F. The acetylmethylcarbinol contents of the samples stored at 50° F. are shown in Figures 43 through 48. The acetylmethylcarbinol content of the samples inoculated with Pen. frequentans (Figure 43), Ps. desmolyticum and Ps. tralucida (Figure 44), M. flavus and M. candidus (Figure 45), T. candida (Figure 46), E. freundii (Figure 47), Alc. metalcaligenes, B. firmus, and Bact.

erythrogenes (Figure 48) did not vary significantly from the control. The acetylmethylcarbinol content of samples inoculated with Ps. fragi and Ps. fluorescens (Figure 44), R. flava (Figure 46), Ach. eurydice (Figure 47), and C. filamentosum (Figure 48) decreased uniformly during the sampling period. The acetylmethylcarbinol content of the control decreased on the third day and increased on the sixth day. The acetylmethylcarbinol contents of samples inoculated with G. candidum and Mucor plumbeus (Figure 43) and M. conglomeratus (Figure 45) were higher at three days than the control but decreased below the control on the sixth day. The acetylmethylcarbinol content of the sample inoculated with E. coli (Figure 47) increased tremendously to 21.6 milligrams per 100-gram sample on the sixth day. The cheese sample inoculated with Ach. butyri (Figure 47) also had an acetylmethylcarbinol content greater than the control on the third and sixth days.

The Biacetyl Content of Cottage Cheese as Affected by Adding Organic Acids and Starter to the Creaming Mixture

The biacetyl contents of cheese samples which contained additions of 0.1 percent citric, sorbic, or lactic acid or 1.0 percent commercial starter to the creaming mixture, are shown in Figures 49 and 50. The additions to the creaming mixture did not affect the

biacetyl content of samples stored at 40° F. (Figure 49). The biacetyl contents of all samples were approximately the same throughout the holding interval.

When stored at 50° F., the biacetyl content of the sample containing 1.0 percent commercial starter was 0.9 milligrams per 100-gram sample higher than the control on the twelfth day. The biacetyl contents of all other samples were approximately the same as the control. The biacetyl content of the cheese sample containing starter increased as the pH decreased within the range observed (Table 5). At three days, with a pH of 4.40, this cheese was not marketable because of the extremely high acid content. The pH values of all other samples, stored both at 40° and 50° F., ranged between 4.95 and 4.63 except the control on the twelfth day which was 4.45.

Table 5. pH of creamed cheese containing 1.0 percent commercial starter or 0.1 percent lactic, citric, or sorbic acid added to the creaming mixture, under different storage conditions.

Additives to the Creaming Mixture	pH Values of Cheese Containing Additives to the Creaming Mixture at Various Storage Times and Temperatures								
	0 da.	3 da.		6 da.		9 da.		12 da.	
		40° F.	50° F.	40° F.	50° F.	40° F.	50° F.	40° F.	50° F.
Control	4.90	4.85	4.80	4.85	4.85	4.80	4.95	4.83	4.45
0.1% citric acid .	4.90	4.80	4.73	4.78	4.83	4.72	4.95	4.79	4.92
0.1% lactic acid .	4.90	4.78	4.75	4.75	4.85	4.63	4.65	4.78	4.78
0.1% sorbic acid	4.90	4.78	4.70	4.80	4.85	4.71	4.71	4.80	4.88
1.0% starter . .	4.90	4.80	4.40	4.82	4.38	4.68	4.35	4.82	4.40

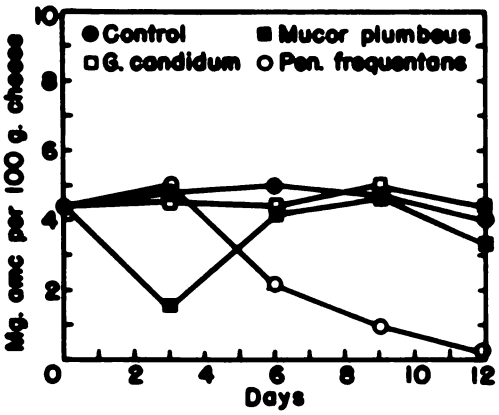

Fig. 37 Variations in the amc content of creamed cottage cheese inoculated with G. candidum, Mucor plumbeus or Pen. frequentans.

