c. 2

3 3

March 7, 1953

To the Graduate Council:

I am submitting herewith a thesis written by David Henderson Thomas entitled "Influence of Starter Incubation Temperature of Thirty-two Degrees Centigrade upon the Associate Organisms and Slit Openness 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.

Woodrow T. Overcast
Major Professor

We have read this thesis
and recommend its acceptance:

Eric W. Swanson

Thomas W. Albrecht

Accepted for the Council:

E. R. Waters
Dean of the Graduate School

INFLUENCE OF STARTER INCUBATION TEMPERATURE OF THIRTY-TWO DEGREES CENTIGRADE UPON THE ASSOCIATE ORGANISMS AND SLIT OPENNESS IN CHEDDAR CHEESE

A THESIS

Submitted to
The Graduate Council
of
The University of Tennessee
in
Partial Fulfillment of the Requirements
for the degree of
Master of Science

---

by

David Henderson Thomas

March 1953

ACKNOWLEDGMENT

The writer wishes to express his gratitude to Dr. W. W. Overcast for directing this work and for guidance in the preparation of this manuscript.

TABLE OF CONTENTS

LIST OF TABLES

CHAPTER I

INTRODUCTION

Slit openness in Cheddar cheese is a texture defect of some concern to the cheese industry. This texture defect lowers the quality of the cheese, making a less desirable product for the market. Therefore, an economic problem arises with the occurrence of slits in Cheddar cheese. It is believed that the associate organisms, Leuconostoc citrovorum and Leuconostoc dextranicum, present in a commercial cheese culture may be a source of slit openness. By reducing their presence in the culture, it was felt that a better textured cheese could be produced without impairing the composition or flavor of the cheese.

The optimum growth temperature for L. citrovorum and L. dextranicum is lower than the optimum for the predominating lactic acid producing organisms, Streptococcus lactis and/or Streptococcus cremoris, in the commercial culture. By incubating the culture at a higher temperature than is commonly practiced by cheese plants, it was hoped that the number of associate organisms could be reduced.

CHAPTER II

STATEMENT OF PROBLEM

The purpose of this investigatinn was to study the influence of a commercial cheese culture incubation temperature of 32° C. upon the associates and slit openness in Cheddar cheese.

CHAPTER III

REVIEW OF LITERATURE

Van Slyke and Price (34) have reported three common types of openness occurring in American Cheddar cheese, mechanical openings, round gas holes, and slit openings. More advancement has been made toward correcting the mechanical openings and the round gas holes than the slit openness.

Enlightening information on slit openness has been contributed by New Zealand investigators (30, 36). This texture defect which is very common and troublesome in New Zealand cheese is also frequently observed in American Cheddar cheese. Overcast and Albrecht (28) have observed slit openness in commercial and experimental lots of Cheddar cheese. In some cases this texture defect was so serious as to cause a bulging of the cheese surfaces.

Slit openness in Cheddar cheese does not appear as the typical gas holes found in gassy cheese, but as irregular openings more or less following the curd particles. The surfaces of the slits are moist and may have a shiny or glazed appearance which helps to distinguish them from mechanical openings (28, 34).

Van Slyke and Price (34) report that pasteurization of cheese milk has been effective in reducing the number of rapid gas producing organisms, belonging to the Escherichia-Aerobacter group, thereby resulting in a better textured cheese. However, Whitehead and Jones (36) have observed that pasteurization of cheese milk has been ineffective in controlling the development of slit openness. These workers found that cheese made from

good quality pasteurized milk showed slit openness at fourteen days of age.

Ashe (2) reported slit openness in Cheddar cheese to be a seasonal texture defect occurring primarily in the fall and winter. Investigations were made to determine the factors responsible for the development of the defect. Soluble calcium ions, in the form of calcium chloride, and citrates, in the form of anhydrous citric acid, were added to the cheese milk without any appreciable effect upon the development of slit openness. Comparisons were made of cheese containing different concentrations of salt. Indications were that the greater amounts of salt added to the milled curd produced less development of slit openness. Little importance was given to the pasteurization temperature, making procedure, or the acidity at the time of milling upon the control of slit openness.

Overcast and Albrecht (28) showed that very distinct slits could be produced in Cheddar cheese by inoculating the cheese milk with L. citrovorum. Sherwood (30) also showed that slit openness in Cheddar cheese developed when certain Leuconostoc species were added to the cheese milk. Commercial cheese cultures were considered as a possible source of slit openness since they contained these organisms.

Prouty and Golding (29) have reported that commercial cheese cultures are not pure cultures. In addition to the predominating lactic acid producing organisms, S. lactis and/or S. cremoris, their associate organisms are invariably found. Other investigators (14, 15, 30, 33, 34) have reported the presence of two species of organisms as associates in commercial cheese cultures to be significant.

