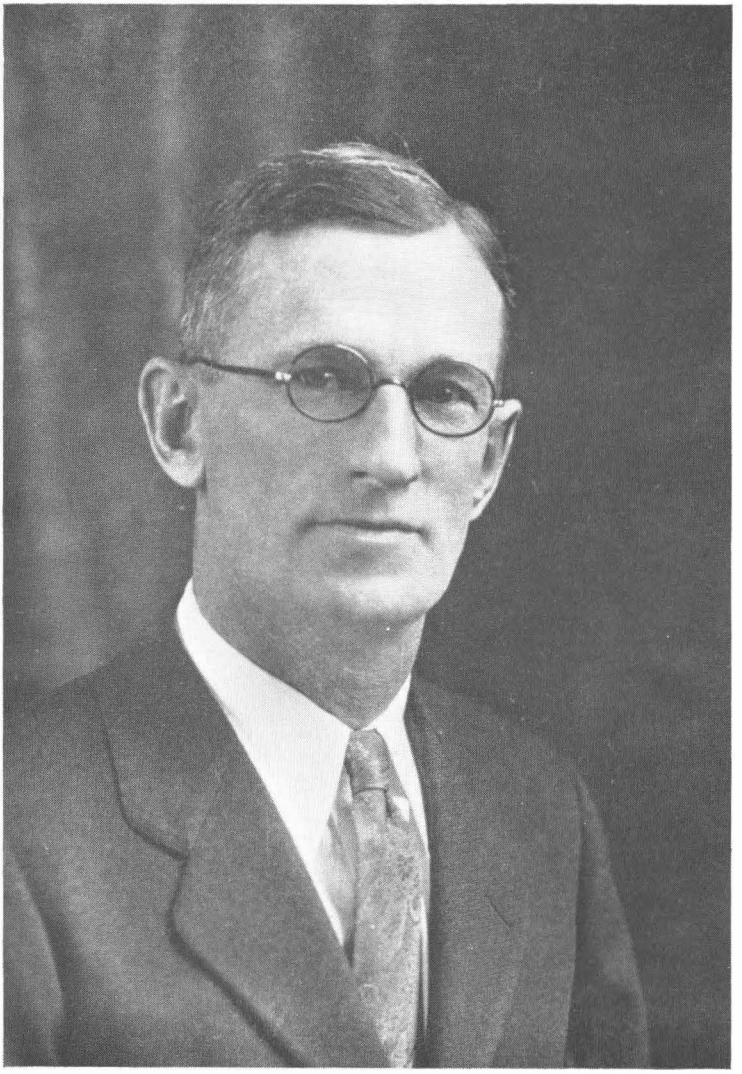

BERNARD WERNICK HAMMER

B. W. HAMMER

PANEGYRIC

By

His Former Students
at the Iowa State College

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TO

BERNARD WERNICK HAMMER

IN APPRECIATION OF HIS WORK IN TEACHING
AND RESEARCH WE DEDICATE THIS BOOK

PREFACE

In the spring of 1911 a conference was held on the campus of Iowa State College that proved to be of unusual significance to Iowa and particularly to Iowa's dairy industry. Prof. M. Mortensen, as head of the Department and Section of Dairy Industry, and the writer, as head of the Department and Section of Bacteriology, conferred long and carefully on the general problem of the future of teaching and research in the field of dairy bacteriology. A course of action was charted. It was decided that recommendations should be prepared looking toward securing for Iowa State the best trained, forward-looking young man that could be secured to develop our work in dairy bacteriology. The recommendation was approved by Dean C. F. Curtiss and by Pres. R. A. Pearson.

For our purpose it was highly desirable to secure the right man with the right training from the right school. At that time the list of institutions that could give adequate training was a short one. It did not take long to secure a list of available men who might prove satisfactory. Then came a trip to interview these men.

The man finally selected came from an institution, the University of Wisconsin, with a fine tradition of accomplishment in agricultural research, and eminence, particularly, in bacteriology and in dairying. Men such as Babcock, Russell, Hastings, Ravenel and Frost could not fail to leave their impress on their students. We followed their recommendations and invited B. W. Hammer to head our work at Iowa State College.

Dairy Industry probably exemplifies better than any other department at Iowa State College the motto of the institution, "Science with Practice." With due credit to the cordial backing and assistance of Professor Mortensen and other members of the staff, much of the success in this accomplishment is to be attributed to the point of view, training, ability and energy of Dr. Hammer. He has now served in his present position for 25 years. It is indeed fitting that his success as teacher and investigator should be celebrated by this acknowledgment from students and associates.

Formal tribute to Dr. Hammer's contributions to commercial, educational and research phases of dairying have been prepared by others for this volume. Yet it is appropriate that at least one comment be made here in an effort to diagnose the reasons for Dr. Hammer's success in his chosen field. Foremost has been his insistence upon research which is not superficial but which goes into fundamentals. No progress can be made in the solution of a problem until it has been broken down into its elements.

The kinds of organisms present, the exact chemical changes which they induce, the strict control of environment, the recognition of the complexity of the reactions involved, the stabilization of conditions so that one factor only will vary at a time, the recognition and separation of significant factors—these are among the techniques which have brought success to his research. In teaching we find an unusual ability to classify and organize material, to separate the important from the trivial and to present material in a straightforward manner and in as simple form as the nature of the complex material will permit.

In Dr. Hammer's relationships to industry there has been recognition that there are many varied and important problems to be solved and that the solution of these problems requires the marshalling of all the facts and techniques of all the sciences. In this attempt he has been remarkably successful. He speaks with authority alike to the buttermaker in the creamery and to the dairy bacteriologist in his national gatherings.

Iowa State College wishes to felicitate Dr. Hammer upon the completion of 25 years of service and to insist that what he has thus far accomplished may be but the merest fraction of what he will still be able to accomplish.

R. E. BUCHANAN
Dean of Graduate College, Professor and Head of Bacteriology, Iowa State College.

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BIOGRAPHY

HENRY GILMAN
Iowa State College

BERNARD WERNICK HAMMER was born Oct. 7, 1886, at Hillsboro, Wis., the son of Robert and Emma (Armbruster) Hammer. After graduation from the Hillsboro High School in 1904 he entered the University of Wisconsin, graduating with the B. S. A. degree in 1908. In 1909 he married Edna Quammen of Madison, Wis. Their son, Robert Q., was graduated from Iowa State College and married an alumna of the school a year ago.

Subsequent to graduation, Dr. Hammer continued his studies at the University of Wisconsin under Dr. E. G. Hastings and was assistant in agricultural bacteriology in 1908-1909. Then he was bacteriologist, associated with Dr. M. P. Ravenel, at the Wisconsin State Hygienic Laboratory, at the University of Wisconsin, from 1909 to 1911, after which he joined the Department of Dairy Bacteriology at Iowa State College in 1911. Since 1916 he has been chief in dairy bacteriology, of the Agricultural Experiment Station, and professor of dairy bacteriology.

He managed to find time from a busy academic life to continue graduate study and research; and in December, 1920, he was awarded the degree doctor of philosophy from the University of Chicago.

The foregoing is a bare recital of his *vita* up to the time of writing. It is difficult to go on and supply any personal touches, for the writer is confident that Dr. Hammer has too much becoming reserve to have anyone speak or write of him in a biographical sense. In short, it is our studied opinion that the subject of this sketch is being imposed on!

The significant contributions of Dr. Hammer in the field of dairy bacteriology need no recital here. We can speak confidently of his catholicity of scholarly interests, of his great enthusiasms, of his sustained scientific curiosity and of his almost untiring energy which have made it

possible to accomplish so much in a short period without any sacrifice of meticulous work. No individual working alone could have managed the large volume of research results, not to mention the editorial work which comprised among other things contributions to Bergey's manual of determinative bacteriology and the writing of a successful text in dairy bacteriology. Most fortunately, Dr. Hammer is not only a researcher in his own right, but a highly successful director of research as evidenced by the numerous important academic and industrial positions held by students who had their training with him.