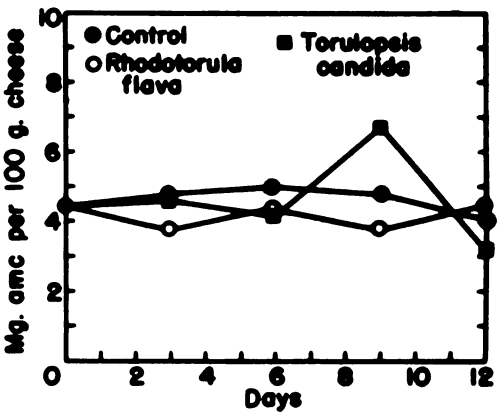

Fig. 40 Variations in the amc content of creamed cottage cheese inoculated with R. flava or T. candida.

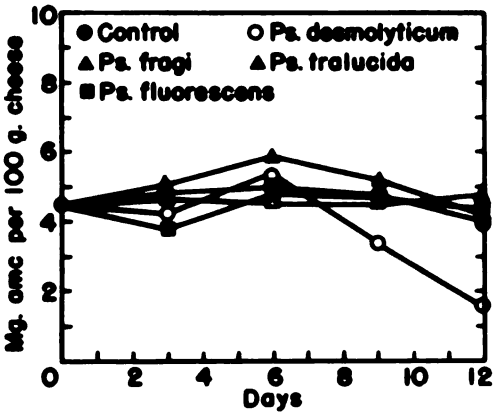

Fig. 38 Variations in the amc content of creamed cottage cheese inoculated with Ps. fragi, Ps. fluorescens, Ps. tralucida or Ps. desmolyticum.

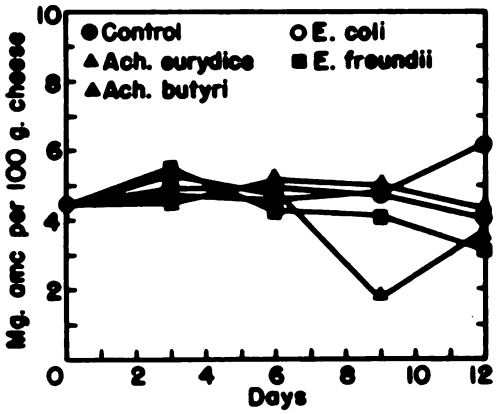

Fig. 41 Variations in the amc content of creamed cottage cheese inoculated with Ach. eurydice, Ach. butyri, E. coli or E. freundii.

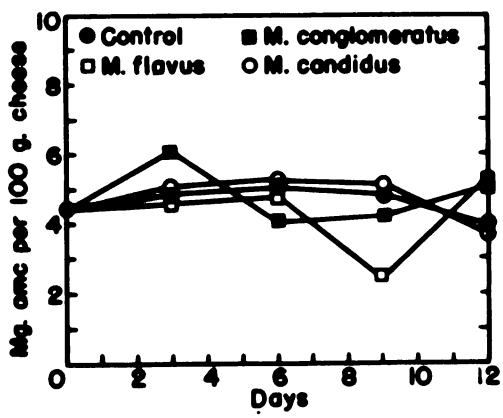

Fig. 39 Variations in the amc content of creamed cottage cheese inoculated with M. flavus, M. conglomeratus or M. candidus.

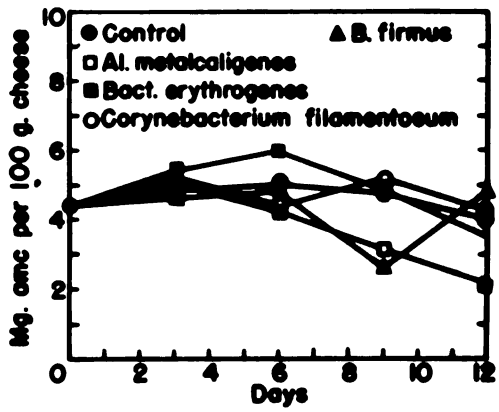

Fig. 42 Variations in the amc content of creamed cottage cheese inoculated with Alc. metalcaligenes, C. filamentosum, Bact. erythrogenes or B. firmus.

Effect of microorganisms on acetylmethylcarbinol content of creamed cottage cheese stored 12 days at 40°F.

