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Population changes of citratefermenting bacteria in cheddar cheese

Hyun Joong Kim

To the Graduate Council:

I am submitting herewith a thesis written by Hyun Joong Kim entitled "Population changes of citratefermenting bacteria in cheddar cheese." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Husbandry.

W. W. Overcast, Major Professor

We have read this thesis and recommend its acceptance:

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Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

May 20, 1968

To the Graduate Council:

I am submitting herewith a thesis written by Hyun Joong Kim entitled "Population Changes of Citrate-fermenting Bacteria in Cheddar Cheese." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Dairying.

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We have read this thesis and recommend its acceptance:

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POPULATION CHANGES OF CITRATE-FERMENTING BACTERIA IN CHEDDAR CHEESE

A Thesis

Presented to

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In Partial Fulfillment

of the Requirements for the Degree

Master of Science

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by

Hyun Joong Kim

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ABSTRACT

Population changes of citrate-fermenting bacteria in commercial Cheddar cheese and flavor developments of the cheese were studied. Ten freshly made Cheddar cheese were purchased from five different manufacturers and the cheese were examined for citrate-fermenting bacteria at regular monthly intervals during a twelve month curing period. The citrate-fermenting bacterial populations were enumerated on tomato juice agar containing 0.5 per cent calcium lactate and 0.6 per cent colloidal calcium citrate. After three months of curing, the examination included organoleptic tests for the flavor and texture of the cheese.

Eight of ten cheese contained citrate-fermenting bacteria throughout curing in variable numbers and two cheese contained the bacteria until eleven months of curing. The populations of citrate-fermenting bacteria of all cheese decreased during curing. Six of ten cheese developed a bitter flavor during curing. Two of the remaining cheese developed a fermented flavor and the other two a flat flavor. All cheese showed slight to distinct openness in texture when

examined after three months of curing and there was no change in the degree of openness throughout the curing.

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CHAPTER I

INTRODUCTION

Cheddar cheese is the most popular of all cheeses in the United States and, hence, is manufactured and consumed in great quantities. A large portion of the Cheddar cheese is consumed in its natural state, and a considerable amount is utilized as pasteurized, process cheese.

Changes which occur in cheese during the manufacturing and ripening process have intrigued dairy scientists for many years. In modern times cheese starters are used to bring about the acid development during the manufacturing operation and develop desirable flavors during the curing of the cheese. These starters are commonly mixed cultures of selected strains or a single strain of lactic acid-forming bacteria (Streptococcus lactis and Streptococcus cremoris) and certain selected strains or a strain of aroma-producing bacteria which ferment citrates in milk.

The citrate-fermenting bacteria are known to contribute to the flavors of Cottage cheese and cultured buttermilk through production of biacetyl (2,3-butanedione) and other

metabolic products. Therefore, one could expect a similar contribution to the flavor of Cheddar cheese. Likewise these organisms are capable of producing small quantities of gas which has been implicated as a source of slit openness, a texture defect of Cheddar cheese.

The present study was carried out to determine the relationship of changes in population of the citrate-fermenting bacteria and their metabolic activities to the resulting flavor characteristics and textures of Cheddar cheese, made from cultures containing these organisms, occurring throughout a 12 month curing period.

CHAPTER II

REVIEW OF LITERATURE

I. DEFINITIONS

The organisms normally associated with Streptococcus lactis in lactic cultures are typical streptococci which are not distinguishable from Streptococcus lactis microscopically. In pure cultures these organisms often are somewhat smaller than Streptococcus lactis (28). The outstanding characteristic of the organisms is the ability to ferment citrates (4, 17).

The citrate-fermenting bacteria normally present in lactic cultures have been placed in various genera by different investigators. Hammer (26) named the organisms Streptococcus citrovorus and Streptococcus paracitrovorus. Hucker and Pederson (31) suggested that they belong to the genus Leuconostoc and recognized two species, Leuconostoc citrovorum and Leuconostoc dextranicum. This genus was also known as Betacoccus or X-bacteria in early days (28, 31, 35).

Several investigators (22, 59, 67) isolated strains

of streptococci which produce relatively large amounts of lactic acid in milk as well as volatile acid, carbon dioxide, acetylmethylcarbinol (acetoin or 3-hydroxy-2-butanone), and biacetyl. In respect to lactose fermentation, these organisms resemble Streptococcus lactis, but the flavor compounds produced suggest a relationship to the citrate-fermenting streptococci (5, 67). Swartling (59) isolated 35 strains of streptococci which rapidly produce lactic acid in milk and vigorously ferment citric acid with production of acetic acid, carbon dioxide, acetylmethylcarbinol, and biacetyl. The strains showed a tendency to form a homogenous group which resembled Streptococcus lactis in morphology, growth temperature, tolerance to sodium chloride, and carbohydrate fermentation. The organisms differed from Streptococcus lactis in the ability to ferment citrates. This worker suggested that the organisms be given species rank under the name Streptococcus diacetilactis.

Because of differences in nomenclature, common usage refers to all these citrate-fermenting bacteria characteristic of lactic culture as "associates" or the aroma-producing bacteria (17, 28).

II. CITRATE-FERMENTING BACTERIA

Occurrence

Hammer (26) in 1920 found that Streptococcus citrovorus and Streptococcus paracitrovorus were frequently associated with the lactic acid-producing bacteria of dairy cultures. Hammer and Babel (28) stated that the aroma-producing bacteria regularly were present in lactic cultures of satisfactory flavor quality.

Abd-El-Malek and Gibson (1) isolated Streptococcus kefir from 19 of 23 samples of raw milk and Streptococcus citrovorus from three samples. Streptococcus kefir was isolated from 5 of 54 samples of freshly pasteurized milk whereas Streptococcus citrovorus was not detected.

Galesloot and Hassing (22) reported that the starters used in the Netherlands for butter and cheese making contained, in addition to the ordinary lactic streptococci, citrate-fermenting bacteria. Initially the only aroma-producing bacteria identified belonged to the genus Leuconostoc. Later when the examination was repeated they also found starters containing Streptococcus diacetilactis.

Zielinska and Hiscox (67) isolated some strains of

aroma-producing streptococci from Cheddar cheese curd. The organisms produced volatile acids and acetylmethylcarbinol in varying degrees in milk culture. The acetylmethylcarbinol-producing strains closely resembled the type strains of Streptococcus diacetilactis but differed from them in producing acetylmethylcarbinol from lactose broth.

Franklin and Sharpe (18, 19) stated that Leuconostoc species were found in some of every different type of milk tested but were never found in any of the Cheddar cheese samples examined. Dacre (13) isolated strains of Leuconostoc species in small numbers from New Zealand Cheddar cheese.

Overcast and Skean (46) found that all of 21 cultures used in making Cheddar cheese contained citrate-fermenting bacteria ranging in number from 2.7 million to 200 million per milliliter.