Through it all there have been the usual accessory academic commitments: First, general teaching, which must be a pleasure to him, otherwise it is difficult to see how he can teach so effectively and excellently; second, the inevitable committee and administrative duties, for which, like a true student of research, he may have something short of a deep affection, but which have been executed with the same care and thoroughness that stamps all of his undertakings.

There is, of course, the high and warm regard which those trained with him have for one who is something more than a mere teacher. Those with whom Dr. Hammer has been associated do not fail to appreciate his deep interest in their welfare and pride in their success. All of which confirms our idea that this commemorative volume is in a way not necessary but perhaps an inevitable tangible expression of the good will of his many co-workers and colleagues.

Dr. Hammer's wide interests have carried him out of the classroom and laboratory, particularly to a diversity of athletic interests and sports. In these activities he has made the time for active participation—with his son, his students and colleagues. His intensity of purpose and interests are splendidly blended with a delightful sense of humor. Finally, it must be acknowledged that his happy family life has had much to do with the man; and the many friends of Dr. and Mrs. Hammer wish them long years of continued happiness and know that the crest of his good work is not yet in sight.

A Tribute from Associates

M. MORTENSEN
Iowa State College

IT SEEMS only a short time since Dr. Hammer, as a young man, came to Iowa State College; but 25 years have already passed since then, and during that period of time many things have taken place and much work has been done.

Comparatively few will be able to look back upon 25 years of service that has been as highly appreciated by an industry as the service rendered by Dr. Hammer during these 25 years. Unlike so many of our scientists, he has from the beginning realized that he was not merely a bacteriologist but the servant of a great industry. It was not long after his arrival in Ames before he began to study the needs of that industry, and he outlined his research so as to be able to render service where it was most needed.

Dr. Hammer has always been a strong advocate of quality, and his most important researches center around quality. It is doubtful whether there has been any piece of research turned out during the past 25 years that has been of as great importance to the dairy industry as his work on butter flavors. This work alone has greatly aided the butter manufacturers in improving the quality of their products.

It would be misleading to state that Dr. Hammer's desire for quality has been satisfied with the improvement of quality in dairy products. His desire for quality is much farther reaching. He is an ardent advocate of quality in research and in teaching; in fact, there is quality in all of the work that comes from his hands.

It is difficult to find exceptional ability in research and teaching in the same person. Dr. Hammer is an exception to that rule, since he is recognized as outstanding in both fields. The students at the Iowa State College, both graduates and undergraduates, admire him, not merely because of his knowledge of the subject he teaches, but also for his ability to

transmit that knowledge to the students as well as his many other fine qualities and the interest he takes in each individual student.

We, who have been associated with Dr. Hammer for the past 25 years, are happy for the privilege of knowing him so well. As a colleague and co-worker, no better man is to be found. He is a great asset, not merely to the Department of Dairy Industry and Iowa State College, but to the State of Iowa and the cause of dairying the world over.

A Tribute from Education and Research

E. G. HASTINGS
University of Wisconsin

THE records of the University of Wisconsin show that Dr. Hammer was a student in its College of Agriculture during the period of 1904-1908, receiving his bachelor's degree in the latter year, and that he was a member of its staff for the next 4 years. In 1912 Dr. Buchanan came to the campus, searching for a man to do research and instructional work in dairy bacteriology. We were glad to recommend Dr. Hammer to his consideration. The result is a matter of history. The issuance of this volume in commemoration of the 25 years of service to the State College of Iowa is evidence of a successful record, successful in every respect, bringing well-deserved honor to him, to his college and to his state. Wisconsin urges its claim to some small fraction of the honor.

Schools exert varying kinds of influences on their students; they supply varying types of contacts; they arouse varying interests and create varying aspirations. If one admits that Dr. Hammer's record reflects in some degree the impress the University made on him, it is content with its work.

One's memory does not recreate with any degree of clarity the pictures of 25 years ago. I cannot recall the specifications demanded by Dr. Buchanan. I am certain that he emphasized the need of ability in investigational work, and I am sure our recommendation was based on our judgment that Dr. Hammer would do research work. Such demands the qualifications of curiosity, of continued self-education and of judgment. If one has not curiosity concerning "what makes the wheels go round," he will never try to find out why they do. He will be satisfied with the explanation of his day. If the mechanism is as complicated as the one our cosmos provides, he will need to be a student all his life. His efforts will need be confined to a tiny zone of nature. He will know that the processes

active in other zones will influence those in his sphere of interest. Thus, he must, in order to do effective work, keep abreast of the advances in other fields. Adult education is no new thing in scientific research. His selection of the questions he would like to answer reflects his judgment. The field should bring abundant material to his hand, which is really saying that his research work should be on subjects which are of significance to his region and to his clientele. The long list of published papers given in this book is firsthand proof of curiosity, of continued education, of industry and of judgment, because they all refer to dairy bacteriology and primarily to butter, the chief dairy product of Iowa.

It is not needful that I should attempt to measure the extent to which Dr. Hammer's work has revealed the biological and chemical processes significant in butter, nor to indicate the practical importance of his findings. The work has been done because he wished to do it. The State of Iowa gave him the opportunity. It certainly has and will continue to receive a great return on its investment.

Every individual is immortal through the impress he makes on other individuals. Shakespeare expresses, in his sixth sonnet, the role of the parent. The role of the teacher is a similar one, many fold magnified. The worker in the creative arts and the worker in scientific research create their impress in a more evident and in a more continuing way. Two thousand years ago Horace wrote, "As long as the Pontiff climbs the Capitol with the silent Vestal by his side, I shall be famed and far beyond the boundaries of Rome I shall travel far, Barbarians unknown my name shall know." The influence of the research worker is certain to endure and be evident to those who follow, for he cuts the steps on which they rest to cut still others in order to obtain a position from which a broader and truer view of nature can be seen.

Some one has said that intelligence is adaptation to the environment. In order to adapt ourselves to nature, we must know the processes thereof. The research man is the agency that slowly reveals to us the intricate relations that exist in every field. He is, therefore, the prime factor in increasing intelligence. As a friend, a teacher and a colleague of Dr. Hammer, I congratulate him on his aid in helping us to be more intelligent.

A Tribute from the Industry

H. C. HORNEMAN
Sugar Creek Creamery Company, Danville, Ill.

TO WATCH the rising sun usher in a new day is to experience the thrill and awe of a great spectacle. The image is enduring. Its speed is phenomenal. The power of a ray of light is truly overwhelming. The smallest leaf whose tip lies in the path of its radiation is suddenly awakened. A miracle is about to be performed.

Instantly, as if by magic, the whole leaf assumes form as it flashes into vibrant action stimulated by this elixir of life. Before the senses have time to comprehend what has come to pass, every other leaf, every twig, every branch, every fiber of the tree almost simultaneously palpitates with a vigor seemingly new-born. While the mind is gloating over this discovery, the forest, the fields, the hills and the valleys spring into being. The grandeur of the onset of a new day gives way to its fruition. Little wonder that primitive people exalted the sun as a deity.

Yet beyond the horizon of the eye that one stimulus imparted to the very tip of the smallest leaf sets in motion a myriad of vital processes that no mortal will ever completely catalog. That one stimulus is only the beginning of a nation's industry, economics and life. To the Master, it is all orderly and ordained; while to man, it seems chaotic and confused unless His reason is sought, "Seek and ye shall find."

The noblest station in life is that of His servant. The character of the Great Teacher and Scientist is reflected in those who sit at His knee and receive knowledge, inspiration and leadership. Just as He sets nature in motion by a ray of light, so also does His truth impel His servants. Just as the rain, sleet and wind assail the forest, so also do misunderstanding and doubt visit upon mankind. As the rigors of nature strengthen the forest, so does adversity temper mankind.

These forces of the Master are constantly at work. One generation

fails to reap the harvest which becomes the bounty of succeeding ones. He continues to work His wondrous ways through men who labor in pursuit of the truth. The laboratory frock is as holy in His eye as the surplice of the preacher. The dominie strives to bring hope and faith to his flock. The scientist is unlocking secrets which turn factory wheels and guide the economics of industries and nations into the safe haven of progress. Has it not been truthfully stated, "Work is prayer"?