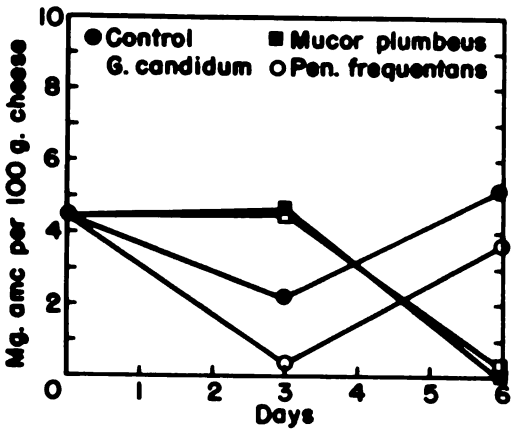

Fig. 43 Variations in the amc content of creamed cottage cheese inoculated with G. candidum, Mucor plumbeus or Pen. frequentans.

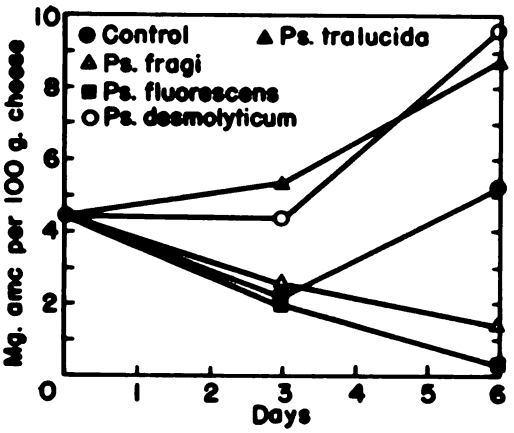

Fig. 44 Variations in the amc content of creamed cottage cheese inoculated with Ps. fragi, Ps. fluorescens, Ps. tralucida or Ps. desmolyticum.

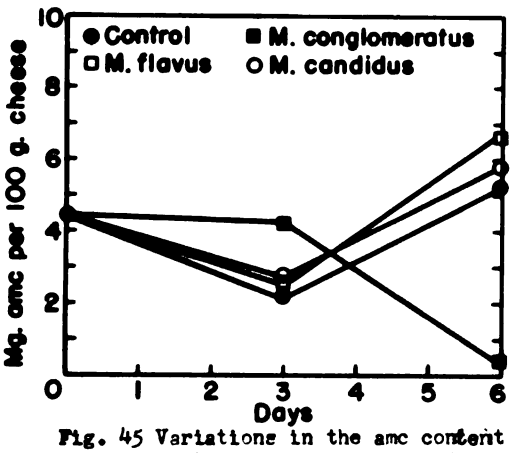

Fig. 45 Variations in the amc content of creamed cottage cheese inoculated with M. flavus, M. conglomeratus or M. candidus.

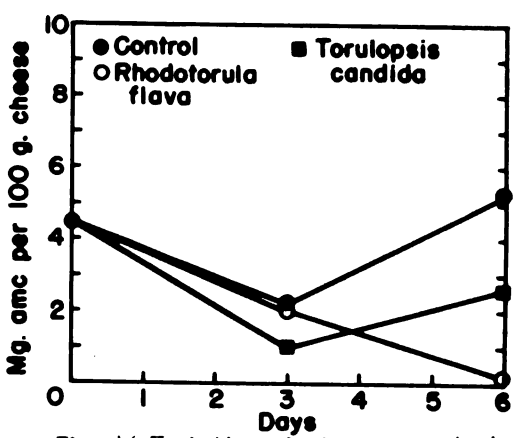

Fig. 46 Variations in the amc content of creamed cottage cheese inoculated with R. flava or T. candida

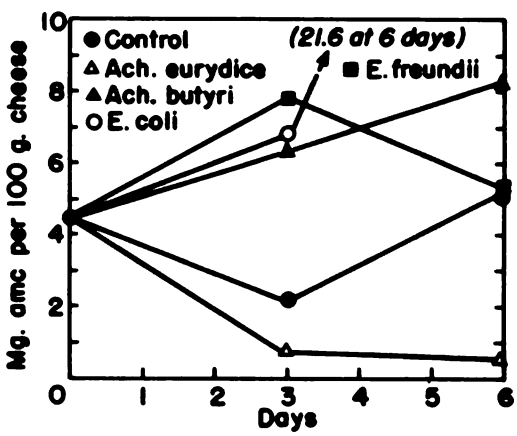

Fig. 47 Variations in the amc content of creamed cottage cheese inoculated with Ach. eurydice, Ach. butyri, E. coli or E. freundii.