The nomenclature of the associates or citric acid fermenting organisms has been the subject of considerable debate, but Bergey's Manual (16) has designated these organisms as Leuconostoc citrovorum and Leuconostoc dextranicum. These names will be applied hereafter in this paper.

Hucker and Pederson (23) compared S. lactis with their associates as to the amount of carbon dioxide produced. S. lactis produced no more than 6 per cent carbon dioxide of the total products resulting from their fermentation processes; whereas the associate organisms produced from 20-26 per cent carbon dioxide. Hucker (21) has previously reported similar results.

Abd-El-Malek and Gibson (1) have done considerable work in the identification of the streptococci present in milk. The associate organisms were considered to be heterofermentative; whereas S. lactis and S. cremoris were found to be homofermentative. From the tests conducted, the associates showed several characteristics that were different from S. lactis and S. cremoris. Of these the ability to produce carbon dioxide was most important in their differentiation.

Hammer and Baker (18) attempted to detect gas formation of L. dextranicum by sealing freshly inoculated milk with several different materials. Some of the results were unsatisfactory due to gas escaping between the seal and the wall of the tube. However, Hammer (16) later showed proof of gas production of the associates by making the container of the culture air tight. When a freshly inoculated milk culture was sealed with a mixture of vaseline and paraffin, the seal was pushed up as

a result of gas production. A similar test was shown by inoculating cans of evaporated milk with the associates and soldering the opening through which the inoculation was made. Bulging of the cans resulted from gas production.

Prouty and Golding (29) made a study to determine the gas production of the associates by the extent of vacuum changes in vacuum packed American Cheddar cheese. When L. citrovorum and L. dextranicum were used in conjunction with S. lactis and Lactobacillus bulgaricus cultures, a greater loss of vacuum occurred than when S. lactis and L. bulgaricus were used alone. A greater loss of vacuum was associated with L. dextranicum than with L. citrovorum. When the cheese was ripened at 45°-50° F., more loss of vacuum occurred than when ripened at 40° F.

Dorn and Dahlberg (10) reported that the flavor and aroma producing bacteria are a source of gas production in Cheddar cheese. Cheese containing these organisms produced gas most rapidly during the first four weeks of ripening, but continued even after 48 weeks of ripening. Sherwood (31) concluded, from his detailed study of the flora of New Zealand Cheddar cheese, that certain strains of the Leuconostoc species are capable of producing open texture when they are present in large numbers.

Galesloot (12) reported that the Leuconostoc species are capable of producing an early gas defect in Cheddar cheese. The species primarily responsible for the defect were L. dextranicum and L. mesenteroides. However, when these organisms were used in cultures in conjunction with very active streptococci, the defect occurred only to a slight extent.

The associates produce relatively large amounts of volatile acids as shown by Hucker (20). Of the total acids produced by the associates, 40 per cent was volatile which was primarily acetic and propionic. S. lactis converts lactose almost entirely into lactic acid with not more than 10 per cent volatile acids of the total acid produced. Hammer (15) and Hammer and Bailey (17) showed high volatile acid production to be a characteristic of L. citrovorum and L. dextranicum.

The value of the associate organisms in the cheese starter for the production of flavor and aroma in the cheese has been questioned by Kelly (25). He considered acid production to be the primary function of a cheese starter. Satisfactory cheese was made when certain strains of either S. lactis or S. cremoris were used. Hucker and Marquardt (22) reported that L. dextranicum produced a more characteristic Cheddar cheese flavor than did L. citrovorum when they were added to the pasteurized cheese milk. One strain of S. lactis alone produced a cheese with a slight acid flavor, but it compared favorably with the commercial cultures containing the associates. Other workers (16, 19, 33) have considered the associates to have little importance in determining the flavor of Cheddar cheese.

Van Slyke and Price (34) have emphasized the control of the incubation temperature of cheese cultures. The temperature of incubation has an influence upon the acid development, activity of the organisms, uniformity and quality in terms of flavor and other physical characteristics.

Hammer (16) states that the incubation temperature influences the species present and their rate of growth in the culture. By varying the

temperature of incubation of the culture above or below the optimum for S. lactis and S. cremoris, the presence of other organisms becomes apparent. Good cultures were obtained at an incubation temperature as low at 18° C. (64.4° F.) and as high as 32° C. (89.6° F.). Relatively high incubation temperatures may greatly reduce the numbers of L. citrovorum and L. dextranicum in the culture. A few transfers at 37° C. may completely remove the associate organisms. Overcast and Albrecht (28) have suggested that an incubation temperature of 80°-85° F. for 6-8 hours may reduce the number of associates without affecting S. lactis or S. cremoris strains.

The associate organisms have a lower optimum temperature for growth than S. lactis and S. cremoris. Hucker and Pederson (23) reported the optimum growth temperature of the Leuconostoc species to be between 21°-25° C.; whereas S. lactis and S. cremoris were found to be most active between 30°-40° C. (16).