The world sorely needs the leadership of scientists. We are not blinded by friendship when we seek to elect Dr. Hammer to that hallowed circle of the Master's servants. In fact, we would be vain indeed to presume it was within our province to do so. His sane search, his humble mien, his stern duty, his kindly counsel and his scientific accomplishments provide Dr. Hammer with his credentials for such exaltation. We, as mortals, cannot do him adequate honor because of the inalienable fact that "virtue is its own reward."

The thought that God works through man has always impressed me. Particularly, in my opinion, is this true of a man who engages in research and dedicates his findings to the advancement of his fellow man. Power and wealth are puny forces, indeed, when compared with the influence of a good teacher. No one who has had the good fortune to know Dr. Hammer as friend or counselor will ever forget the warmth and glow of his personality. To know Dr. Hammer is to understand that the doctrine of "Peace on Earth, Good Will towards Man" can only become a reality when science performs its true function of furthering the knowledge that the Master reveals to those who seek truth.

The greatest tribute we can pay Dr. Hammer is to revere his mission and follow his leadership with unfaltering devotion.

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Scientific Treatises
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Bernard Wernick Hammer

Further Observations on the Quantitative Changes in the Microflora of Cream and Butter During Manufacture, Storage and Shipment[1]

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A STUDY was made in the summer of 1927 in connection with investigations to determine the changes in the microflora of cream and butter during the processing of the cream and the manufacture, storage and shipment of the butter and in an attempt to ascertain whether mold, yeast or bacterial counts of cream or butter could be used as indices of the market grade or keeping quality of the butter.

With the co-operation of an Eastern buyer, the studies were made at nine typical Minnesota co-operative creameries and at the terminus on the Eastern seaboard. The creameries were grouped into three sets, each set consisting of three creameries selected because shipments from them were made in the same car. The observations were made during the month of August, and a member of the Dairy Husbandry staff was assigned to each creamery to make the necessary laboratory tests and to record other pertinent information such as manufacturing records, temperature readings and scorings.

At each creamery, during the week of observation, each lot of cream was subjected to analyses from the time it was received until the butter was placed in the car for shipment. All laboratory tests, chemical and microbiological, were made at the creamery, where the necessary equipment had been provided from the University laboratories. The raw cream was tested for fat, acidity, mold, yeast and bacterial counts and then graded. Immediately after pasteurization and cooling, samples were taken for mold, yeast and bacterial counts. Where flash pasteurization was used, a composite sample was collected during the process. When

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starter was used, an additional sample was taken for analysis just before the cream was pumped to the churn. A sample of the finished butter was taken from the churn, following working, for complete Kohman analysis and microbiological examination. The day the butter was shipped a sample was taken from each churning for analysis. Each churning of butter was scored when fresh and when shipped.

The butter in all creameries was printed, wrapped in parchment and placed in 60-pound boxes for shipment. The print from which the sample was taken before shipment was marked and placed in a regular box for examination in the East.

A maximum-minimum registering thermometer was fastened securely in a separate box, which was placed near the center of the refrigerator car before the car door was sealed.

Complete manufacturing records for each churning, temperature of atmosphere inside and outside the creamery, and of the butter storage room during the day, were kept. Observations were also made daily on the weather.

At the Eastern market, when the butter arrived, samples were taken by the author from each churning for scoring, chemical analysis and mold, yeast and bacterial counts. The readings of the thermometers in the cars were also recorded.

All plates were poured with whey agar and acidulated with tartaric acid in the case of mold and yeast analyses. For mold and yeast counts incubation was at room temperature for three days; for bacterial counts, seven days.

The data showing the changes in the microflora during manufacture, storage and shipment are presented in tables 1-3.

Table 1 shows that pasteurization (165° F. at creameries 1, 4, 6, 7, 8 and 9, 145° F. in coil vats at creameries 2 and 3, and 180° F. flash at creamery 5) was especially efficient in destroying molds in the cream at creameries 1, 2, 3 and 4, ranging from 99.6 to 100 percent effective. Results were fair in this respect at creameries 6 and 8 but least satisfactory at creamery 5, where the flash system was used. With the high-acid cream at creameries 7 and 9 the percentage reduction of molds was marked where the original counts were high, but the final counts were not as low as might be desired. As indicated in table 2, creameries 1, 2, 3, 4 and 6 had the best records for the destruction of yeasts by pasteurization. The remarkable reduction in bacterial counts as a result of pasteurization is clearly demonstrated in table 3. Altogether it is apparent that proper pasteurization may be relied upon to reduce the numbers of molds, yeasts and bacteria in cream in a striking manner.

Tables 1 and 2 indicate that certain creameries using starter were carrying cultures notably contaminated with molds and yeasts.

The data definitely demonstrate that the churn was a very important source of molds, yeasts and bacteria because in the majority of cases the

TABLE 1. *Changes in mold counts of cream and butter*

Creamery number	Percent acidity of cream	Mold counts — number per ml.						Score of butter at market
		Raw cream	Pasteurized cream	Pasteurized cream + starter	Fresh butter	Butter when shipped	Butter at market	
1	.16	170	0	....	1	4	0	91
1	.16	170	0	....	20	8	3	92
1	.16	190	0	....	2	1	3	91½
1	.14	70	0	....	0	2	1	91½
1	.14	70	0	....	10	9	5	92½
1	.15	270	0	....	3	3	1	91
1	.15	270	0	....	6	3	4	91½
1	.16	160	0	....	5	3	4	91
1	.14	600	0	....	1	2	0	90
1	.14	600	0	....	10	3	2	91
2	.16	1200	1	0	2	3	2	92
2	.16	1100	0	0	1	0	0	91½
2	.16	1100	0	0	0	0	1	90½
2	.13	70	0	....	1	1	0	92
2	.14	20	0	....	10	0	0	91
2	.14	20	0	....	0	2	0	93
2	.15	110	0	0	12	2	2	92
2	.13	180	0	0	2	18	24	92
3	.20	270	0	13	3	5	10	92
3	.20	270	0	6	0	6	20	92½
3	.20	60	0	....	2	0	2	92
3	.17	90	0	10	70	8	10	92
3	.17	90	0	4	70	8	6	91
3	.19	700	0	4	4	3	20	92
3	.19	50	0	....	460	3	1	90½
4	.14	190	0	0	5	6	2	92
4	.20	1600	0	....	5	9	1	91½
4	.22	1700	1	....	10	2	3	91
4	.14	180	0	....	3	10	5	92½
4	.14	600	1	....	6	49	8	91
4	.17	330	0	....	1	6	3	92
4	.13	330	0	....	26	28	1	91½
4	.21	230	0	....	12	14	2	90½
4	.23	230	1	....	6	21	9	90
4	.13	480	0	....	0	15	2	92
5	.17	120	4	4	20	190	4	91
5	.19	100	70	60	14	170	3	91
5	.12	90	1	10	180	56	11	92½
5	.12	90	0	....	19	9	2	92½
5	.11	300	5	....	760	120	4	92
5	.13	400	0	1	0	80	4	90½
5	.11	90	1	....	4	10	2	92

TABLE 1. (Continued)

Creamery number	Percent acidity of cream	Mold counts — number per ml.						Score of butter at market
		Raw cream	Pasteurized cream	Pasteurized cream + starter	Fresh butter	Butter when shipped	Butter at market	
6	.16	30	1	....	3300	4610	1700	92
6	.12	30	0	....	2200	2460	900	92½
6	.12	70	0	....	....	....	....	.....
6	.10	230	0	....	....	3630	1860	92
6	.12	.....	0	....	3200	3300	6	91½
6	.10	250	3	....	4200	2670	2	92
7	.28	5500	0	....	5	1	3	92
7	.34	2900	80	....	11	14	7	92
7	.32	2900	0	....	40	2	2	91
7	.30	5000	1	....	0	4	2	92
7	.26	1400	0	....	4	2	5	92
7	.30	4300	1	....	6	10	12	92
7	.28	5200	15	....	7	6	0	92
7	.32	4500	4	....	4	1	2	92
7	.32	4000	0	....	7	4	4	91½
7	.32	7800	2	....	1	1	1	92
8	.19	700	0	....	12	15	21	92
8	.19	90	0	....	11	8	4	92
8	.19	480	1	....	5	10	3	92
8	.14	6	0	....	9	10	4	92
8	.20	380	10	....	13	19	10	92
8	.21	110	0	....	9	13	11	92
8	.24	170	0	....	16	38	11	92
9	.26	3900	5	....	8	2	....	.....
9	.33	6700	7	....	8	1	....	.....
9	.39	22500	8	....	10	3	....	.....
9	.25	1000	0	....	4	2	....	.....
9	.27	1000	2	....	1	6	....	.....
9	.29	500	4	....	4	50	....	.....
9	.30	2600	3	....	7	3	....	.....

fresh butter gave higher mold and yeast counts and in some instances higher bacterial counts than the cream from which the butter was made. The very bad condition of the churn in creamery 6 is particularly impressive.