Overcast and Rao (47) observed that 27 of 40 samples of Cheddar cheese contained citrate-fermenting bacteria. Hamamoto et al. (23, 24) found that 29 of 38 raw milk samples contained Leuconostoc species. They also reported that 49 of 86 ripened cheese contained between 1,000 and 10 million colonies of Leuconostoc species per gram of cheese.

In Lactic Culture

Hammer and Bailey (25) in 1919 observed that lactic cultures produce considerable volatile acids in milk whereas Streptococcus lactis did not. However, certain isolates from the cultures yielded considerable volatile acid. Hammer (26) in 1920 suggested that citric acid was the primary source of the volatile acid formed in lactic cultures by the organisms associated with Streptococcus lactis. Michaelian et al. (42) demonstrated the importance of acetylmethylcarbinol and biacetyl in lactic cultures and showed that cultures having a satisfactory flavor contained comparatively large amounts of these materials, whereas cultures lacking in flavor contained relatively small amounts, or none.

Hammer and Babel (28) stated that dairy cultures of Streptococcus lactis ordinarily do not contain significant amounts of acetylmethylcarbinol or biacetyl so that the relatively large amounts of these compounds in lactic cultures are the result of associative action of two types of organisms: lactic acid-producing and aroma-producing species. Since the studies of Hammer (26) in 1920, the symbiotic relationship between lactic acid-producing bacteria and the aroma-producing bacteria in starter cultures has been explained by

several investigators (10, 17, 28, 36, 37, 42). In mixed starter cultures containing lactic acid-producing bacteria and aroma-producing bacteria, the function of the first species is to produce acid from lactose, and the function of the latter species is to ferment the citric acid in milk to volatile compounds (17). Streptococcus lactis grown alone in milk produce only small amounts of volatile compounds, and the aroma-producing bacteria are unable to ferment citric acid until a low pH is obtained. Together these organisms perform a function that neither can carry out alone (28). Lundstedt (35) and Foster et al. (17) stated that the lactic streptococci to be used in a mixed-strain culture in association with citrate-fermenting bacteria must be able to combine with these "associates" to give maximum flavor and aroma production without inhibition of acid production. The association must be stable and give a consistently good culture, even after many transfers.

Occasionally, a pure culture of a lactic acid-producing streptococci yields sufficient flavor compounds in milk to affect the flavor (33) but the majority of cultures do not (15, 26, 33, 42). Hammer and Babel (28) stated that in some instances in which pure cultures of Streptococcus lactis have

been carried through a series of transfers in dairy plants, the cultures eventually acquired the flavor of typical mixed-strain culture. These workers suggested that the phenomenon was due to contamination in the plants and indicated that the aroma-producing organisms were widely distributed. This explained why commercial cultures of Streptococcus lactis or Streptococcus cremoris sometimes were able to produce satisfactory flavor.

In Citrate Fermentation

Hammer (26) in 1920 was the first to prove that the aroma-producing bacteria fermented the citrates normally present in milk. In practice over the past 70 years evidence has been accumulated that milk does not develop the typical aroma prior to coagulation and aroma production generally is associated with visible evidence of carbon dioxide production. Michaelian et al. (42) in 1933 found that the aroma-producing bacteria in pure cultures produce large amounts of acetylmethylcarbinol and biacetyl if enough acid is added to the milk to lower the pH to 4.3 to 4.0. These investigators reported that the aroma constituents were produced rapidly in the cultures only when the pH was

lowered. Van Beynum and Pette (62) observed that the characteristic aroma of a good culture was the result of the combined action of lactic acid-producing bacteria and Leuconostoc species. In a pure culture of the Leuconostoc species in milk, no aroma was observed, so that the associated growth of lactic acid-producing bacteria was necessary for the aroma formation.

Various investigators (17, 28, 32, 44, 60, 62) explained that citrate was fermented by Leuconostoc species or Streptococcus diacetilactis to give biacetyl, acetylmethylcarbinol, 2,3-butylene glycol (2,3-butanediol), carbon dioxide, acetic acid, and propionic acid as the principal fermentation end-products. Traces of alcohols, aldehydes, and similar compounds were also formed in the fermentation.

Foster et al. (17) stated that citrate-fermentation by aroma-producing bacteria usually was so complete that all of the 0.13 to 0.18 per cent of citric acid present in normal mixed-milk was utilized. Henning et al. (30) studied citrate-utilization by Streptococcus diacetilactis in mixed-strain starter cultures and indicated that about 80 per cent of milk citrate was utilized by Streptococcus diacetilactis within 24 hours at 21°C. while Streptococcus lactis and Streptococcus cremoris failed to fermented any citrate.

Pack et al. (48) observed that in commercial mixed-strain lactic cultures incubated at 21°C., biacetyl increased for 12 hours, and then decreased. The rate of the aroma loss after 12 hours varied. Some lost 80 per cent biacetyl in the next 6 to 12 hours, whereas others gradually lost this amount over a period of 60 hours. Loss of the aroma could be retarded by refrigerating (2°C.) the cultures before biacetyl destruction began. Pack et al. (49) suggested that by a careful combination of time and temperature of incubation and prompt cooling, the biacetyl in milk cultures could be stabilized and improved. These workers found that peak level of biacetyl production in mixed-strain lactic cultures was higher at 21°C. than at 30°C. At the higher temperature, however, an earlier initiation of the biacetyl synthesis and an earlier attainment of the peak level occurred.

Seitz et al. (55) indicated that an average of 1.5 parts per million biacetyl was produced by 16 strains of Streptococcus diacetilactis cultured in nonfat milk. The same 16 strains of the organisms increased average biacetyl production to 8.5 parts per million in an improved cultural procedure in which the strains were cultured in sterile homogenized milk containing 1 per cent sodium citrate.

Sandine et al. (54) found that crude sonic extracts of Streptococcus diacetilactis cells possessed an active citritase enzyme with a direct relationship between enzyme protein concentration and amount of citrate broken down. Collins and Harvey (9) isolated some strains of Streptococcus diacetilactis that did not produce acetylmethylcarbinol and gas from citrates. The reason given for this loss of ability to ferment citrates was mutation of the strains.

In Manufacturing and Curing of Cheese

Dawson and Feagan (14) investigated the trend of the populations of starter bacteria (Streptococcus lactis, Streptococcus diacetilactis, Streptococcus cremoris) during the manufacture and maturing of Cheddar cheese. These investigators showed that the three species of starter organisms attained quite different maximum populations at different stages of the cheese making process. Streptococcus lactis strains maintained a high but declining population during maturing of the cheese, beginning to die out gradually after eight weeks. Streptococcus diacetilactis strains showed similar trends but at lower population levels. Streptococcus cremoris, on the other hand, gave much lower populations, dying out rapidly

after two weeks, and virtually disappearing at eight weeks. Feagan and Dawson (16) observed that individual cheese of similar age and milk source showed considerable variability in the type of starter bacteria and non-starter bacteria present. Reiter et al. (53) reported that Cheddar cheese flavor was present not only in the Cheddar cheese made with lactic starter and added floras derived from the curd of the commercial cheese, but also in the cheese made with pure cultures of lactic starter bacteria only.