Hammer and Bailey (17) have found that the volatile acid producers may be unable to grow at 37° C. Cultures incubated at 37° C. produced only 5.3-9.8 per cent volatile acid of the total acid produced; whereas at an incubation temperature of 20° C., 32.6-39.3 per cent of the acid was volatile. Hammer (15) reports that L. dextranicum may grow to a limited extent at 37° C. while L. citrovorum will not grow at all.

Hammer and Baker (18) compared the rate of growth of L. dextranicum when incubated at 21° and 37° C. Of the 116 different strains of L. dextranicum used in the study, 17.2 per cent grew better at 37° C., 48.3 per cent made better growth at 21° C., and with 34.5 per cent no observable difference occurred. When the incubation temperature varied above or below

the optimum growth temperature of the organisms, there was no sudden change in their growth rate but a gradual change with all degrees of response.

Bang (5) reported that the associate organisms produce a greater amount of diacetyl at 20° C. than at any other incubation temperature.

Golding et al. (13) have shown the rate of acid development in cheese making to be similar to the rate occurring in the cultures. Three commercial cultures were used to inoculate pasteurized skim milk. Maximum acid development occurred in 8 hours between 85°- 90° F. When the cultures were incubated at either 77° or 95° F., acid production was reduced to about half of that occurring at 85°-90° F. Incubation temperatures of either 60° or 100° F. showed insignificant acid development during the 8-hour test.

Babel (4) recognized the relationship of incubation temperature to the acid development in both cultures and cheesemaking. The rate of acid development was studied in seven different cultures when incubated at 86°, 98°, 101°, and 104° F. Three cultures produced acid most rapidly at 86° F. while four produced acid most rapidly at 98° F.

Whitehead and Cox (35) made a detailed study to determine the effect of the incubation temperature upon acid production of streptococci. Strains of streptococci differed in the temperature requirements for maximum acid production. Some strains produced acid quite rapidly at 20°-30° C. and decolorized methylene blue at 37° C. Other strains produced acid rapidly at 20°-30° C. but were slow in reducing methylene blue at 37° C. Some strains of streptococci survived 37° C. with difficulty which may help to

explain why some starters are so slow in producing acid in the latter stages of cheese making. Starters suitable for Cheddar cheese making must be active acid producers at 20°-30° C. and must be able to grow at 37° C. (35).

Toens and Hammer (32) compared cultures that were incubated at various temperatures. Incubation temperatures of 18°, 21°, 25°, and 32° C. were found to produce good cultures. The maximum temperature above which good cultures could not be produced was found to be between 32°-37° C.

Better cultures were obtained when the amount of inoculum was adjusted to produce coagulation within 6-8 hours at 21° C. (32). Dahlberg and Ferris (9) found little difference in freshly pasteurized milk inoculated with 1 per cent starter and incubated at 86° F. for 6-8 hours than when the milk inoculated with 1 per cent starter was incubated at 70° F. for 18 hours.

CHAPTER IV

EXPERIMENTAL PROCEDURE

Preparation of Cheese Cultures. In preparing milk for the mother cultures, approximately 100 milliliters of skim milk was poured into 6-ounce screw cap bottles and placed in flowing steam for one hour. After steaming, the milk was stored in a refrigerator at 4° C. until the cultures were transferred. The culture milk was raised to the incubation temperature of the cultures before the transfers were made. The same procedure was used in preparing milk for the bulk starters, except quart milk bottles were used instead of 6-ounce bottles.

The commercial cultures, FD and HP, were selected for the experiment. A comparison of each culture was made by using two temperatures of incubation. These cultures incubated at 21° C. for 15 to 18 hours served as the control cultures. The experimental cultures were obtained by incubating the cultures at 32° C. for 6 to 8 hours.

Before the experimental cultures were used in making cheese, they were transferred every day, except Sunday, for 37 days, using a 1 per cent inoculum. After this period the cultures were transferred every other day throughout the remaining portion of the experiment.

Cheese Manufacturing Methods. Six lots of surplus Grade A milk, purchased from the Knoxville Milk Producers Association, was used in making the experimental cheese. The milk was pasteurized at 144° F. for 30 minutes. The milk was then cooled to approximately 40° F. and transferred to four cheese vats. Three were 50-gallon vats, and the fourth was a 100-gallon vat.

The cheese milk was adjusted to 86° F. in the cheese vats. In each trial two vats of cheese milk were inoculated with 1 per cent of the control culture and the other two were inoculated with 1 per cent of the experimental culture. These bulk cultures were incubated at 21° C.

The method used for manufacturing the cheese was the slow heat-low heat treatment outlined by Jarman (24).