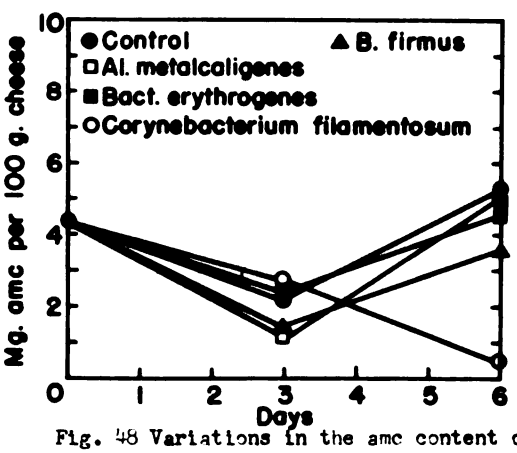

Fig. 48 Variations in the amc content of creamed cottage cheese inoculated with Alc. metalcaligenes, C. filamentosum Bact. erythrogenes or B. firmus.

Effect of microorganisms on acetylmethylcarbinol content of creamed cottage cheese stored 6 days at 50°F.

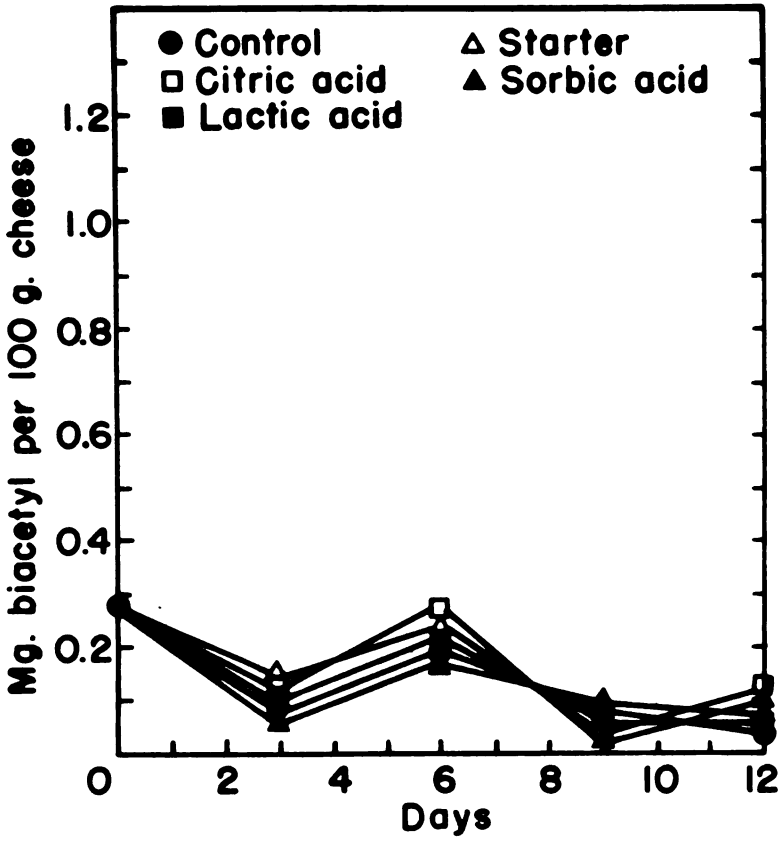

Fig. 49 Cheese stored at 40°F.