In Texture Defect of Cheese

Sherwood (56) observed that slit openness in Cheddar cheese developed when certain strains of Betacoccus species were added to the cheese milk. In a later study (57), this investigator concluded that certain strains of Betacoccus species were capable of producing open texture when they were present in large numbers. Galesloot (20) reported that Betacoccus species were capable of producing an early gas defect in texture of Cheddar cheese. However, when these organisms were used in cultures in conjunction with very active lactic acid-producing streptococci, the defect occurred only to a slight extent. Hucker and Pederson (31) compared

Streptococcus lactis with Leuconostoc species as to the amount of carbon dioxide produced. Gas produced by a Streptococcus lactis culture was no more than 6 per cent carbon dioxide of the total products resulting from the fermentation process, whereas the Leuconostoc organisms produced from 20 to 26 per cent carbon dioxide. Prouty and Golding (51) made a study to determine the carbon dioxide production of Streptococcus citrovorus and Streptococcus paracitrovorus by the extent of vacuum changes in vacuum packed Cheddar cheese. A greater loss of vacuum occurred in the Cheddar cheese made with Streptococcus citrovorus and Streptococcus paracitrovorus than the cheese made with Streptococcus lactis and Lactobacillus bulgaricus. Overcast and Albrecht (45) reported that several strains of Leuconostoc citrovorum were isolated from Cheddar cheese showing slit openness. The Cheddar cheese made with single-strain of Streptococcus lactis showed little or none of this texture defect. However, when the Streptococcus lactis culture was used in conjunction with the isolated strains of Leuconostoc citrovorum, the defect was very pronounced. The cheese with the texture defect did not show the typical gas hole, but rather irregular openings following the curd particles,

giving the appearance of a mechanical opening that had split the curd. Thomas (61) observed that a commercial cheese culture incubated at 32°C. for 6 to 8 hours had fewer citrate-fermenting organisms, Leuconostoc citrovorum and Leuconostoc dextranicum, than when incubated at 21°C. for 15 to 18 hours. Cheese made from the latter culture, which had the greater number of Leuconostoc organisms, contained a greater number of gas-producing organisms than the cheese made from the culture incubated at 32°C. The Cheddar cheese made from the culture incubated at 32°C. showed little or no slit openness, whereas the cheese made from the culture incubated at 21°C. showed distinct slit openness.

III. ISOLATION AND ENUMERATION METHODS

Prouty and Glenn (52) developed a plate culture method for differentiation and enumeration of Leuconostoc citrovorum. The colonies of Leuconostoc citrovorum were very small and round to irregular or angular in shape on this medium containing brom cresol purple indicator. The numbers of colonies developing on this medium were comparable to those developing on tomato juice agar when identical dilutions of a pure culture of Leuconostoc citrovorum were plated. The yellow

colonies of lactic acid-producing streptococci on this medium were readily differentiated from the colonies of Leuconostoc citrovorum.

Lundstedt (37) used the citrated starter whey agar medium for the differentiation between lactic acid-producing bacteria and aroma-producing bacteria in starters. The colonies of lactic acid-producing bacteria had diameters of approximately one millimeter while the colonies of aroma-producing bacteria had diameters of three millimeters. The colonies of aroma-producing bacteria on this medium showed the spectral colors of the rainbow in transmitted light.

More recently Galesloot et al. (21) developed an agar medium which was made turbid by means of calcium citrate. Aroma-producing bacteria of the starters, Betacoccus cremoris and Streptococcus diacetilactis, fermented the citrate and produced clear zones around the colonies. The conditions were such that the ordinary, non-citrate-fermenting starter streptococci produced insufficient acid to give rise to clear zones around the colonies.

Mayeux et al. (40) reported the use of a medium containing 75 parts per million of sodium azide for detecting Leuconostoc species in mixed-strain starter cultures. On

this medium, Streptococcus lactis, Streptococcus cremoris, and Streptococcus diacetilactis were inhibited, and colonies which were opaque and white to yellow in color appeared after incubation of four days. The colonies of Leuconostoc citrovorum were 0.5 to 2.0 millimeters in diameter, translucent, and exhibited a bluish iridescence. The colonies of Leuconostoc dextranicum were larger (1 to 5 millimeters in diameter), transparent, and slimy.

McDonough et al. (41) found that Leuconostoc species were much more resistant to small amounts of tetracycline than either Streptococcus lactis or Streptococcus cremoris. Consequently, these investigators developed a selective plating medium consisting of a tomato juice agar base containing 0.15 micrograms per milliliter of tetracycline. The medium inhibited the growth of most lactic acid-producing bacteria but permitted normal growth of the Leuconostoc species.

Skean and Overcast (58) modified the medium developed by Galesloot et al. (21). The lactic acid-producing bacteria inhibitor, calcium lactate, and the aroma-producing bacteria indicator, calcium citrate, were retained as in the original medium. The modified medium substituted commercial tomato

juice agar for the whey agar containing casaminoacids. This modified medium was found to be much easier to prepare for the isolation and enumeration of citrate-fermenting bacteria than the medium containing whey agar and casaminoacids.

IV. CARBONYL COMPOUNDS AND THE FLAVOR OF CHEESE

The nature of the compounds responsible for the typical flavor of Cheddar cheese has not yet been established in spite of a considerable amount of investigation by many workers in recent years. Failure to attribute Cheddar cheese flavor to any of the major constituents of ripened cheese has led investigators to examine more closely the minor volatile compounds of cheese and, in particular, the carbonyl compounds (38, 39).

Carbonyl compounds identified as present in Cheddar cheese can be grouped into acidic and neutral carbonyls. Neutral carbonyl compounds, considered to be degradation products of the acidic compounds and indicated as present in Cheddar cheese, include: biacetyl, acetylmethylcarbinol, 2,3-butylene glycol, butyraldehyde, acetaldehyde, acetone, methyl ethyl ketone, 3-hydroxybutanone, pentanone-2, heptanone-2, nonanone-2, and propionaldehyde (17, 28, 32, 39).

Hammer (27) in 1935 described the creatine test for the quantitative determination of acetylmethylcarbinol plus biacetyl in starter cultures. Prill and Hammer (50) in 1939 described the colorimetric method for the quantitative measurement of biacetyl.