Before the cheese was paraffined, samples were taken for the determination of fat, moisture, total nitrogen, water soluble nitrogen, and pH. After the cheese aged for three months, samples were again taken for water soluble nitrogen and pH determinations.

Methods of Analysis. The samples for analysis were taken with a cheese trier. Four plugs were taken from a cheese of each vat. These samples were placed in an airtight glass jar and stored at 40° F. until the determinations were made. The moisture determinations were made immediately after sampling, and the fat, total nitrogen, water soluble nitrogen, and pH determinations were made within three days of sampling. All tests were made in duplicate.

The Babcock method outlined by Burke (8) was used in determining the fat content of the cheese, except a 20 per cent Paley cheese test bottle was used instead of a 50 per cent cream test bottle. A 4.5 gram sample was used and the results were multiplied by two. The moisture was determined by the method outlined in Official Methods of Analysis of the Association of Official Agricultural Chemists (27), except the vacuum varied from 17 to 28 inches and the time dried was 12 to 15 hours. The total nitrogen of the cheese was determined by the Kjeldahl method outlined

by Markley and Hann (26). The method outlined by Zimmerman (37) was used to determine the water soluble nitrogen.

Twenty-five grams of ground cheese was weighed to the nearest tenth on a butter-moisture balance. The cheese was then added to 240 milliliters of distilled water and mixed for 10 minutes in a Waring Blender. The resulting suspension was poured into a 300-milliliter Erlenmyer flask and incubated at 60° C. for one hour. During the incubation the suspension was shaken at 15-minute intervals. At the end of one hour of incubation, the suspension was mixed thoroughly and filtered through a No. 12 fluted filter. A 25-milliliter sample of the resulting filtrate, equivalent to 2.5 grams of the original cheese, was transferred to a Kjeldahl flask containing 10 grams of sodium sulfate and 0.1 gram of copper sulfate. Twenty milliliters of concentrated sulfuric acid was added, and the nitrogen was determined according to the Kjeldahl method (26). The results were expressed as the soluble nitrogen as per cent of the total protein.

The Quinhydrone Electrode method described by Van Slyke and Price (34) was used in obtaining the first pH determination of the experimental cheese. The Beckman pH meter was used for the other pH determinations.

The cheese was examined for slit openness at 3 weeks of age. The examinations were made by taking plugs from each lot of cheese with a cheese trier and observing under natural light.

Microbiological Examinations. The activity of the cultures was determined by the H. Leber activity test (2).

The method used for determining the associates present in the cultures was based upon their inability to produce noticeable changes in litmus milk. The cultures, propagated at 21° C. and 32° C., were plated on tomato juice agar at dilution rates to produce approximately 100 colonies per plate. The plates were then incubated at 21° C. for 5 days. From a plate of each culture, containing approximately 100 colonies per plate, the colonies were picked into test tubes containing 6-8 milliliters of litmus milk. The inoculated litmus milk was then incubated at 21° C. for 5 to 10 days. The tubes showing no acid or reduction were examined microscopically for growth. If organisms were found present, they were assumed to be associates. Those tubes showing acid, reduction, and coagulation were regarded as S. lactis and/or S. cremoris. From this the approximate percentage of the associates occurring in the cultures were determined.

The technique described by Overcast and Albrecht (28) was used for the detection of the gas producing Leuconostoc species present in the cheese. Glucose milk was used as the medium for the gas producing organisms. The glucose milk was tubed at a depth of 5-6 centimeters and sterilized by intermittent steaming for one hour on 3 successive days.

Samples were taken asceptically from each lot of cheese examined. The outer one-eighth to one-fourth inch of the surface layer of the cheese was removed with a sterile spatula before the sample was taken. The sample was removed with a sterile cheese trier and placed in a sterile petri dish. Five grams of the sample were weighed into a petri dish on a Torison Balance. A 1:2 dilution was prepared by emulsifying the 5 grams of cheese in 9.5 milliliters of 2 per cent sterile sodium citrate solution,

using a sterile mortar and pestle. The tubes of glucose milk, melted and cooled at 45° C., were inoculated with the emulsified cheese of various dilutions. An agar plug was poured on top of the inoculated glucose milk after the glucose milk had solidified. About 5 milliliters of sterile water was placed on top of the agar plug. The three-tube limiting dilution technique described by Buchanan and Fulmer (7) was used for an estimation of the associates present in the cheese.

CHAPTER V

EXPERIMENTAL RESULTS

This experiment was designed to show the effect of an incubation temperature of 32° C. upon the associate organisms in commercial cultures and upon the development of slit openness in Cheddar cheese. Commercial cultures, FD and HP, were used in making the experimental cheese. The control of each of these cultures was incubated at 21° C. for 15-18 hours, and the experimental culture was obtained by incubating at 32° C. for 6-8 hours. Eleven comparisons were made with these cultures, making a total of 22 vats of cheese. The cheese was made on six different days in the winter, spring, and summer seasons.