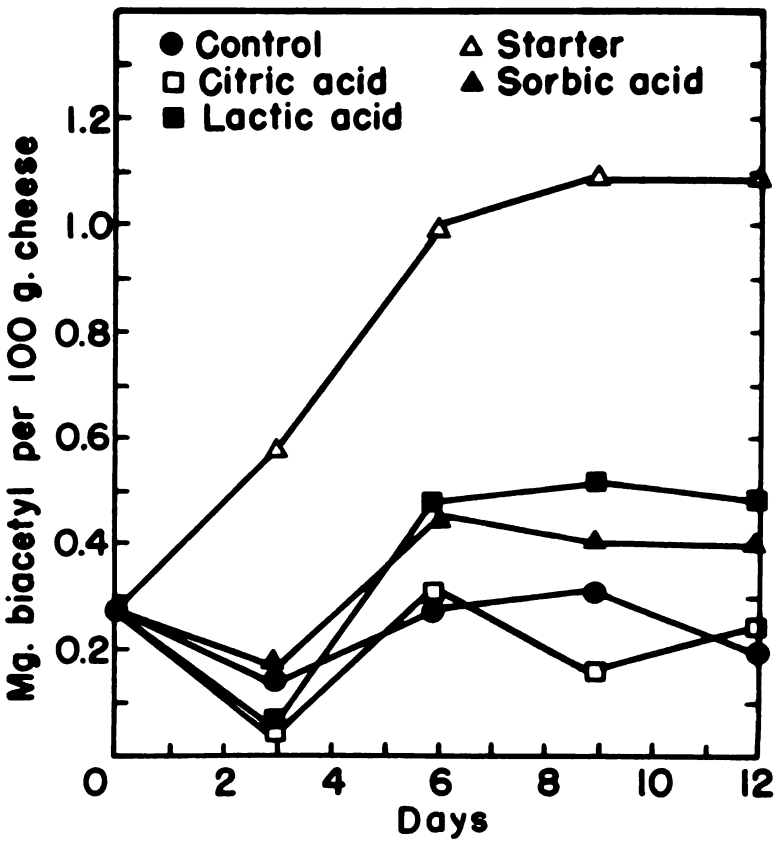

Fig. 50 Cheese stored at 50°F.

Variations in the biacetyl content of creamed cottage cheese stored 12 days when 1.0 percent commercial starter, or 0.1 percent citric, lactic or sorbic acids were added to the creaming mixture.

DISCUSSION

Effect of Various Organic Acids Used to Acidulate Cottage Cheese Wash Waters on the Biacetyl and Acetylmethylcarbinol Content of the Cottage Cheese

The initial biacetyl values of fresh cottage cheese analyzed in this experiment usually ranged from 0.3 to 0.8 milligrams per 100-gram sample (3 to 8 parts per million) and most samples were within the range of 0.4 to 0.6 milligrams (4 to 6 parts per million). These results are somewhat higher than those obtained by Krishnaswamy and Babel (1951) who reported 1.06 to 2.25 parts per million of biacetyl in cottage cheese curd. In results not reported herein, initial biacetyl values ranged from 0.07 to 1.04 milligrams per 100-gram sample (0.7 to 10.4 parts per million) however, extremely high and low values were seldom observed.

The biacetyl and acetylmethylcarbinol content of cottage cheese usually increased when lactic acid was used to acidify cottage cheese wash waters. When the cheese was stored at 40° F., all samples washed with waters containing lactic acid attained a biacetyl and acetylmethylcarbinol content significantly higher than the control. The rate of biacetyl and acetylmethylcarbinol production was much greater than the control on the third day, but was

only slightly higher on the sixth, ninth, and twelfth days. However, in the samples washed with lactic acid, the early increase in rate of biacetyl and acetylmethylcarbinol plus the continued production of these compounds at a rate nearly equal to the control, maintained biacetyl and acetylmethylcarbinol quantities above the control. Addition of lactic acid to the wash water appeared to increase the biacetyl and acetylmethylcarbinol content of the cheese stored at 40° F. just as any additional intermediate compound would tend to increase the total end product of a fermentation. These results are supported by Virtanen and Kontio (1941), Evenhuis et al. (1952) and Folke Bange (1943a, 1943b, 1944a, 1944b, 1945) who reported that lactic and pyruvic acids are intermediate products of the citric acid and lactose fermentation by Leuconostoc organisms. Pyruvic and lactic acids are closely related, being interchangeable through a reversible oxidation-reduction reaction.

The addition of lactic acid to the wash waters did not increase the biacetyl and acetylmethylcarbinol content of cheese stored at 50° F. Biacetyl and acetylmethylcarbinol contents of the samples stored at 50° decreased more rapidly and completely than those of the samples stored at 40° F. Also, at the same sampling interval, the pH values of all samples stored at 50° F. were lower than those of the corresponding sample stored at 40° F. Apparently organism

activity was much higher at 50° than 40° F. This increased organism activity probably resulted in greater destruction or utilization of biacetyl by organism metabolism. Any increase in biacetyl and acetylmethylcarbinol which may have occurred as a result of added lactic acid was probably offset by the increased destruction of these flavor components.

No significant increase in biacetyl content was found in the samples washed with waters containing citric acid. Other investigators who worked with butter and butter cultures reported increased biacetyl when citric acid was added. Farmer and Hammer (1931), Ritter and Christen (1935), Ruehe (1937), Prill and Hammer (1939), and Gehrke and Weiser (1938), all obtained large increases in biacetyl content when they added citric acid to butter cultures. Nelson and Brence (1953) added citric acid to cottage cheese and obtained increased volatile acidity and improved flavor, but they did not determine acetylmethylcarbinol and biacetyl. The quantity of citric acid that remained in the cottage cheese after draining may have been insufficient to permit an increase in biacetyl.