Calbert and Price (6) stated that a small quantity of biacetyl was essential in the typical flavor and aroma of Cheddar cheese and in a later study (7), using the colorimetric method (50), found that all lots of cheese examined contained biacetyl. These workers found that Cheddar cheese with flavor defects contained from 0.2 to 3.35 parts per million of biacetyl, whereas 78 per cent of cheese with no flavor defects contained less than 0.5 parts per million of biacetyl. These workers concluded that larger amounts of biacetyl than 0.5 parts per million frequently appeared to be associated with flavor defects. Calbert and Price (8) suggested that the biacetyl in Cheddar cheese was produced by the action of microorganisms upon the citrates in a manner similar to the production of biacetyl in starter cultures. These workers stated that a gradual decrease in the amount of biacetyl in Cheddar cheese was noted during the early stages of curing. These workers suggested the decrease was caused

by volatilization of the biacetyl and by the reduction of biacetyl to acetylmethylcarbinol and 2,3-butylene glycol as a result of chemical reaction or the action of microorganisms.

Walker and Harvey (66) observed that flavor of mature Cheddar cheese was apparent after 8 to 12 weeks of curing and afterward the flavor of Cheddar cheese became more pronounced as the concentration of carbonyl compounds increased.

Kristoffersen and Gould (34) examined commercial Cheddar cheese for carbonyl compounds and found that the numbers and concentration of the carbonyl compounds varied between different cheese and in individual cheese during 12 months of ripening. These investigators concluded that, in general, a relationship could not be established between flavor and amount of the individual compounds present in Cheddar cheese.

Dacre (12) examined the volatile compounds in Cheddar cheese and concluded that the components of typical flavor of Cheddar cheese were volatile and were present in the cheese in concentration of only a few parts per million.

Vedamuthu et al. (63) observed that the Cheddar cheese with fruity, fermented flavor defects after three months of

curing contained 1.3 to 1.6 times greater concentration of carbonyl compounds than the cheese with normal flavor. These workers (64, 65), in later studies, demonstrated that cultures associated with high production of carbonyl compounds in milk also were repsonsible for fruity off-flavor defects in Cheddar cheese.

Dacre (11) showed that cultures of Leuconostoc species, when added to cheese milk in addition to lactic acid-producing bacteria, brought about an increase in flavor intensity in the resultant Cheddar cheese.

CHAPTER III

EXPERIMENTAL METHODS

Ten freshly made Cheddar cheese were purchased from five different manufacturers. The cheese were made from five different commercial starter cultures. Immediately after purchasing, the cheese were examined for citrate-fermenting bacteria. These bacterial counts were recorded as the population of first month. Thereafter, samples were repeatedly examined for the population of citrate-fermenting bacteria at a regular interval of one month to the end of the curing. After three months of curing, the examinations included organoleptic tests for the flavor and texture of the cheese. The organoleptic examinations were performed by two or more experienced judges following the guide of Nelson and Trout (43). All cheese were kept in a 10°C. (50°F.) curing room throughout the curing period.

The medium described by Skean and Overcast (58) was employed for the enumeration of citrate-fermenting bacteria. Five grams of calcium lactate were added to a liter of tomato juice agar and sterilized in an autoclave at 15 pounds

pressure for 15 minutes. The stabilized calcium citrate suspension was prepared by adding 1.5 grams of carboxymethyl cellulose to 200 milliliters of warm distilled water at 45°C. and it was allowed to stand overnight at this temperature. To this carboxymethyl cellulose gel, 20 grams of finely ground calcium citrate was added and the mixture was homogenized at low speed for approximately two minutes. The calcium citrate suspension was placed in 100 milliliter flasks which were plugged with cotton and sterilized in an autoclave. Immediately prior to pouring the plates six milliliters of the warm calcium citrate suspension was added to each 100 milliliters of the modified tomato juice agar and mixed thoroughly.

Two ounces of the cultures from which the cheese were manufactured, were stored at approximately 4°C. until the cultures were used. Each culture was transferred with 2 per cent inoculum into 100 milliliters of steamed skimmilk in six ounce screw-cap bottles and incubated at 21°C. for 16 hours or until coagulation occurred. From these cultures, total bacterial populations were enumerated on tomato juice agar and citrate-fermenting bacterial populations were determined on the tomato juice agar containing 0.5 per cent

calcium lactate and 0.6 per cent colloidal calcium citrate. The plates were incubated at 21°C. for six days.

In the examinations of citrate-fermenting bacterial populations in cheese the packages of cheese were opened in the laboratory and the outer layers were removed with a sterile spatula. Approximately ten grams of the interior portion was pulled out by a sterile cheese trier and one gram was weighed into a sterile mortar containing nine milliliters of warm sterile 2 per cent sodium citrate solution. With a sterile pestle, the sample was triturated into a smooth and uniform suspension. From this suspension the appropriate dilutions for plating were prepared using standard 99 milliliter water blanks. The plates were poured with 12 to 15 milliliters of the prepared medium and after solidification the plates were incubated at 21°C. for seven days. After the incubation time the colonies, encircled by a clear zone or halo, were counted with the aid of a Quebec Colony Counter. When possible, plates were selected for counting and counted according to Standard Procedure (2).

CHAPTER IV

RESULTS AND DISCUSSION

This experimental work was designed to explore the relationship of changes in population of the citrate-fermenting bacteria and their metabolic activities to the resulting flavor characteristics and texture of Cheddar cheese. Cultures with the same designation in this study originated from the same culture supplier and were presumed to be identical at the time each cheese manufacturer received the cultures.

The data in Table I show cheese A#1, A#2, S7/19, and S8/3 were made from culture M-34 by two different manufacturers and cheese B7/31 and B8/3 were made from culture M-56 by the same manufacturer. Cheese C#2 and K#44 were made from culture H-44 by different manufacturers.

Total bacterial counts and citrate-fermenting bacterial counts of the cultures are presented in Table II. All cultures used in manufacture of the cheese contained variable numbers of citrate-fermenting bacteria. When the citrate-fermenting bacteria per milliliter were expressed as per

TABLE I

SOURCES, CULTURES, AND SIZES OF CHEESE

Cheese Designation	Culture Used	Source of Cheese	Size of Cheese(lbs.)
A#1	M-34	Plant A	40
A#2	M-34	Plant A	40
B7/31	M-56	Plant B	20
B8/3	M-56	Plant B	20
C#1	H-5	Plant C	20
C#2	H-44	Plant C	20
K#44	H-44	Plant K	40
K#57	H-57	Plant K	40
S7/19	M-34	Plant S	12
S8/3	M-34	Plant S	12

TABLE II

TOTAL BACTERIAL COUNTS AND CITRATE-FERMENTING BACTERIAL COUNTS OF CULTURES

Culture Number	Colony Counts Per ml. of Cultures		% of Citrate-fermenting Bacterial Counts
	Total Bacterial Counts	Citrate-fermenting Bacterial Counts	
	In Millions		
M-34	700	450	64
M-34[a]	N.G.	N.G.	
H-44	240	4.7	2
H-44[b]	150	5.6	4
M-56	750	180	24
H-5	61	15	25
H-57	350	230	66

[a]M-34 culture from Plant A failed to grow when transferred after its receipt.