The control cultures had a higher percentage of associates in their flora than did the experimental cultures as shown by the inability of the associates to produce noticeable changes in litmus milk. The flora of the FD control culture consisted of 6-19 per cent associates; whereas the FD experimental culture had no associates present in the representative 1-milliliter sample. The HP control culture contained from 7-36 per cent associates and the HP experimental culture had from none to 2 per cent associates in the 1-milliliter sample.

The FD culture was more active than the HP culture based upon the resazurin reduction time. Both the FD control and experimental cultures reduced the resazurin within 45 to 50 minutes; whereas the HP control required from 55 to 60 minutes for complete reduction and the HP experimental culture required from 50 to 55 minutes to completely reduce the resazurin.

A comparison of slit openness in Cheddar cheese, made from cultures incubated at 21° and 32° C., is shown in Table I. Six comparisons were made with the HP culture and five comparisons with the FD culture. The degree of slit openness in cheese made from the FD and HP control cultures ranged from questionable evidence of slits to distinct slits; whereas the degree of slit openness in cheese made from the experimental cultures showed from no evidence of slits to questionable evidence of slits. The third comparison with the HP culture and the first comparison with the FD culture showed questionable evidence of slit openness in the cheese made from both control and experimental cultures. The other comparisons showed slit openness to be more pronounced in the cheese made from the control cultures than in cheese made from experimental cultures.

The number of gas producing organisms present in the three different cheese examined is presented in Table II. Cheese (6-4-52) 1 made with the control FD culture showed an estimate of gas producing bacteria of 125,000 while the cheese (6-4-52) 2 made with the experimental culture showed less than 200 gas producing bacteria per gram. Similar results are shown in cheese (6-4-52) 3 and (6-4-52) 4 using the HP cultures. Comparisons are also shown of the same cheese at 4 and 16 weeks of age for both HP and FD cultures. In the ten comparisons that were made, the control cultures showed a greater number of gas producing organisms per gram of cheese since no evidence of gas production was shown in the lowest dilution, which was 1:200, in the three-tube limiting dilution technique described by Buchanan and Fulmer (7).

A summary of the composition of all the experimental cheese is given in Table III. No significant difference in the composition is

TABLE I

THE DEGREE OF SLIT OPENNESS IN CHEESE MADE FROM CULTURES INCUBATED AT 21° AND 32° C. AND RIPENED AT 50° F.

Date Made	Cheese	Culture	Incubation Temperature of Culture (° C.)	Degrees of Slit Openness[a]
2-22-52	3	HP	21	++
4-17-52	1	HP	21	++
5- 1-52	3	HP	21	±
6- 4-52	3	HP	21	++
9- 4-52	3	HP	21	+++
9-11-52	3	HP	21	+++
2-22-52	4	HP_s	32	+
4-17-52	2	HP_s	32	±
5- 1-52	4	HP_s	32	±
6- 4-52	4	HP_s	32	±
9- 4-52	4	HP_s	32	±
9-11-52	4	HP_s	32	±
2-22-52	1	FD	21	±
5- 1-52	1	FD	21	++
6- 4-52	1	FD	21	++
9- 4-52	1	FD	21	++
9-11-52	1	FD	21	+++
2-22-52	2	FD_s	32	±
5- 1-52	2	FD_s	32	±
6- 4-52	2	FD_s	32	±
9- 4-52	2	FD_s	32	±
9-11-52	2	FD_s	32	−

[a] − = no evidence of slits; ± = questionable evidence of slits; + = very slight evidence of slits; ++ = slight evidence of slits; +++ = distinct slits.

TABLE II

NUMBER OF GAS PRODUCING ORGANISMS PER GRAM AS DETERMINED BY THE LIMITING DILUTION TECHNIQUE IN GLUCOSE MILK

Date Made	Cheese	Age of Cheese (Weeks)	Culture	Temperature of Incubation (° C.)	Number of Bacteria/g. Cheese
6- 4-52	1	10	FD	21	125,000
9- 4-52	1	4	FD	21	140
9- 4-52	1	16	FD	21	19,000
9-11-52	1	4	FD	21	600
9-11-52	1	16	FD	21	600
6- 4-52	2	10	FD_s	32	Less than 200
9- 4-52	2	4	FD_s	32	Less than 200
9- 4-52	2	16	FD_s	32	Less than 200
9-11-52	2	4	FD_s	32	Less than 200
9-11-52	2	16	FD_s	32	Less than 200
6- 4-52	3	10	HP	21	80,000
9- 4-52	3	4	HP	21	60,000
9- 4-52	3	16	HP	21	180
9-11-52	3	4	HP	21	60,000
9-11-52	3	16	HP	21	280
6- 4-52	4	10	HP_s	32	Less than 200
9- 4-52	4	4	HP_s	32	Less than 200
9- 4-52	4	16	HP_s	32	Less than 200
9-11-52	4	4	HP_s	32	Less than 200
9-11-52	4	16	HP_s	32	Less than 200