It will be noted that there is no consistency in the changes taking place in the mold, yeast or bacterial counts from the time the butter was made until it reached the market.

An interesting relationship between the acidity and the mold, yeast and bacterial counts of the raw cream is revealed in table 4. It is clear that there is the expected correlation between the total number of microorganisms and the acidity. The important fact is, however, that even so-

Creamery number	Percent acidity of cream	Yeast counts — number per ml.					
		Raw cream	Pasteurized cream	Pasteurized cream + starter	Fresh butter	Butter when shipped	Butter at market
1	.16	370	0	.....	15	15	11
1	.16	370	0	.....	2,500	45	56
1	.17	420	0	.....	150	20	7
1	.14	380	0	.....	27	31	47
1	.14	380	0	.....	29	16	28
1	.15	820	0	.....	30	25	23
1	.15	820	0	.....	70	19	155
1	.16	1,550	0	.....	7	1	2
1	.14	620	0	.....	0	4	2
1	.14	620	0	.....	11	4	11
2	.16	600	0	0	40	3	58
2	.16	450	0	0	0	2	7
2	.16	450	0	0	0	1	14
2	.13	2,020	0	.....	5	11	51
2	.14	160	0	.....	120	7	23
2	.14	160	0	.....	6	13	11
2	.15	700	0	0	65	0	86
2	.13	1,290	0	0	13	2	17
3	.20	240	0	620	51	63	198
3	.20	240	0	76	18	16	216
3	.20	510	0	.....	8	2	28
3	.17	880	0	280	290	10	115
3	.17	880	0	19	30	34	66
3	.19	200	0	14	26	17	310
3	.19	350	0	.....	600	90	200
4	.14	700	0	0	250	270	98
4	.20	2,300	0	.....	39	10	14
4	.22	1,800	7	.....	6	126	15
4	.14	230	0	.....	34	480	41
4	.14	1,700	0	.....	11	70	14
4	.17	540	0	.....	3	4	0
4	.13	580	0	.....	15	7	29
4	.21	790	0	.....	5	7	0
4	.23	790	0	.....	4	12	2
4	.13	1,200	0	.....	17	5	7

TABLE 2. (Continued)

Creamery number	Percent acidity of cream	Yeast counts — number per ml.					
		Raw cream	Pasteurized cream	Pasteurized cream + starter	Fresh butter	Butter when shipped	Butter at market
5	.17	1,320	0	0	74	100	110
5	.19	820	10	10	10	70	55
5	.12	750	0	0	780	66	20
5	.12	850	0	.....	37	22	15
5	.11	610	6	.....	510	460	220
5	.13	1,290	3	0	0	580	340
5	.11	120	0	.....	16	33	10
6	.16	750	0	.....	150	30	130
6	.12	79	0	.....	510	130	200
6	.12	120	0	.....	.....	.....	.....
6	.10	590	0	.....	.....	140	160
6	.12	.....	0	.....	4,500	320	32
6	.10	370	0	.....	200	220	65
7	.28	4,000	0	.....	20	9	150
7	.34	1,500	20	.....	3	5	1,680
7	.32	200	0	.....	70	5	25
7	.30	100	8	.....	1,400	1	20
7	.26	300	0	.....	20	4	10
7	.30	1,900	0	.....	5	3	13
7	.28	1,200	4	.....	20	4	30
7	.32	4,400	70	.....	18	1	10
7	.32	1,700	0	.....	12	24	36
7	.32	4,500	0	.....	0	3	10
8	.19	320	8	.....	45	16	45
8	.19	1,500	8	.....	6	10	9
8	.19	1,400	1	.....	11	2	28
8	.14	430	2	.....	1	10	37
8	.20	1,100	0	.....	6	19	48
8	.21	330	0	.....	0	38	61
8	.24	430	0	.....	3	23	60
9	.26	3,200	0	.....	3	0	.....
9	.33	6,200	0	.....	2	0	.....
9	.39	20,000	0	.....	15	1	.....
9	.25	700	0	.....	4	0	.....
9	.27	600	2	.....	0	0	.....
9	.29	840	10	.....	1	0	.....
9	.30	2,800	7	.....	4	0	.....

TABLE 3. *Changes in bacterial counts of cream and butter*

Creamery number	Percent acidity of cream	Bacterial counts — number per ml.					
		Raw cream	Pasteurized cream	Pasteurized cream + starter	Fresh butter	Butter when shipped	Butter at market
1	.16	127,000,000	26,000	.....	2,800	200	600
1	.16	127,000,000	26,000	.....	6,500	1,800	3,250
1	.17	134,000,000	300	.....	2,100	500	366
1	.14	44,000,000	5,300	.....	3,600	400	630
1	.14	44,000,000	5,300	.....	2,700	400	2,300
1	.15	78,000,000	28,900	.....	5,500	200	1,100
1	.15	78,000,000	28,900	.....	9,800	2,600	21,200
1	.16	67,000,000	28,000	.....	6,700	900	1,120
1	.14	175,000,000	20,500	.....	3,100	700	70
1	.14	175,000,000	20,500	.....	9,600	2,300	850
2	.16	90,000,000	1,300,000	1,100,000	1,130,000	1,000,000	82,000
2	.16	30,000,000	.....	1,500,000	970,000	1,000,000	48,000
2	.16	30,000,000	.....	580,000	1,860,000	1,000,000	740,000
2	.13	32,000,000	1,950,000	.....	26,200	12,500	5,600
2	.14	101,000,000	1,100	.....	1,700	2,200	1,900
2	.14	101,000,000	1,100	.....	1,100	45,000	1,100
2	.15	86,000,000	200	2,800,000	32,000	500	34,000
2	.13	560,000,000	240,000	10,700,000	17,000	9,900	12,200
3	.20	246,000,000	7,300	.....	217,000	6,900	2,300
3	.20	246,000,000	7,300	.....	132,000	8,500	33,000
3	.20	118,000,000	.....	.....	240,000	29,100	20,000
3	.17	92,000,000	2,200	.....	930,000	8,500	26,000
3	.17	92,000,000	2,200	.....	110,000	77,000	21,400
3	.19	189,000,000	1,800	.....	169,000	29,300	128,000
3	.19	167,000,000	23,600	.....	16,000	9,600	14,200
4	.14	62,000,000	3,800	73,000	1,300	2,400	6,100
4	.20	534,000,000	9,500	.....	5,400	1,800	3,800
4	.22	446,000,000	4,600	.....	3,700	1,200	10,200
4	.14	56,000,000	1,300	.....	900	2,200	12,100
4	.14	145,000,000	6,100	.....	4,900	1,400	2,100
4	.17	171,000,000	2,600	.....	.....	.....	.....
4	.13	75,000,000	.....	.....	11,300	4,500	1,300
4	.21	391,000,000	3,100	.....	2,600	400	100
4	.23	393,000,000	2,000	.....	380	600	330
4	.13	119,000,000	100	.....	400	100	290

TABLE 3. (Continued)