[b]H-44 culture from Plant K.

cent of each corresponding total bacterial counts, the percentage ranged from 2 to 66 per cent. One M-34 culture used in the manufacture of cheese A#1 and A#2 failed to grow when transferred into milk on the day of arrival at the laboratory. Culture H-44 carried in different plants showed little difference between plants in the total and citrate-fermenting bacterial counts.

All cheese in this study showed slight to distinct openness when examined after three months of curing and there was no change in the degree of openness throughout the curing regardless of the population change of citrate-fermenting bacteria.

The data in Tables III to XII show the population changes of citrate-fermenting bacteria in ten different cheese and the changes of flavor of the cheese. Eight of ten cheese contained citrate-fermenting bacteria throughout the curing period in variable numbers and two cheese contained the citrate-fermenting bacteria until 11 months of curing. All cheese decreased in the population of citrate-fermenting bacteria at the end of curing compare to the early stages of curing. Six of the ten cheese developed a bitter flavor at the end of curing. Two of the remaining cheese

TABLE III

COUNTS OF CITRATE-FERMENTING BACTERIA AND FLAVOR CRITICISMS OF CHEESE A#1

Curing Period (Month)	Bacterial Counts (In Thousands)	Flavor Criticisms
1	1,500	- - -[a]
2	10	- - -
3	4	- - -
4	330	flat
5	540	flat
6	45,000	flat
7	2,200	sl. acid
8	2,000	sl. acid
9	100	sl. fermented
10	3,000	sl. unclean acid
11	700	acid
12	N.G.[b]	fermented acid

[a] No examinations were made until the cheese was three months old.

[b] No growth.

TABLE IV

COUNTS OF CITRATE-FERMENTING BACTERIA AND FLAVOR CRITICISMS OF CHEESE A#2

Curing Period (Month)	Bacterial Counts (In Thousands)	Flavor Criticisms
1	80	- - -[a]
2	59	- - -
3	1	- - -
4	33	flat
5	23	flat
6	500	sl. unclean
7	60	acid
8	N.G.[b]	acid
9	5	sl. fermented
10	1	sl. fermented
11	40	fermented acid
12	N.G.	fermented acid

[a] No examinations were made until the cheese was three months old.

[b] No growth.

TABLE V

COUNTS OF CITRATE-FERMENTING BACTERIA AND FLAVOR CRITICISMS OF CHEESE B7/31

Curing Period (Month)	Bacterial Counts (In Thousands)	Flavor Criticisms
1	30,000	- - -[a]
2	74,000	- - -
3	7,500	- - -
4	39,000	flat
5	39,000	flat
6	3,200	flat
7	27,000	sl. flat
8	35,000	fermented
9	18,000	sl. flat
10	30,000	sl. bitter
11	15,000	sl. bitter
12	10,000	sl. bitter

[a]No examinations were made until the cheese was three months old.

TABLE VI

COUNTS OF CITRATE-FERMENTING BACTERIA AND FLAVOR CRITICISMS OF CHEESE B8/3

Curing Period (Month)	Bacterial Counts (In Thousands)	Flavor Criticisms
1	55,000	- - -[a]
2	88,000	- - -
3	370,000	- - -
4	56,000	flat
5	43,000	flat
6	800	flat
7	9,000	flat
8	40,000	sl. fermented
9	20,000	flat
10	40,000	flat
11	2,000	sl. bitter
12	12,000	sl. bitter

[a]No examinations were made until the cheese was three months old.

TABLE VII

COUNTS OF CITRATE-FERMENTING BACTERIA AND FLAVOR CRITICISMS OF CHEESE C#1

Curing Period (Month)	Bacterial Counts (In Thousands)	Flavor Criticisms
1	6,400	- - -[a]
2	6,300	- - -
3	1,800	- - -
4	300	flat
5	2,800	flat
6	10,000	flat
7	3,000	acid
8	9,500	bitter
9	200	sl. bitter
10	100	bitter
11	60	bitter
12	10	bitter

[a]No examinations were made until the cheese was three months old.

TABLE VIII

COUNTS OF CITRATE-FERMENTING BACTERIA AND FLAVOR CRITICISMS OF CHEESE C#2

Curing Period (Month)	Bacterial Counts (In Thousands)	Flavor Criticisms
1	13,000	- - -[a]
2	3,100	- - -
3	680	- - -
4	440	flat
5	230	flat
6	50	flat
7	44	sl. acid
8	100	sl. acid
9	2.5	sl. acid
10	2	sl. acid
11	4	sl. bitter
12	3	sl. bitter

[a]No examinations were made until the cheese was three months old.

TABLE IX

COUNTS OF CITRATE-FERMENTING BACTERIA AND FLAVOR CRITICISMS OF CHEESE K#44

Curing Period (Month)	Bacterial Counts (In Thousands)	Flavor Criticisms
1	N.G.[a]	- - -[b]
2	N.G.	- - -
3	N.G.	- - -
4	28	flat
5	26	flat
6	150	sl. unclean
7	N.G.	flat
8	17	bitter
9	4	bitter
10	2	bitter
11	80	bitter
12	4	bitter

[a] No growth.

[b] No examinations were made until the cheese was three months old.

TABLE X

COUNTS OF CITRATE-FERMENTING BACTERIA AND FLAVOR CRITICISMS OF CHEESE K#57

Curing Period (Month)	Bacterial Counts (In Thousands)	Flavor Criticisms
1	30,000	- - -[a]
2	45,000	- - -
3	6,000	- - -
4	300	flat
5	14,000	flat
6	15,000	flat
7	34,000	flat
8	33,000	bitter
9	2,000	bitter
10	5,000	bitter
11	600	bitter
12	2,000	bitter

[a]No examinations were made until the cheese was three months old.

TABLE XI

COUNTS OF CITRATE-FERMENTING BACTERIA AND FLAVOR CRITICISMS OF CHEESE S7/19

Curing Period (Month)	Bacterial Counts (In Thousands)	Flavor Criticisms
1	4,000	- - -[a]
2	70	- - -
3	40	- - -
4	10	flat
5	1	flat
6	170	sl. unclean
7	7	flat
8	12	flat
9	2	flat
10	1	flat
11	2	flat
12	1	flat

[a]No examinations were made until the cheese was three months old.