TABLE III

SUMMARY OF THE COMPOSITION OF THE ELEVEN COMPARISONS

Date Made	Cheese No.	Culture	Per Cent Fat	Per Cent Moisture	Total Protein	Water Soluble Protein[a] 3 Days Old	Water Soluble Protein[a] 3 Months Old	pH 3 Days Old	pH 3 Months Old
2-22-52	1	FD	32.4	39.17	23.64		30.54		5.3
	2	FD_s	32.6	39.67	23.73		30.88		5.25
	3	HP	32.4	38.30	23.80		30.79		5.2
	4	HP_s	32.0	38.62	23.77		31.29		5.2
4-17-52	1	HP	31.7	39.58	24.37	8.69	24.78	5.2*	5.2
	2	HP_s	31.9	38.85	25.30	9.29	24.34	5.19*	5.1
5-1-52	1	FD	31.8	38.18	24.83	10.23	24.80	5.2	5.2
	2	FD_s	32.2	37.38	24.89	10.57	25.39	5.2	5.2
	3	HP	31.8	38.88	24.46	10.38	25.83	5.3	5.2
	4	HP_s	31.6	37.70	24.95	9.41	25.13	5.1	5.1
6-4-52	1	FD	31.4	36.29	25.05	11.17	25.90	5.2	5.3
	2	FD_s	32.6	37.22	24.83	11.47	27.50	5.1	5.1
	3	HP	31.6	39.24	23.90	11.00	25.27	5.3	5.2
	4	HP_s	32.4	38.06	24.28	9.88	25.82	5.2	5.2
9-4-52	1	FD	31.2	38.41	24.55	18.24	35.84	5.1	5.1
	2	FD_s	31.2	38.63	24.52	21.00	34.70	5.1	5.2
	3	HP	30.8	39.34	23.88	19.93	36.09	5.3	5.2
	4	HP_s	30.8	39.58	24.05	20.91	33.76	5.2	5.2
9-11-52	1	FD	32.0	38.46	25.06	9.35	29.48	5.2	5.1
	2	FD_s	31.8	38.88	23.97	8.41	29.20	5.1	5.1
	3	HP	31.6	38.13	22.88	7.86	30.33	5.3	5.3
	4	HP_s	31.7	38.17	23.17	8.45	34.31	5.2	5.1

[a] Expressed as per cent of total protein.

s Experimental culture, incubated 32° C. for 6-8 hours.

* Quinhydrone electrode pH determination.

shown in favor of either the control or experimental cultures. The fat content of all the cheese varied from 30.8-32.6 per cent and the fat calculated and expressed as the per cent fat in dry matter ranged from 50.65-54.03 per cent which met the legal requirements as set by the Federal Register (11). The total protein content ranged from 22.88-25.30 per cent. The per cent water soluble nitrogen, calculated and expressed as per cent of total protein, varied from 6.86-21.0 per cent at 3 days of age and from 24.34-36.09 per cent at 3 months of age. The pH of the cheese varied from 5.1-5.3 at both 3 days and 3 months of age.

There was no observable difference in the flavor of cheese made from the control and experimental cultures.

The cheese made from the control cultures in some cases showed a slight bulging of the cheese surfaces. The control cultures produced a softer and a more spongy body than the experimental cultures. By pressing the surface of the cheese, the cheese made from the control cultures could be identified.

CHAPTER VI

DISCUSSION

The flora of commercial cultures that are commonly used in Cheddar cheese making consist of various strains of S. lactis and/or S. cremoris plus their associate organisms, L. citrovorum and L. dextranicum. The associates produce a relatively large amount of carbon dioxide which is the primary method for differentiating them from S. lactis and S. cremoris (12). This characteristic of the associates gives reason to suspect them as a source of slit openness since their presence in commercial cultures is quite significant. Slit openness was produced by inoculating the cheese milk with the associate organisms (28, 30). This was further proof that the associates may be a possible source of slit openness. These findings of Sherwood (30) and Overcast and Albrecht (28) have been confirmed and extended. The data presented showed that in 9 comparisons out of 11, the culture containing the greater number of associates produced the greater degree of slit openness.

The flora of a commercial culture may be influenced by the incubation temperature (16). The growth of the organisms may be slowed down or completely stopped depending on the extent the temperature is varied above or below their optimum for growth. S. lactis and S. cremoris require a higher temperature for optimum growth than do L. citrovorum and L. dextranicum. The incubation temperature that is commonly used by cheese plants was used for the control culture. These cultures were incubated at 21° C. which is optimum for the growth of the associates. The experimental culture was incubated at 32° C. which was near the optimum for S.

lactis and S. cremoris and the maximum for the associates. It seems that this would account for the removal or the reduction of the associates from the experimental culture.