The high biacetyl and acetylmethylcarbinol values attained in the sample washed with water adjusted to pH 5.2 and 0.25 percent sorbic acid and stored at 50° F., were attributed to the ability of sorbic acid to inhibit fermentations which destroy biacetyl. The

amount of sorbic acid remaining after drainage, in the cheese washed with waters adjusted to pH 6.2 and 5.8, may have been insufficient to inhibit the fermentation which destroys biacetyl. The pH values of the samples washed with waters containing sorbic acid and stored at 50° F., did not decrease as low as the control. The greater the concentration of sorbic acid, the less the pH of the cheese decreased. These results indicate that the bacteriological agent producing the acid in the cheese was inhibited by sorbic acid. Sorbic acid did not appear to inhibit production of biacetyl. Leu. citrovorous and/or Leu. dextranicum tolerated all concentrations (0.05 to 0.25 percent) or sorbic acid added to the cheese wash water. When stored at 40° F., the cheese samples containing sorbic acid showed no inhibition of the fermentation which destroyed biacetyl. The results suggested that the microorganisms causing this fermentation were not active at 40° F.

Organoleptic quality was proportional to biacetyl values in only a few of the cheese samples. Some samples with high biacetyl contents also had a high flavor score. However, this was not always true; several samples containing relatively high biacetyl contents had poor organoleptic quality. This was particularly true in high acid cheese. Occasionally, high biacetyl values were found in low-scoring cheese samples during the last three days of storage;

especially samples having stale, yeasty, and fruity off-flavors. These results indicate that high biacetyl values do not necessarily denote high scoring cottage cheese. However, there was a definite correlation between flat flavor and low biacetyl values. All cheese samples criticized as flat had low biacetyl contents and no sample with a high biacetyl content was criticized as flat. These results concur with those of Wiley et al. (1939) who stated that biacetyl content is not a sure guide to flavor because of off-flavors which may be present. Parker and Elliker (1953) stated that cheese criticized as high acid frequently had a high biacetyl content.

The pH values of the cheese decreased slowly and regularly until visible spoilage defects appeared; thereafter the pH increased as spoilage progressed during the period observed. Cheese stored at 50° had lower pH values at the same sampling interval and spoiled earlier than the corresponding samples stored at 40° F. The decrease in pH values was attributed to acid production by the natural lactic fermentation. When the organisms causing spoilage overgrew the normal lactic fermentation, the pH frequently increased. Davis and Babel (1954) noted that cottage cheese at pH 4.7 developed slime and as slime formed, the pH of the slimy portion increased. The results indicate that a storage temperature of 40° F. is much

more desirable than 50° F. for maintaining a high level of biacetyl and acetylmethylcarbinol.

The data suggest a general relationship between biacetyl and acetylmethylcarbinol. The tendency for high and low biacetyl values to be associated with high and low acetylmethylcarbinol values was apparent and has been observed by other workers. Davies (1933), Hammer (1934), and Michaelian et al. (1933) reported a general relationship between acetylmethylcarbinol and biacetyl. Hammer (1934) stated that the acetylmethylcarbinol content of butter was usually ten times greater than the biacetyl content. Results, in this experiment, indicated that the quantity of acetylmethylcarbinol usually is nearly ten times as large as the biacetyl content of the same sample of cheese.

Destruction of Biacetyl and Acetylmethylcarbinol by Microorganisms in Cottage Cheese

The reduced biacetyl contents in samples inoculated with G. candidum, Pen. frequentans, Ps. desmolyticum, Ps. fragi, Ps. fluorescens, Ps. tralucida, T. candida, R. flava, Ach. eurydice, E. freundii, and Alc. metalcaligenes were attributed to destruction or utilization of the biacetyl by these organisms. Reduction of biacetyl occurred at 40° and 50° F., but was more pronounced at 50° F.