TABLE XII

COUNTS OF CITRATE-FERMENTING BACTERIA AND FLAVOR CRITICISMS OF CHEESE S8/3

Curing Period (Month)	Bacterial Counts (In Thousands)	Flavor Criticisms
1	30,000	- - -[a]
2	12,000	- - -
3	800	- - -
4	2,600	flat
5	130	flat
6	210	flat
7	21	flat
8	300	flat
9	20	flat
10	0.6	flat
11	4	flat
12	0.4	flat

[a]No examinations were made until the cheese was three months old.

developed a fermented flavor and two other cheese had a flat or green flavor at the end of curing. The cheese with fermented flavor criticism showed no growth of citrate-fermenting bacteria at the end of curing.

In spite of the fact that cheese A#1 and A#2 were made from the same culture in the same plant on the same date, the data in Tables III and IV show considerable differences in the population changes of citrate-fermenting bacteria. Cheese A#1 maintained high counts of the bacteria until the cheese was 11 months old while cheese A#2 maintained relatively low counts of the bacteria throughout the curing. Considerable fluctuations in the population of citrate-fermenting bacteria occurred in cheese A#1 and a fermented flavor developed at nine months of age. Cheese A#2 maintained relatively constant level of the bacterial counts and developed the fermented flavor defect at nine months of age. These differences between cheese A#1 and A#2 may have been due to failure in the cheese making to maintain uniform sanitary conditions of the plant and uniform quality of the milk from which these cheese were made.

The data in Tables V and VI show the population changes of citrate-fermenting bacteria and flavor developments in

cheese B7/31 and B8/3. These cheese were made from the same culture by the same manufacturer and the culture contained a relatively high percentage of citrate-fermenting bacteria (24 per cent of total bacteria). These cheese contained very high numbers of citrate-fermenting bacteria and the population maintained this high level throughout the curing. Cheese B7/31 developed fermented flavor at the age of eight months, then bitter flavor developed at ten months of age. Cheese B8/3 developed fermented flavor at the age of eight months and bitter flavor at 11 months of age. The similarity of these two cheese in changes of the bacterial counts and flavor development may indicate that these cheese were manufactured from uniform quality of milk in the same procedure of cheese making.

The data in Table VII, pg. 33, show cheese C#1 made from the culture with low count of total bacteria but high percentage of citrate-fermenting bacteria (24 per cent of total bacteria) contained high numbers of citrate-fermenting bacteria decreasing after eight months of curing. This cheese developed bitter flavor at eight months of age. The differences in the trend of citrate-fermenting bacterial population changes among cheese made from different cultures may have been due to different species or strains.

Cheese C#2 and K#44 were made from the same culture by different manufacturers. The culture used for the manufacture of these cheese contained a very low percentage of citrate-fermenting bacteria (4 and 2 per cent of total bacteria respectively). The date in Tables VIII and IX, pgs. 34 and 35, show cheese C#2 contained high counts of citrate-fermenting bacteria and the counts decreased consistently throughout the curing while cheese K#44 contained very low counts of the bacteria in early stages of curing and maintained the low population of bacteria to the end of curing. Cheese C#2 developed bitter flavor at 11 months of age and cheese K#44 developed the bitter flavor at eight months of age. The different practices of carrying culture and cheese making by cheese manufacturers could be the explanation for the differences in citrate-fermenting bacterial populations and the flavor development of these cheese made from the same culture. Slightly different time and temperature of incubation of cultures or time and temperature of cooking of the cheese making could lead to such different characteristics of the resulting cheese.

The data in Table X, pg. 36, show cheese K#57 made from a culture with high content of total bacteria and high

percentage of citrate-fermenting bacteria (66 per cent of total bacteria) generally contained high populations of citrate-fermenting bacteria throughout the curing. However, the bacterial population decreased to a slight extent at nine months of age. This cheese developed the bitter flavor at the age of eight months.

Cheese S7/19 and S8/3 were made from the same culture with high total bacterial counts and a high percentage of citrate-fermenting bacteria (64 per cent of total bacteria) by the same manufacture. The date in Tables XI and XII, pgs. 37 and 38, show the population changes of citrate-fermenting bacteria and the flavor development of these cheese. Cheese S7/19 contained a high population of citrate-fermenting bacteria at one month of age, then decreased to a low level at two months of age and remained low throughout the curing while cheese S8/3 with a higher population decreased more consistently. These cheese resulted in a flat flavor at the end of curing. The flat flavor defect of these cheese may have been due to failure by the cheese maker to control acid production by lactic acid-producing bacteria during the cheese making. Foster et al. (17) stated that excessive acidity during cheese making could cause a short, crumbly

body and flavor development could not occur in such cheese.

The variations in citrate-fermenting bacterial counts from month to month may be explained on the basis that microorganisms are not evenly distributed in cheese. Likewise, growth of microorganisms in curing of cheese develops as colonial growth which in turn could account for considerable variation. To reduce this variation, many samples would have had to been taken from over the entire cheese at each sampling period which would have interfered with the normal curing of the cheese.

Six of ten cheese in this experiment developed a bitter flavor before the end of curing. This is a common flavor defect in Cheddar cheese and has been reported to be associated with poor quality of milk, poor starters, and unsanitary practices in the cheese factory. Likewise, certain starter cultures have been implicated as causing bitter flavor. The four cheese in this work that did not develop a bitter flavor were all made from the same starter culture M-34. The non-bitter cheese were manufactured in two different plants and from casual observation no great difference existed in sanitary practices or sources of milk. This would

appear as additional evidence that the flavor defect may be associated with the species or strains of bacteria in the starter culture. Hansen et al. (29) reported that Cheddar cheese made with pure cultures of citrate-fermenting bacteria (Streptococcus paracitrovorus and Streptococcus citrovorus) produced a distinctly bitter flavor and decidedly open texture while well balanced mixed cultures produced cheese having a satisfactory flavor.

CHAPTER V

SUMMARY AND CONCLUSIONS

Population changes of citrate-fermenting bacteria in commercial Cheddar cheese and the flavor developments of the cheese were studied. Ten freshly made Cheddar cheese were purchased from five different manufacturers and the cheese were examined for citrate-fermenting bacteria for twelve months of curing period at a regular interval of one month. After three months of curing, the examinations included organoleptic tests for the flavor and texture of the cheese.

Eight of ten cheese contained citrate-fermenting bacteria throughout curing in variable numbers and two cheese contained the bacteria until eleven months of curing. The populations of citrate-fermenting bacteria of all cheese decreased during curing. Six of ten cheese developed a bitter flavor during curing. Two of the remaining cheese developed a fermented flavor and the other two a flat flavor. All cheese showed slight to distinct openness in texture when examined after three months of curing and there was no change in the degree of openness throughout the curing.

Since most commercial starter cultures for Cheddar cheese making contain variable numbers of citrate-fermenting bacteria, further work is necessary in this area to determine the role of these bacteria in the manufacture and curing of Cheddar cheese.

LIST OF REFERENCES

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(1) Abd-El-Malek, Y., and Gibson, T. 1948. The Streptococci in Milk. J. Dairy Res., 15:233.