Creamery number	Percent acidity of cream	Bacterial counts — number per ml.					
		Raw cream	Pasteurized cream	Pasteurized cream + starter	Fresh butter	Butter when shipped	Butter at market
5	.17	304,000,000	25,100	40,000,000	940,000	96,000	13,600
5	.19	206,000,000	1,000	100,000,000	1,000,000	520,000	2,700
5	.12	87,000,000	10,000	2,300,000	12,200	10,800	7,600
5	.12	40,000,000	4,000	.....	3,700	3,000	1,700
5	.11	68,000,000	12,000	.....	4,700	2,900	5,600
5	.13	93,000,000	800	100,000,000	1,000,000	182,000	4,800
5	.11	500,000,000	1,000,000	.....	560,000	12,600	6,000
6	.16	218,000,000	10,800	.....	88,000	1,500	30,000
6	.12	11,000,000	300	.....	27,200	3,300	37,000
6	.12	19,000,000	9,800	.....	.....	.....	.....
6	.10	7,000,000	400	.....	39,000	2,900	52,000
6	.12	.....	100	.....	26,500	600	6,000
6	.10	9,700,000	20,500	.....	24,800	3,500	14,000
7	.28	500,000,000	2,000	.....	4,100	15,400	8,300
7	.34	400,000,000	384,000	.....	82,000	14,000	460,000
7	.32	500,000,000	500	.....	4,000	7,800	8,700
7	.30	300,000,000	2,400	.....	208,000	7,600	8,600
7	.26	224,000,000	1,200	.....	2,800	1,000	1,900
7	.30	390,000,000	1,500	.....	2,500	2,400	2,500
7	.28	480,000,000	73,000	.....	8,100	3,400	1,800
7	.32	480,000,000	1,000,000	.....	187,000	12,700	3,400
7	.32	292,000,000	200	.....	2,000	5,300	2,800
7	.32	460,000,000	24,600	.....	300	700	1,400
8	.19	172,000,000	3,700	.....	3,900	900	6,100
8	.19	150,000,000	11,000	.....	5,800	600	7,000
8	.19	208,000,000	9,500	.....	3,900	400	5,500
8	.14	51,000,000	19,600	.....	5,600	2,000	4,800
8	.20	288,000,000	15,700	.....	26,500	37,000	104,000
8	.21	144,000,000	1,000	.....	1,400	300	2,500
8	.24	233,000,000	22,100	.....	10,700	16,000	34,000
9	.26	330,000,000	2,000	.....	1,200	200	.....
9	.33	267,000,000	13,200	.....	1,900	.....	.....
9	.39	370,000,000	700	.....	500	200	.....
9	.25	211,000,000	4,600	.....	600	200	.....
9	.27	156,000,000	800	.....	2,000	200	.....
9	.29	208,000,000	29,000	.....	12,000	100	.....
9	.30	252,000,000	1,000,000	.....	81,000	200	.....

TABLE 4. *Relation between acidity of cream and numbers of microorganisms*

Number of samples	Percent of acidity	Mold count per ml.			Yeast count per ml.			Bacterial count per ml.		
		Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum	Average
41	.10-.20	6	1,600	304	79	2,300	762	7,000,000	560,000,000	145,000,000
6	.21-.25	110	1,700	573	330	1,800	807	144,000,000	446,000,000	303,000,000
9	.26-.30	500	5,500	3,265	100	4,000	1,660	156,000,000	500,000,000	315,000,000
7	.31-.39	2,900	22,500	7,330	200	20,000	5,500	267,000,000	500,000,000	396,000,000

called "sweet cream" below 0.20 percent acidity contained large numbers of bacteria.

Table 5 indicates that there was a greater tendency for samples of butter with higher salt contents to show decreasing mold and yeast counts immediately after manufacture. On the other hand, this salt effect was largely lost after a few days. In the case of bacterial counts, the salt effect is not as noticeable in the early stages but becomes more pronounced with the passage of time. The bacterial counts showed a tendency to decrease in the fresh butter regardless of the salt content, while the survivors later were apparently under the influence of the brine. These data agree in general with those presented by others and offer some suggestive explanation for changes in the microflora of butter.

An analysis of the data to determine any possible relationships between the temperature of creamery coolers, or of refrigerator cars in transit, and the changes in the microflora or quality of the butter did not yield any positive relationships. The temperatures in creamery coolers ranged from 30-45° F. during the period of investigation, while the minimum-maximum temperatures of the refrigerator cars from the time they

TABLE 5. *Effect of salt content of butter on the quantitative changes in the microflora during storage and shipment*

Number of samples	Salt percentage	Mold counts: Percent of samples showing changes in count — Fresh butter to time of shipment: Increase	Decrease	No change	From time of shipment to arrival at market: Increase	Decrease	No change
7	1.0-1.9	71.4	28.6	0.0	0.0	100.0	0.0
26	2.0-2.4	46.2	50.0	3.8	30.4	60.9	8.7
28	2.5-2.9	53.6	38.6	7.8	18.5	77.8	3.7
10	3.0-3.4	33.3	55.6	11.1	40.0	30.0	30.0
		Yeast counts					
7	1.0-1.9	42.9	42.9	14.2	75.0	25.0	0.0
26	2.0-2.4	42.3	53.9	3.8	65.2	34.8	0.0
28	2.5-2.9	39.3	60.7	0.0	59.3	40.7	0.0
10	3.0-3.4	33.3	66.7	0.0	90.0	10.0	0.0
		Bacterial counts					
7	1.0-1.9	28.6	71.4	0.0	100.0	0.0	0.0
26	2.0-2.4	12.0	88.0	0.0	59.1	40.9	0.0
28	2.5-2.9	14.8	85.2	0.0	61.5	38.5	0.0
10	3.0-3.4	30.0	70.0	0.0	40.0	60.0	0.0

left the siding at the creamery until they reached the market were as follows: 48-50° F., 46-52° F., 47-59° F., 42-63° F. and 35-65° F. The butter reached the market from 8 to 15 days after it was made.

Scorings of the butter upon its arrival at the market proved that the quality of the butter at that time was slightly better in those cases where the mold, yeast and bacterial counts of the raw cream were low. The scores showed a tendency to be somewhat higher when the butter was made from cream of the lower acidities. On the other hand, there was no relation (a) between the final butter quality and the mold, yeast or bacterial counts of the pasteurized cream nor (b) between the mold, yeast or bacterial counts of the fresh butter and the change in score during storage or transit. The changes in mold, yeast or bacterial counts of the butter were not influenced by the days in storage before shipment nor the days in transit.

SUMMARY

1. Data were obtained at nine Minnesota creameries and at an Eastern market on the quantitative changes in the microflora of cream and butter during manufacture and shipment.

2. Pasteurization of the cream was effective in most instances in reducing the numbers of microorganisms.

3. The churn in many cases was shown to be an important source of contamination.

4. Changes in the microflora of the butter did not follow any consistent pattern.

5. The number of microorganisms was usually greater in the more acid cream, although the number present in so-called "sweet cream" was often remarkably high.

6. The amount of salt in the butter often influenced the trend of the mold, yeast and bacterial counts but this effect was not uniform.

7. No positive relationship was established between the temperature of the creamery coolers or refrigerator cars involved in these studies and the changes in the microflora or market grade of the butter.

8. Neither a mold, yeast nor bacterial count of the butter would have served as a reliable index of the market or keeping quality of the butter made at these creameries. There was a tendency toward higher scores, however, when the butter was made from cream which was low in mold, yeast or bacterial count before pasteurization.

Churn Contamination as a Source of Yeasts and Molds in Butter

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YEAST and mold counts on butter have been of value in indicating the efficiency of pasteurization of the cream and the sanitary condition of the equipment—vats, pipe lines and churns—in which the pasteurized cream and the butter are handled. That yeasts and molds do not play a significant part in determining the flavor score and keeping quality of butter of medium or high salt content has been shown by several investigators. Thus, Grimes (3) graded and examined 135 samples of butter for total bacterial count, yeasts and molds but found no correlation between the flavor score of the butter when two weeks old and the results of any of the microbiological examinations.