Cheese samples inoculated with Mucor plumbeus, M. conglomeratus, and E. coli and stored at 50° F. had lower biacetyl contents than the control. The lower amounts occurring in samples inoculated with M. conglomeratus and E. coli may have been caused by another contaminant present in this particular group of cheese samples. Mucor plumbeus appeared to reduce biacetyl at 50° but not at 40° F. and seemed to grow slowly at 40° F., which may have been the reason biacetyl was not destroyed at the lower temperature. Biacetyl was not completely destroyed in any sample; cheese showing heavy slime formation still contained biacetyl in amounts ranging from 0.04 to 0.8 milligrams per 100-gram sample. Parker and Elliker (1952, 1953) reported that Ps. fragi, Ps. viscosa, and Alc. metalcaligenes destroyed biacetyl and stated that all biacetyl was destroyed prior to the formation of the gelatinous defect associated with these organisms. They stated that disappearance of biacetyl may be a symptom of spoilage in cottage cheese. Elliker (1945) reported that Ps. fluorescens, Ps. fragi, and some unidentified strains of Pseudomonas destroyed biacetyl. Virtanen and Kontio (1941) found that Ps. fluorescens destroyed biacetyl but very little acetylmethylcarbinol.

M. flavus, M. candidus, C. filamentosum, Bact. erythrogenes and B. firmus did not appear to influence the biacetyl content of

cottage cheese. The environmental conditions seemed to reduce the growth rate of these organisms. Results indicated that B. firmus, C. filamentosum, and Bact. erythrogenes were inhibited by the pH encountered in cottage cheese. M. flavus and M. candidus also appeared to have a reduced growth rate.

Acetylmethylcarbinol was not as subject to destruction by organisms as biacetyl; reduction usually occurred during the last half of the storage period. Acetylmethylcarbinol is probably utilized after more available sources of carbon are metabolized. Slight reductions in acetylmethylcarbinol content occurred in samples inoculated with Pen. frequentans, Ps. desmolyticum, and Alc. metalcaligenes and stored at 40° F. Reductions also were noticeable in samples inoculated with Ps. fluorescens, Ps. fragi, R. flava, Ach. eurydice and C. filamentosum, and stored at 50° F. C. filamentosum does not grow well in cottage cheese and the reduced amounts of acetylmethylcarbinol in the sample inoculated with C. filamentosum may have been due to a natural contaminant. The reduced acetylmethylcarbinol content in the samples inoculated with the other organisms mentioned above was attributed to destruction or utilization of the acetylmethylcarbinol by these organisms. The great increase in acetylmethylcarbinol in the sample inoculated with E. coli and stored at 50° F. could not be explained. It is recognized that A.

aerogenes produces acetylmethylcarbinol. The reliability of the identification of the E. coli organism used in this experiment was considered. However, the culture of E. coli used corresponded to the characteristics described in Bergey's Manual (1948) except that no indole was formed and gelatin was liquefied. This organism gave a negative Voges-Proskauer reaction when tested in the laboratory. With the exception of C. filamentosum, all organisms that destroyed acetylmethylcarbinol, also destroyed biacetyl. The abilities of organisms to reduce acetylmethylcarbinol and biacetyl appear to be correlated.

Generally, all organisms associated with cottage cheese spoilage reduced the biacetyl content while organisms not associated with spoilage did not reduce biacetyl. G. candidum, Pen. frequentans, Mucor plumbeus, Ps. tralucida, Ps. fragi, Ps. fluorescens, Ps. desmolyticum, R. flava, T. candida, Alc. metalcaligenes, and Ach. eurydice are associated with cottage cheese spoilage and all appeared to reduce biacetyl. The rate and amount of reduction varied. Samples inoculated with T. candida, Pen. frequentans, and Ps. desmolyticum appeared to have the greatest rate of destruction. The amount of reduction was the most consistent in samples inoculated with Pseudomonas organisms. Samples inoculated with E. freundii, E. coli, M. conglomeratus and Ach. butyri were exceptions to the above

correlation. The biacetyl contents were lower than the control in samples inoculated with E. freundii and stored at both temperatures and those inoculated with M. conglomeratus and E. coli and stored at 50° F., even though these organisms are not associated with cottage cheese spoilage. Ach. butyri causes spoilage of cottage cheese but did not destroy biacetyl. E. coli, E. freundii, and M. conglomeratus were probably inhibited by the low pH of the cheese and any growth which may have occurred was at a substantially reduced rate. The possible presence of a contaminant may be the reason for the lower biacetyl values in samples inoculated with E. coli, E. freundii, and M. conglomeratus.

The pH values could not be correlated with the biacetyl and acetylmethylcarbinol content either in the cheese washed with acidified waters or in the cheese inoculated with microorganisms. Results showed that the pH range which might have resulted in greater biacetyl production was below the pH range encountered in normal cottage cheese. Michaelian et al. (1938) obtained maximum yields of biacetyl in butter cultures at pH 3.7 to 3.9. Cox (1945) stated that biacetyl-producing ability per cell unit increased with decreasing pH between pH 5.5 and 4.4. Results indicated that fresh cheese with a very low pH had a greater biacetyl content than cheese of normal pH.