(2) American Public Health Association. 1967. Standard Methods for the Examination of Dairy Products. 12th Ed. American Public Health Association, New York.

(3) Badings, H. T., and Galesloot, T. E. 1962. Studies on the Flavor of Different Type of Butter Starters with Reference to the Defect "Yoghurt Flavor" in Butter. 16th Int. Dairy Congr., B:199.

(4) Breed, R. S., Murray, E. G. D., and Smith, N. R. 1957. Bergey's Manual of Determinative Bacteriology. 7th Ed. The Williams and Wilkins Co., Baltimore.

(5) Briggs, C. A. E. 1952. A Note on the Serological Classification of Streptococcus diacetilactis. J. Dairy Res., 19:167.

(6) Calbert, H. E., and Price, W. V. 1948. Studies of Sources of the Typical Flavor in Cheddar Cheese. J. Dairy Sci., 31:713.

(7) Calbert, H. E., and Price, W. V. 1949. A Study of the Diacetyl in Cheese. I. Diacetyl Content and Flavor of Cheddar Cheese. J. Dairy Sci., 32:515.

(8) Calbert, H. E., and Price, W. V. 1949. A Study of the Diacetyl in Cheese. II. The Changes in Diacetyl Content of Cheddar Cheese During Manufacturing and Curing. J. Dairy Sci., 32:521.

(9) Collins, E. B., and Harvey, R. J. 1961. Failure in Production of Acetoin and Gas by Cultures of Streptococcus diacetilactis. J. Dairy Sci., 44:1158.

(10) Cox, G. A. 1945. The Effect of Acidity on the Production of Diacetyl by Betacocci in Milk. J. Dairy Res., 14:28.

(11) Dacre, J. C. 1953. Cheddar Cheese Flavor and Its Relation to Tyramine Production by Lactic Acid Bacteria. J. Dairy Res., 20:217.

(12) Dacre, J. C. 1955. A Chemical Investigation of the Volatile Flavor Principle of Cheddar Cheese. J. Dairy Res., 22:219.

(13) Dacre, J. C. 1958. Characteristics of a Presumptive Pediococcus Occurring in New Zealand Cheddar Cheese. J. Dairy Res., 25:409.

(14) Dawson, D. J., and Feagan, J. T. 1957. Bacteriology of Cheddar Cheese; A Study of Starter Organisms in Manufacture and Maturing. J. Dairy Res., 24:210.

(15) Elliker, P. R., Sandine, W. E., Hauser, B. A., and Moseley, W. K. 1964. Influence of Culturing Cottage Cheese Dressing with Different Organisms on Flavor and Keeping Quality. J. Dairy Sci., 47:680.

(16) Feagan, J. T., and Dawson, D. J. 1959. Bacteriology of Cheddar Cheese; Some Observations on the Micro-flora During Maturing. Aust. J. Dairy Technol., 14:59.

(17) Foster, E. M., Nelson, F. E., Speck, M. L., Doetsch, R. N., and Olson, J. C., Jr. 1964. Dairy Microbiology. Prentice-Hall, New Jersey.

(18) Franklin, J. G., and Sharpe, M. E. 1962. The Bacterial Content of Milk Used for Making Cheddar Cheese and Its Effect on the Flora and Quality of the Cheese Produced. 16th Int. Dairy Congr., C:305.

(19) Franklin, J. G., and Sharpe, M. E. 1963. The Incidence of Bacteria in Cheese Milk and Cheddar Cheese and Their Association with Flavor. J. Dairy Res., 30:87.

(20) Galesloot, T. E. 1950. An Early Gas Defect in Cheese Caused by Betacoccus Species. Neth. Milk and Dairy J. 4:274.

(21) Galesloot, T. E., Hassing, F., and Stadhouders, J. 1961. Agar Media for the Isolation and Enumeration of Aroma Bacteria in Starters. Neth. Milk and Dairy J., 15:145.

(22) Galesloot, T. E., and Hassing, F. 1961. Some Differences in Behavior Between Starters Containing as Aroma Bacterium either Streptococcus diacetilactis or Betacoccus cremoris. Neth. Milk and Dairy J., 15:242.

(23) Hamamoto, M., Kanauchi, T., and Mino, K. 1964. Studies on Leuconostoc Organisms in Cheese. I. On the Distribution of Leuconostoc Organisms in Raw Milk, Starter Cultures, and Cheese. Jap. J. Zootech. Sci., 35:317. Cited from Dairy Sci. Abst. No. 1512. 1965. 27:247.

(24) Hamamoto, M., Kanauchi, T., and Mino, K. 1965. Studies on Seuconostoc Organisms in Cheese. II. Changes in Number and Flora of Leuconostoc Organisms in Gouda Cheese. Jap. J. Zootech. Sci., 36:334. Cited from Dairy Sci. Abst. No. 567. 1966. 28:87.

(25) Hammer, B. W., and Bailey, D. E. 1919. The Volatile Acid Production of Starters and of Organisms Isolated from Them. Iowa Agr. Expt. Sta. Research Bull., 55.

(26) Hammer, B. W., 1920. Volatile Acid Production of Streptococcus lacticus and the Organisms Associated with It in Starters. Iowa Agr. Expt. Sta. Research Bull., 63.

(27) Hammer, B. W. 1935. The Creatine Test for Acetylmethylcarbinol Plus Diacetyl in Butter Cultures. J. Dairy Sci., 18:579.

(28) Hammer, B. W., and Babel, F. J. 1957. Dairy Bacteriology. 4th Ed. John Wiley and Sons Inc., New York.

(29) Hansen, H. C., Bendixen, H. A., and Theophilus, D. R. 1933. Influence of Different Starters for the Quality of Cheddar Cheese. J. Dairy Sci., 16:121.

(30) Henning, D. R., Vedamuthu, E. R., Sandine, W. E., and Elliker, P. R. 1964. Citrate Utilization and Overgrowth by Streptococcus diacetilactis in Mixed-strain Starter Cultures. J. Dairy Sci., 47:674.

(31) Hucker, G. J., and Pederson, C. S. 1930. Studies on the Coccaceae. XVI. The Genus Leuconostoc. N. Y. (Geneva) Agr. Expt. Sta. Tech. Bull., No. 167.

(32) Kosikowski, Frank V. 1966. Cheese and Fermented Milk Foods. Edwards Brothers Inc., Michigan.

(33) Krishnaswamy, M. A., and Babel, F. J. 1951. Biacetyl Production by Cultures of Lactic Acid Producing Streptococci. J. Dairy Sci., 34:374.

(34) Kristoffersen, T., and Gould, I. A. 1958. Carbonyl Compounds in Cheddar Cheese and Their Possible Relationship to Flavor. 15th Int. Dairy Congr., 2:720.