Slit openness appears to be more serious at certain seasons of the year than at others. The cheese made in September showed a greater degree of slit openness than did the cheese made in the late winter, spring, and summer months. Since many of the farmers in Tennessee breed their cows to freshen in the early spring, many of the cows were probably in the later part of their lactation in September. The pastures were very short in this month due to the extremely dry summer. These two factors may have caused a variation in the composition of the milk, thereby influencing the nature of the curd to become more susceptible to slit openness. More research is evidently needed to determine the cause for the seasonality of slit openness.

The associates, L. citrovorum and L. dextranicum, are responsible for the production of the flavor and aroma compounds, acetylmethylcarbinol and diacetyl, in the commercial cheese culture, but the value of these compounds in Cheddar cheese, added by the commercial culture, is insignificant. This indicates that the associates have little value in determining the Cheddar cheese flavor.

The cheese made from the control culture showed a greater number of gas producing organisms than did the cheese made from the experimental culture. The degree of slit openness was more pronounced in the cheese containing the high numbers of gas producing organisms. It appears that a better textured cheese may be made by using certain strains of S. lactis

and/or S. cremoris with fewer associates which may be obtained by incubating the commercial culture at 32° C.

CHAPTER VII

SUMMARY

A commercial cheese culture incubated at 32° C. for 6 to 8 hours has fewer associate organisms, L. citrovorum and L. dextranicum, than when incubated at 21° C. for 15 to 18 hours. Cheese made from the latter culture, which had the greater number of associates, contained a greater number of gas producing organisms than did the cheese made from the culture incubated at 32° C. The presence of the gas producing organisms showed an influence upon the development of slit openness. The degree of slit openness in the cheese made from cultures incubated at 32° C. ranged from no evidence of slits to questionable evidence of slits, whereas the degree of slit openness in the cheese made from the culture incubated at 21° C. ranged from questionable evidence of slits to distinct slits.

There was no significant difference in the composition and flavor of the cheese made from the culture incubated at 21° C. or 32° C.

BIBLIOGRAPHY

BIBLIOGRAPHY

1. Abd-El-Malek, Y., and Gibson, T. "The Streptococci in Milk," Journal of Dairy Research, 15 (March 1948), 233-248.

2. Angevine, N. C. "H. Leber Activity Test and Angevine Adaptation of this Test for Detecting Inhibitory Substances in Milk, or Other Conditions Retarding Bacterial Development of Lactic Acid." Meyer-Blanke Company, Laboratory Division, St. Louis, Missouri, Lab. No. 20, January 1951 (Mimeographed).

3. Ashe, Farris E. "A Study of Slit Openness Defect in Cheddar Cheese," Unpublished M. S. Thesis, Department of Dairy, The University of Tennessee, June 1952.

4. Babel, F. J. "Factors Influencing Acid Production by Cheese Cultures. I. Effect of Cooking Temperatures on Acid Production in the Manufacture of Cheddar Cheese," Journal of Dairy Science, 29 (September 1946), 589-596.

5. Bang, Folke. "Temperaturens inflytande pa Streptococcus citrovorus amnesomsattning." (andning oct jasning) Suenska Mejeritidningen, 3 fig. 37 (36) (1945), 417-422. Biological Abstracts Sec. G. 21 (1947), 22973.

6. Breed, R. S., Murray, E. G. D., and Hitchens, P. A. Bergey's Manual of Determinative Bacteriology. 6th ed., Baltimore: The William and Wilkins Company, 1948. 346-348.

7. Buchanan, R. E., and Fulmer, E. I. Physiology and Biochemistry of Bacteria. Baltimore: The Williams and Wilkins Company, 1928. 8-12.

8. Burke, A. D. Practical Dairy Testing and Fundamentals of Dairying. Milwaukee: Olson Publishing Company, 1935. 188-189.

9. Dahlberg, A. C., and Ferris, F. "Influence of the Frequency of the Transfer of Lactic Starters Upon the Rate of Acid Development and Quality of Cheddar Cheese," Journal of Dairy Science, 28 (October 1945), 771-778.

10. Dorn, F. L., and Dahlberg, A. C. "Gas Production by Cheddar and Limburger Cheese Ripened in Tin Cans," New York (Geneva) Agricultural Experiment Station Bulletin No. 266 (1942).

11. Federal Register, Division of Federal Register, The National Archives, Washington, D. C., 6 (January-March 1941), 195-197.

12. Galesloot, Th. E. "An Early Gas Defect in Cheese Caused by Betacoccus (Leuconostoc) Species," Netherlands Milk and Dairy Journal, 4 (1950), 274-288.