Biacetyl Content as Affected by Adding Citric, Lactic, or Sorbic Acid or Commercial Starter to the Creaming Mixture

At each sampling interval, the biacetyl content of the cheese containing 1.0 percent commercial starter and stored at 50° F., was substantially above the control. However, due to an extremely low pH the cheese was not marketable at three days. The low pH probably was the result of acid production by Streptococcus lactis and provided an excellent environment for Leuconostoc organisms to produce biacetyl. The biacetyl content of cheese containing additions of 0.1 percent sorbic, lactic, or citric acids to the creaming mixture remained approximately the same as the control during the entire storage period.

SUMMARY AND CONCLUSIONS

This experiment was divided into two parts: (a) an effort to increase the biacetyl and acetylmethylcarbinol content of cottage cheese and (b) a study of destruction of these flavor components by microorganisms. Three concentrations of sorbic, citric, and lactic acids were used to acidify cottage cheese wash water in an effort to increase the biacetyl and acetylmethylcarbinol content of the cheese. One-tenth of one percent of citric, lactic, or sorbic acid, or one percent commercial starter was added to creaming mixtures to determine their effect on the biacetyl and acetylmethylcarbinol content of cottage cheese.

Organisms inoculated into cottage cheese to determine their effect upon the biacetyl and acetylmethylcarbinol were: Pseudomonas fluorescens, Pseudomonas fragi, Pseudomonas desmolyticum, Micrococcus candidus, Achromobacter eurydice, Achromobacter butyri, Escherichia coli, Escherichia freundii, Bacillus firmus, Bacterium erythrogenes, Corynebacterium filamentosum, Alcaligenes metalcaligenes, Geotrichum candidum, Penicillium frequentans, Mucor plumbeus, Rhodotorula flava, and Torulopsis candida. All of these organisms had been isolated from spoiled cottage cheese.

When used to acidify cottage cheese wash waters, lactic acid produced increases in biacetyl and acetylmethylcarbinol in cheese samples stored at 40° but not at 50° F. The increases in biacetyl and acetylmethylcarbinol ranged from 0.10 to 0.50 and 1.1 to 3.1 milligrams per 100-gram sample, respectively (1 to 5 and 11 to 31 parts per million).

Cottage cheese washed with water acidulated to pH 5.2 with sorbic acid attained a biacetyl content of 1.1 milligrams and an acetylmethylcarbinol content of 4.4 milligrams per 100-gram sample, higher than the control after twelve days' storage at 50° F. The substantially greater amount of biacetyl and acetylmethylcarbinol was due to the inhibition, by sorbic acid, of a fermentation that destroyed biacetyl.

Leuconostoc citrovorous and/or Leuconostoc dextranicum grew well in trypticase soy broth containing 0.25 percent sorbic acid and adjusted to pH 4.0.

G. candidum, Pen. frequentans, Ps. fragi, Ps. fluorescens, Ps. desmolyticum, Ps. tralucida, R. flava, T. candida, E. freundii, Ach. eurydice, and Alc. metalcaligenes appeared to destroy biacetyl. Reduction of biacetyl occurred both at 40° and 50° F. but was more pronounced at 50° F. M. flavus, M. candidus, C. filamentosum, Bact.

erythrogenes and *B. firmus* did not cause a decrease in biacetyl in cottage cheese at either temperature.

Biacetyl was not completely destroyed in any sample and cheese showing heavy slime formation still contained biacetyl in amounts ranging from 0.04 to 0.8 milligrams per 100-gram sample.

Acetylmethylcarbinol is not as subject to reduction by organisms as biacetyl. Cottage cheese samples inoculated with *Pen. frequentans*, *Ps. desmolyticum*, and *Alc. metalcaligenes* showed moderate acetylmethylcarbinol reduction, while those inoculated with *Ps. fluorescens*, *Ps. fragi*, *R. flava*, *Ach. eurydice*, and *C. filamentosum* exhibited slight reduction in acetylmethylcarbinol.

The ability to reduce flavor components was closely correlated with the ability to produce spoilage in cottage cheese.

Additions of citric, lactic, and sorbic acid and commercial starter to creaming mixtures did not increase biacetyl or acetylmethylcarbinol contents of the cheese.

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