Macy, Coulter and Combs (10) reported that the flavor of salted butter stored for one month at 35° F. was uniformly good and that the majority of samples showed decreasing counts of bacteria, yeasts and molds during storage. For unsalted butter, however, these investigators reported an entirely different situation; there was a noticeable tendency for the bacterial, yeast and mold counts to increase during storage, accompanied by serious deterioration in flavor. The possibility of a relationship between this deterioration and the increases in counts is pointed out. Olson and Hammer (13) found no significant differences in keeping qualities of salted (2.5%) butter from clean and from contaminated churns.

The importance of churn sanitation in the manufacture of unsalted butter and butter of low salt content has, however, been fully recognized. Thus, Olson and Hammer (13) showed that unsalted butter from clean churns possessed keeping qualitity at 45° F. distinctly superior to that of unsalted butter from contaminated churns. Experience of the author in commercial manufacture of unsalted butter from cream ripened to 0.40 percent acidity has amply demonstrated the necessity of low yeast and

mold counts if the butter is to have good keeping quality throughout the ordinary holding period in fresh consumption channels.

The influence of the churn as a source of contamination of the butter has been studied by a number of investigators. Gregory (2), Lund (8), Hood and White (5) and Brown (1) presented some of the early results pointing to the churn as an important source of yeasts and molds in butter. Hunziker (6) states that the churn is the most difficult piece of equipment to sterilize and recommends the use of boiling water. Macy and Combs (9) made a survey of commercial creameries in 1925 and 1926 to investigate sources of mold in butter and found that the churn was one of the principal sources of contamination from equipment. James (7) used both boiling water and chlorine compounds in attempting to sterilize churns and discovered that molds and yeasts were worked from the churns (into a sterile water rinse) after extreme exposures to hot water and to chemicals.

Macy, Combs and Morrison (11) showed that the churn may be an important source of mold in butter. Morrison, Macy and Combs (12) studied the effects of hot water, steam and chlorine compounds on the microflora in churns and found that the hot water treatment was most effective but that it must be administered daily to prevent the churn from becoming a serious source of contamination. Chlorine compounds were not found effective.

Olson and Hammer (13), however, found that treating highly contaminated churns regularly with either sodium hypochlorite or a chloramine preparation resulted in large reductions in the numbers of organisms present. Using either the rinse or agar disc method of judging results, hot water effected "striking" reductions in the numbers of organisms, yeasts and molds being largely eliminated.

The agar disc method for studying contamination from churns, developed and reported by Hammer and Olson (4), consists of allowing a small amount of an agar medium to solidify in contact with the wood of the churn and transferring it to a sterile petri dish for incubation.

While in some cases the type of churn used was not reported in the literature, apparently most, if not all, of the work has been done with churns of the combined churn-and-worker type, with the workers permanently installed in the churn. The present work was done (1) to study the yeast and mold content of butter made in Simplex churns, which have a set of workers to be run into the churn only for the working of the butter, and (2) to determine if some sterilizing treatment could be devised which would leave the churn and workers in satisfactory condition from a bacteriological point of view. This work received impetus from the practical necessity of reducing churn contamination to the point which would permit the manufacture of unsalted butter possessed of keeping quality—that is, the retention of the desired culture flavor and aroma throughout its life in the hands of the consumer.

METHODS

All work herein reported was done in a commercial creamery equipped with 1,000-gallon Jensen vertical-coil vats and No. 12 Simplex churns, the average churning being approximately 1,000 pounds of butter. A common method for determining the extent of contamination left in a churn after a "sterilizing" treatment has been to rinse the churn with sterile water and to make plate counts on the water after agitation in the churn. Inasmuch as the present work was done with commercial creamery equipment, it was not possible to prepare the quantities of sterile water necessary to adequately rinse a churn of the size employed. Ordinary city water, therefore, was used. While bacterial and yeast and mold counts were made on the water after rinsing the churn, and in a number of cases on the water entering the churn also, it was thought highly desirable to follow through and determine the yeast and mold count of the cream in the churn after a short run and of the finished butter. In a number of cases counts were made from the start—the pasteurized cream in the vat—through the several stages to the finished butter.

While it is recognized that there would be some breaking up of clumps of cells in the agitation of cream in the churn, the comparatively low yeast and mold counts secured on the cream after a 10-minute run in the adequately treated churn are believed to indicate that the breaking up of clumps was of minor importance as compared with contamination from the churn barrel in accounting for the increase in yeast and mold count from the cream in the vat to the cream in the churn. In a few preliminary trials, in which a small, sterile, enameled churn was used in the laboratory for churning 1,500-gram quantities of cream, it was not possible to demonstrate by increased plate counts any definite breaking up of clumps of yeast and mold cells. After a churning period of 10 minutes the counts showed slight decreases in two cases and a slight increase in another, the average for the three trials being 52 colonies before churning and 50 colonies after.

Comparisons were made between various churn treatments, each of two churns receiving a different "sterilizing" treatment in any one experiment. Cream from the same vat was used for the two churns so that the butter from them was directly comparable. Yeast and mold counts were made on whey agar with 1 ml. of 1 percent tartaric acid per plate in the early part of the work, and on potato dextrose agar adjusted to pH 3.5 with tartaric acid in the later experiments. All counts reported are the average of duplicate plates. Incubation was for 5 days at 20° to 25° C.

EXPERIMENTAL

At the beginning of the experimental work the churns had been in service for two years, during which time they had received a routine treatment which will be designated as "ordinary" hot water and which consisted of the following:

1. In afternoon after butter was removed.
   a. 50 gallons (approximately) hot water, 150° F., with Wyandotte Cleaner and Cleanser, or tri-sodium-phosphate. Churn run 5 minutes with workers in.
   b. 150 gallons (approximately) water at 210° F. Churn run 15 minutes, workers in.
2. Next morning.
   a. 50 gallons cold water, churn run 5 minutes with workers in.

That this treatment was not efficient in the destruction of germ life in the churns and workers and in the production of butter with a low yeast and mold count had been known. A series of bacterial and yeast and mold counts on the cold churn-rinse water in the morning and yeast and mold counts on the butter was made at the beginning of the experimental work, and it was found that the cold rinse water had a plate count of 12,000 to 50,000 bacterial colonies per ml. and a yeast and mold count varying from 200 to 1,000 per ml. Expressed on another basis, the 50 gallons of rinse water removed from 2,270,000,000 to 9,460,000,000 bacteria and from 37,800,000 to 189,200,000 yeasts and molds (plate counts) from the churns. The yeast and mold count of the butter churned in such churns varied from 100 to 700 per ml. While it was not possible to use sterile water for the routine rinse of 50 gallons of cold water, the bacterial and mold counts on the water used were always very low as shown by numerous check determinations on samples of the water taken as it was going into the churn. The average bacterial count of 47 per ml. by the plate method for 14 determinations on separate days is believed to warrant the assumption that the bacterial content of the water going into the churn was unimportant as a source of the large numbers of organisms found in the same water coming out. The high yeast and mold counts of the water after rinsing the churns were essentially yeast counts, since the yeasts usually greatly outnumbered the molds. Yeasts were never found in the water going into the churns.

VIGOROUS HOT WATER TREATMENT

It seemed likely that the poor results secured with the "ordinary" hot water treatment were to be attributed to the fact that the last rinse of boiling water was used with the workers in the churn, causing a rapid drop in temperature and the loss of a good portion of the water in the first five minutes' run. Table 1 gives a record of the temperatures observed.

The rapid drop in temperature of the water from the boiling point to around 150° F. in five minutes and to only 125° F. in fifteen minutes easily explains the survival and growth of enormous numbers of bacteria, yeasts and molds as revealed by the counts on the cold rinse water the following morning.