(35) Lundstedt, Erik. 1958. Starter Failures and Their Prevention. Milk Dealer, 47:(7)45.

(36) Lundstedt, Erik. 1959. Part I. The Symbiosis Between Lactic Acid Bacteria and The Aroma Bacteria in Starters. Milk Dealer, 48:(3)47.

(37) Lundstedt, Erik. 1959. Part II. The Symbiosis Between Lactic Acid Bacteria and Aroma Bacteria in Starters. Milk Dealer, 48:(4)42.

(38) Mabbitt, L. A. 1961. Review of the Progress of Dairy Science; Section B. Bacteriology. The Flavor of Cheddar Cheese. J. Dairy Res., 28:303.

(39) Marth, E. H. 1963. Microbiological and Chemical Aspects of Cheddar Cheese Ripening; A Review. J. Dairy Sci., 46:869.

(40) Mayeux, J. V., Sandine, W. E., and Elliker, P. R. 1962. A Selective Medium for Detecting Leuconostoc Organisms in Mixed-strain Starter Cultures. J. Dairy Sci., 45: 655.

(41) McDonough, F. E., Hargrove, R. E., and Tittsler, R. P. 1962. A Selective Plating Medium for Leuconostoc in Mixed Lactic Cultures. J. Dairy Sci., 45:656.

(42) Michaelian, M. B., Farmer, R. S., and Hammer, B. W. 1933. Relationship of Acetylmethylcarbinol and Diacetyl to Butter Cultures. Iowa Agr. Expt. Sta. Res. Bull., No. 155.

(43) Nelson, J. A., and Trout, G. M. 1964. Judging Dairy Products. 4th Ed. Olsen Publishing Co., Milwaukee, Wis.

(44) Newlander, J. A., and Atherton, H. V. 1964. The Themistry and Testing of Dairy Products. Third Ed. Olsen Publishing Co., Milwaukee, Wis.

(45) Overcast, W. W., and Albrecht, T. W. 1952. Gas Production in Cheddar Cheese Caused by Leuconostoc citrovorum. J. Dairy Sci., 35:554.

(46) Overcast, W. W., and Skean, J. D. 1964. Population of Citrate-fermenting Bacteria in Lactic Cultures. J. Milk and Food Technol., 27:4.

(47) Overcast, W. W., and Rao, K. M. 1967. The Occurrence of Citrate-fermenting Bacteria in Cheddar Cheese. J. Milk and Food Technol., 30:17.

(48) Pack, M. Y., Sandine, W. E., Elliker, P. R., and Day, E. A. 1964. Diacetyl Production and Destruction Patterns in Mixed-strain Starter Cultures. J. Dairy Sci., 47:674.

(49) Pack, M. Y., Vedamuthu, E. R., Sandine, W. E., and Elliker, P. R. 1968. Effect of Temperature on Growth and Diacetyl Production by Aroma Bacteria in Single and Mixed-strain Lactic Cultures. J. Dairy Sci., 51: 339.

(50) Prill, E. A., and Hammer, B. W. 1939. Production of Diacetyl From Citric Acid in Butter Cultures. J. Dairy Sci., 22:67.

(51) Prouty, C. C., and Golding, N. C. 1946. The Action of the Associate Starter Organisms in American Cheddar Cheese. J. Dairy Sci., 29:495.

(52) Prouty, C. C., and Glenn, Wilburn. 1953. A Plate Culture Technique for the Quantitative Determination of Leuconostoc citrovorum in Cultured Buttermilk with Observations on the Progressive Changes in Numbers During the Fermentation Process. J. Dairy Sci., 36: 564.

(53) Reiter, B., Fryer, T. F., Pickering, A., Chapman, H. R., Lawrence, R. C., and Sharpe, M. E. 1967. The Effect of the Microbial Flora on the Flavor and Free Fatty Acid Composition of Cheddar Cheese. J. Dairy Res., 34:257.

(54) Sandine, W. E., Seitz, E. W., Elliker, P. R., and Day, E. A. 1961. Studies on the Mechanism of Diacetyl Synthesis by Streptococcus diacetilactis. J. Dairy Sci., 44:1157.

(55) Seitz, E. W., Sandine, W. E., Day, E. A., and Elliker, P. R. 1961. Studies on Factors affecting Diacetyl Production by Streptococcus diacetilactis. J. Dairy Sci., 44:1158.

(56) Sherwood, I. R. 1939. The Relation of Certain Lactic Acid Bacteria to Open Texture in Cheddar Cheese. J. Dairy Res., 10:326.

(57) Sherwood, I. R. 1939. The Bacterial Flora of New Zealand Cheddar Cheese. J. Dairy Res., 10:426.

(58) Skean, J. D., and Overcast, W. W. 1962. Another Medium for Enumerating Citrate-fermenting Bacteria in Lactic Cultures. J. Dairy Sci., 45:1530.

(59) Swartling, P. F. 1951. Biochemical and Serological Properties of Some Citric Acid Fermenting Streptococci From Milk and Dairy Products. J. Dairy Res., 18:256.

(60) Thiel, C. C. 1940. The Influence of Various Factors on the Fermentation End Products of the Heterofermentative Streptococci. J. Dairy Res., 11:51.

(61) Thomas, D. H. 1953. Influence of Starter Incubation Temperature of 32°C. Upon the Associate Organisms and Slit Openness in Cheddar Cheese. M.S. Thesis, The University of Tennessee, Knoxville, Tennessee.

(62) Van Beynum, J., and Pette, J. W. 1939. The Decomposition of Citric Acid by Betacoccus cremoris. J. Dairy Res., 10:250.

(63) Vedamuthu, E. R., Sandine, W. E., and Elliker, P. R. 1964. Influence of Strain Composition of Lactic Starters on Flavor and Texture of Cheddar Cheese. J. Dairy Sci., 47:679.

(64) Vedamuthu, E. R., Sandine, W. E., and Elliker, P. R. 1966. Flavor and Texture in Cheddar Cheese; I. Role of Mixed-strain Lactic Starter Cultures. J. Dairy Sci., 49:144.

(65) Vedamuthu, E. R., Sandine, W. E., and Elliker, P. R. 1966. Flavor and Texture in Cheddar Cheese; II. Carbonyl Compounds Produced by Mixed-strain Lactic Starter Cultures. J. Dairy Sci., 49:151.

(66) Walker, J. R. L., and Harvey, R. J. 1959. Some Volatile Compounds in New Zealand Cheddar Cheese and Their Possible Significance in Flavor Formation; I. Identification of the Volatile Carbonyl Fraction. J. Dairy Res., 26:265.

(67) Zielinska, M., and Hiscox, E. R. 1954. Aroma Producing Group "N" Streptococci Isolated From Cheddar Cheese Curd. J. Dairy Res., 21:238.

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