13. Golding, N. S., Amundson, H., and Wagenaar, R. O. "Factors Affecting the Acidity in Pasteurized Skim Milk Inoculated with Commercial Lactic Starters," Journal of Dairy Science, 26 (October 1943), 909-919.

14. Hammer, B. W. "Volatile Acid Production of S. lacticus and Organisms Associated with It in Starters," Iowa Agricultural Experiment Station, Research Bulletin No. 63 (1920).

15. _____. "New Angles to the Starter Makers Problem," Journal of Dairy Science, 4 (July 1921), 227-285.

16. _____. Dairy Bacteriology. Third edition. New York: John Wiley and Sons, Inc., 1948.

17. _____, and Bailey, D. E. "The Volatile Acid Production of Starters and of Organisms Isolated from Them," Iowa Agricultural Experiment Station, Research Bulletin No. 55 (1919).

18. _____, Baker, M. P. "Studies of S. paracitrovorus Group," Iowa Agricultural Experiment Station, Research Bulletin No. 81 (1923).

19. Hansen, H. C., Bendixen, H. A., and Theophilno, D. R. "Influence of Different Starters on the Quality of Cheddar Cheese," Journal of Dairy Science, 16 (March 1933), 121-127.

20. Hucker, G. J. "Studies of the Coccaceae XIV. Certain Biochemical Reactions Produced by the Streptococci," New York (Geneva) Agricultural Experiment Station, Technical Bulletin No. 143 (1928).

21. _____. "Studies of the Coccaceae XIII Production of CO_2 by the Streptococci," New York (Geneva) Agricultural Experiment Station, Technical Bulletin No. 142. (1928).

22. _____, and Marquardt, J. C. "The Effect of Certain Lactic Acid Producing Streptococci Upon the Flavor of Cheddar Cheese," New York (Geneva) Agricultural Experiment Station, Technical Bulletin No. 117 (1926).

23. _____, and Pederson, C. S. "Studies of the Coccacaeae XVI The Genus Leuconostoc," New York (Geneva) Agricultural Experiment Station, Technical Bulletin No. 167 (1930).

24. Jarman, Edgar R. "Influence of Rate and Temperature of Cooking on the Time Required for Manufacture of Cheddar Cheese from Pasteurized Milk," Unpublished M. S. Thesis, Department of Dairy, The University of Tennessee, June 1952.

25. Kelly, C. D. "Lactic Acid Streptococci Associated With the Early Stages of Cheddar Cheese Ripening," New York (Geneva) Agricultural Experiment Station, Technical Bulletin No. 201 (1932).

26. Markley, K. S., and Hann, R. M. "A Comparative Study of the Gunning-Arnold and Winkler Boric Acid Modifications of the Kjeldahl Method for the Determination of Nitrogen," Journal of the Association of Official Agricultural Chemists, 8 (1924-1925), 455-467.

27. Official Methods of Analysis of the Association of Official Agricultural Chemists. Seventh Edition. Washington, D. C.: Association of Official Agricultural Chemists, 1952, 262-264.

28. Overcast, W. W., and Albrecht, T. W. "Gas Production in Cheddar Cheese Caused by Leuconostoc citrovorum," Journal of Dairy Science, 35 (June 1952), 554-558.

29. Prouty, C. C., and Golding, N. S. "The Action of the Associate Starter Organism in American Cheddar Cheese," Journal of Dairy Science, 29 (1946), 495-496 (Abstract).

30. Sherwood, I. R. "The Relation of Certain Lactic Acid Bacteria to Open Texture in Cheddar Cheese," Journal of Dairy Research, 10 (May 1939), 326-335.

31. _____, "The Bacterial Flora of New Zealand Cheddar Cheese," Journal of Dairy Research, 10 (September 1939), 426-448.

32. Toens, P., and Hammer, B. W. "I. Influence on Starters of Air Supply Temperature of Incubation and Rate of Ripening," Iowa Agricultural Experiment Station, Research Bulletin No. 85 (1925).

33. Vaandrager, G. "Acid Development in Cheddar Cheese and the Propagation of Starters in Cheese Factories," Department of Agriculture, Union of South Africa, Bulletin No. 304 (1950).

34. Van Slyke, L. L., and Price, W. V. Cheese. Second Edition. New York: Orange Judd Publishing Company, Inc., 1949.

35. Whitehead, H. R., and Cox, G. A. "Selection of Starter Cultures for Cheese," New Zealand Journal of Agriculture, 50 (2): (1935), 107-109.

36. _____, and Jones, L. J. "The Effect of Hydraulic Pressing on Cheese Texture," New Zealand Journal of Science and Technology, 27 (1946), 406-410.

37. Zimmerman, H. Personal Communication to W. W. Overcast (October 1951).