TABLE 1. *Decrease in temperature of churn wash water*
Temperatures (degrees F.) on separate days

	1	2	3	4	5	6
Water in tank	206	210	210	210	210	210
Water in churn	185	.....	183	187	.....	.....
Water after 6 revolutions	.....	182	.....	.....	.....	.....
Water after 5 min. run (½ water lost)	.....	158	148	146	.....	.....
Water after 15 min. run (very little water left)	125	132	125	122	124	126

It was accordingly decided to try the boiling water rinse (150 gallons) at night in a closed churn and attempt to give the workers treatment outside of the churn with scalding water after they had received the alkali rinse in the churn. A daily comparison was made between two churns, one having the "ordinary" hot water treatment and the other the so-called "vigorous" hot water treatment. Both churns were given only a cold rinse of about 50 gallons of water in the morning before cream was pumped in. One vat of cream was used for each pair of churnings so that the butter counts were directly comparable. The results of this comparison are given in table 2.

TABLE 2. *"Ordinary" treatment vs. "vigorous" hot water treatment*

	Ordinary—workers in			Boiling water (150 gal.) in closed churn, run 15 or 30 minutes		
	Plate count—colonies per ml.			Plate count—colonies per ml.		
	Cold A. M. rinse		Butter—	Cold A. M. rinse		Butter—
Exp. No.	Bacteria	Yeasts & molds	yeasts & molds	Bacteria	Yeasts & molds	yeasts & molds
4	3,800	500	.....	4,300	400	.....
5	6,500	400	.....	9,000	450	.....
6	49,500	550	.....	9,200	400	.....
9	54,000	760	.....	810	16	.....
10[1]	8,700	390	92	45	29	15
11	9,000	220	200	86	60	86
12	11,800	680	180	10,500	40	8
13	18,000	1,060	340	1,050	36	9
14	20,800	1,500	530	1,620	79	31
15	13,200	510	330	11,000	53	100
16	21,100	1,190	600	5,600	68	89
17	21,200	1,070	380	9,300	165	49
18	29,000	700	710	3,000	59	1,470
Ave.	20,507	733	373	5,038	143	206

[1] In experiments 10 to 18, inclusive, cold morning rinse was in closed churn.

The bacterial counts on the cold rinse water in the morning varied from 3,800 to 54,000 per ml. for the "ordinary" hot water treatment and averaged 20,507, compared with limits of 45 and 11,000 and an average of 5,038 per ml. for the "vigorous" treatment. Yeast and mold counts on the rinse water averaged 373 per ml. for the "ordinary" treatment and 143 per ml. for the "vigorous." The yeast and mold counts on the butter showed decidedly the advantage of the closed-churn treatment. Eight of the nine churnings in the direct comparison had a count of 100 per ml. or less and averaged 48. The one high count of 1,470 brought the average up to 206 per ml. On the other hand, the butter from the same cream (each pair from the same vat) but churned in the churn that received the "ordinary" treatment had only one out of nine churnings below 100 per ml. in yeast and mold count, the others varying from 180 to 710 per ml. and averaging 373.

Thus, a definite improvement in the bacteriological condition of the butter was brought about by using a boiling water rinse in a closed churn at night and scalding the workers outside of the churn. In this way the churn barrel itself received the full benefit of the hot water, although the bacterial counts on the cold rinse water left much to be desired in the approach toward sterility. The average bacterial plate count of 5,038 colonies per ml. of cold rinse water in the morning meant that over 950,000,000 cells and clumps were removed from the churn by the 50 gallons of rinse water. This treatment, it was recognized, left the workers as a rather important source of contamination of the butter since it is impossible to secure adequate treatment of the wood through scalding water or steam. Undoubtedly the condition of the workers was responsible for the one high count of 1,470 yeasts and molds per ml. among the "vigorous" treatment counts.

USE OF CHLORINE STERILIZERS

A. *Sodium Hypochlorite*

Because of economic reasons—the large amount of steam required to prepare adequate quantities of water for the boiling water treatment for all churns and the time element involved—it was thought desirable to determine the efficiency of chlorine sterilizers. Accordingly, a number of comparisons were made between hot water and sodium hypochlorite, one churn receiving a hot water treatment for a series of days and another the chlorine rinse in addition to the hot water. The hot water treatment consisted of a final rinse of 50 gallons of boiling water at night and the same in the morning followed by cold water; the sodium hypochlorite rinse (75 p.p.m.) was used either night or morning, or both. When used at night it followed the boiling water, and when used in the morning it was in place of the hot rinse and followed by the cold. Thus, when used in the morning only, there was a direct comparison between hot water

TABLE 3. *Effect of sodium hypochlorite rinse in addition to hot water treatment*

	Boiling hot water (50 gal.) P.M. & A.M.				Sodium hypochlorite rinse (75 p.p.m. av. chlorine) after boiling water		
		Cold A.M. rinse		Butter		Cream in churn after 10 min. run	Butter
Exp. No.	Workers in or out	Bacteria col. per ml.	Y. & M. col. per ml.	Y. & M. col. per ml.	Rinse in closed churn	Y. & M. col. per ml.	Y. & M. col. per ml.
24	In	5,200	940	1,010	A.M. only	.....	900
25	In	3,800	830	650	P.M. & A.M.	380	610
26	Out	3,650	620	550	P.M. & A.M.	1,150	500
27	Out	2,020	490	570	P.M. only	690	970
33	Out	400	107	630	A.M. only	360	210
34	Out	1,350	280	1,100	A.M. only	188	340
48	Out	.....	.....	450	A.M. only	450	420
50	In	.....	.....	71	A.M. only	200	34
Ave.		2,732	544	629		482	498

and sodium hypochlorite since other items in the treatment of the two churns were identical.

Table 3 gives the results obtained. The yeast and mold counts of the butter from the churn which received the hot water treatment varied from 71 to 1,100 and averaged 629 per ml., while the butter from the same vat of cream but from the churn which received the chlorine rinse varied from 34 to 970 yeasts and molds per ml. and averaged 498. In every comparison except one the chlorine rinse resulted in a lower yeast and mold count in the butter, and in that one the chlorine rinse had been used at night only. Apparently the chlorine rinse was more effective when used in the morning, although it by no means resulted in a sterile churn. Yeast and mold counts were made on the cream in the churn after a 10-minute run, and it was found that they varied from 188 to 1,150 per ml. and averaged 482. A churn responsible for such contamination could not be said to be in satisfactory bacteriological condition.

The condition of the cream before its entrance into the churns was checked by making yeast and mold counts on samples out of the 1,000-gallon vats. Through a sterile tube the cream was removed from below the surface into sterile wide-mouth glass-stoppered bottles. The counts varied from 0 to 18 and averaged 6 per ml. for the series of determinations. The entire pasteurizing and cooling system was sterilized with steam and flushed with 190° F. water previous to circulation of the cream, and the sanitary lines between the holding vats and churns were also steamed and flushed. The cream was flashed at 160° F. into a holding vat, where pasteurization was completed by holding it for a period of 20 minutes at a temperature ranging from 150° to 155° F. The cream was then

pumped through an internal tubular cooler and into a 1,000-gallon holding vat of the glass-enamel, vertical-coil type. The holding vat was previously steamed for a period of 15 minutes. The efficiency of this vat treatment was studied by rinsing sides and coils with one gallon of sterile, distilled water. It was found that the vat was not sterile. The plate method on the rinse water showed a bacterial count of 20 colonies per ml. The finding of yeast and *Oospora lactis* cells in the cream in the vat was undoubtedly a result of contamination from pipe lines, connections, pumps and vats which was not eliminated even with the procedures employed.

B. *Diversol*

In a series of trials with Diversol, a chlorine-carrying cleaning compound, essentially the same results were secured as with sodium hypochlorite. The same concentration of chlorine (75 p.p.m.) was used in a rinse both night and morning after a rinse of boiling water. The yeast and mold count of the butter varied from 210 to 380 per ml. in four trials and averaged 275, as compared with an average of 520 per ml. for churnings from the same vats of cream but made in churns which received the "ordinary" hot water treatment night and morning. Counts on the latter butter varied from 360 to 680 per ml. In one case both the churn and the workers were thoroughly scrubbed with the Diversol solution after the alkali rinse; in addition the churn was run closed for 10 minutes with the Diversol rinse of 50 gallons of warm water containing 75 p.p.m. available chlorine. In the morning the churn received a hot rinse of 50 gallons of 210° F. water followed by another Diversol rinse like the first. Two cold rinses were given the churn, the first to wash out the traces of chlorine and the second for sampling. The latter gave a bacterial count of 120 colonies per ml. and a yeast and mold count of 130 per ml., showing that over 22,500,000 bacterial cells and clumps and over 24,500,000 yeasts and molds were removed from the churn by the second cold rinse in spite of the thorough treatment with hot water and chlorine solution and the mechanical removal effected by the several rinses. The cream that went into this churn had a yeast and mold count of 5 per ml. in the vat, but contained 114 yeasts per ml. after it had been run in the churn for 10 minutes; the butter had a yeast and mold count of 280 per ml. The results of two other trials in which the Diversol was used only in rinse water after the hot water rinse night and morning are summarized as follows:

Yeast and mold counts per ml.

Cream in vat	Cream in churn after 10-min. run	Finished butter
9	180	230
8	275	380

The use of Diversol in churn treatment, while it reduced the yeast and mold count of the butter as compared with the "ordinary" hot water treatment, was not successful in lowering the count below 200 per ml.

C. *Chlorinated Lime*

The efficiency of chlorinated lime (bleaching powder) in the destruction of germ life in churns was investigated in a limited way. The "ordinary" hot water treatment, supplemented by a rinse of 50 gallons of boiling water in the morning before the cold rinse, was used on one churn; on another churn the same treatment was employed with the addition of chlorinated lime rinse at night after the hot rinse, 1 pound of the powder being added to 50 gallons of water at 120° F., giving an available chlorine content of approximately 800 p.p.m. This is considerably in excess of usual strengths employed in the sterilization of utensils, pipe lines and vats, but it was thought desirable to use extreme measures in attempting to secure efficiency in churn sterilization. In view of the relative cheapness of chlorinated lime, the use of high concentrations of available chlorine would not be impractical from an economic standpoint if they were found to be effective.

As before, cream from the same vat was used for each comparison between the churns representing the two different treatments. Table 4 gives the data secured. They show that the use of the chlorinated rinse resulted in lower bacterial and yeast and mold counts on the cold morning rinse water, and lower yeast and mold counts on the butter, the only exception to this being the yeast and mold count on the rinse water in experiment 19. The butter in the case of the "ordinary" hot water treatment averaged 1,255 yeasts and molds per ml., while the butter from the same cream but churned in the chlorine-treated churn averaged 420 per ml. While this was an improvement, the latter figure represents considerable contamination and is evidence of the difficulties to be met in at-

TABLE 4. *Effect of a chlorinated lime rinse in addition to "ordinary" hot water treatment*

	"Ordinary" hot water[1], workers in churn			"Ordinary"[1] plus final $CaOCl_2$ rinse in P.M. (800 p.p.m. av. chlorine)		
	Plate count—colonies per ml.			Plate count—colonies per ml.		
	Cold A. M. rinse		Butter	Cold A. M. rinse		Butter
Exp. No.	Bacteria	Y. & M.	Y. & M.	Bacteria	Y. & M.	Y. & M.
19	3,400	200	1,000	2,800	530	400
20	24,300	1,500	1,600	8,300	740	480
21	.....	.....	1,700	.....	.....	500
22	24,000	1,200	720	3,300	540	300
Ave.	17,233	966	1,255	4,800	603	420

[1] Supplemented by a rinse of 50 gallons of boiling water in morning before cold rinse.

tempting to sterilize churns even with extremely high chlorine contents in the sterilizing rinse.

The above results were secured with the butter workers in the churn and receiving all sterilizing rinses—both hot water and chlorinated lime—as well as the cold water rinse. Further trials were made in which the churn was run closed for all rinses except the preliminary hot alkali rinse after the butter was removed, the workers then being treated with scalding water outside of the churn. In one comparison the chlorinated rinse was used in the morning in place of hot water; the churn was run closed for 10 minutes followed by the usual cold rinse. The cream in the vat had a plate count of 4 yeasts and molds per ml.; the cream in the chlorine-treated churn after a 10-minute run had a count of 64 per ml.; and the count on the finished butter was 240. The butter from the same cream but in the churn that received the hot water treatment, however, had a plate count of 1,160 yeasts and molds per ml.

In another comparison with churns run closed for the sterilizing rinses, the chlorinated lime rinse was used both night and morning. In this case the cream in the vat had a plate count of 3 yeasts and molds per ml., while in the chlorine-treated churn after a 10-minute run the count was 170. Thus, a chlorinated lime rinse of excessively high chlorine content was unable to sterilize the churn barrel even when used both in the afternoon as a final rinse and in the morning before the cream was pumped into the churn. The churn barrel still yielded contaminating organisms to the cream.

D. *Chloramine-T*

The more stable nature of the organic form of a chlorine compound and its prolonged action over a period of time suggested the desirability of employing it for the sterilization of churns. The commercial product Santamine was used in a series of trials, either in the final rinse water at night or as a spray after the hot water rinse. When used in the rinse water, the recommended strength of 1 ounce per 30 gallons of water was employed (approximately 35 p.p.m. available chlorine based on a chlorine content in the chloramine-T of 13 percent). When used as a spray, the "standard" Santamine solution of 8 ounces dry powder per gallon of water was sprayed directly into the churn without dilution. While the use of a solution of such a strength would be impractical from an economic point of view because of the cost of the product, it was deemed advisable to determine what results could be obtained when extreme measures were taken. The counts on the cold rinse water and on the butter are given in table 5. In experiment 2 the chloramine was used in the final rinse of warm water, the workers being in the churn for all rinses. The counts on the cold rinse water in the morning and on the butter show no essential difference in churn contamination. Final yeast and mold counts on the

TABLE 5. *Chloramine-T treatment—rinse or spray—after ordinary hot water treatment*

Exp. No.	Ordinary hot water treatment—workers in			Chloramine-T treatment in P.M. in addition to hot water		
	Plate count—colonies per ml.			Plate count—colonies per ml.		
	Cold A. M. rinse water		Butter	Cold A. M. rinse water		Butter
	Bacteria	Y. & M.	Y. & M.	Bacteria	Y. & M.	Y. & M.
2	56,000	170	480	40,000	640	560
3	11,600	180	39	2,120	270	220
4	3,800	500	.....	3,700	310	.....
5	6,500	400	.....	11,600	220	.....
6	49,500	550	.....	30,000	630	.....
	2nd rinse					
6	9,000	300	.....	.....	.....	.....
7	6,300	120	430	9,500	310	280
Ave.	22,283	317	310	16,153	397	353

butter were 480 and 560 per ml. for the ordinary hot water and the chloramine treatment, respectively. The cream, as usual, was from the same vat.

When the chloramine solution was used as a spray, the counts on the cold rinse water were both higher and lower than those for the ordinary hot water treatment. In experiments 6 and 7 the churn was held closed after the spraying for 15 and 35 minutes, respectively, to delay the drying out and to prolong the time of action of the chloramine. This was apparently of no benefit as judged by the counts on the cold rinse water and on the butter. In experiment 7 the butter in the chloramine-treated churn had a final count of 280 yeasts and molds per ml.

The use of chlorine compounds did not prove effective in the methods employed for the sterilization of the churns. The high yeast and mold counts secured on the cream in the churn after a short period of agitation suggest that the chlorine treatments were not effective in eliminating the churn barrel itself as a source of contamination, even though the barrel was exposed to the action of the chlorine solution during a run when the churn was kept closed and worker contamination was not a part of the problem.

USE OF FORMALIN

In a few trials formalin was added to the rinse water (1 quart in 30 gallons of water at 70° F.) after the hot water treatment both night and morning. Results are shown in table 6. While the finished butter churned in the formalin-treated churn showed a lower yeast and mold count than butter from the same vat of cream churned in the hot-water-treated churn, two of the counts were over 200 per ml.; the counts on the cream after a 10-minute run in the churn indicate definite contamination from the bar-

TABLE 6. *Effect of formalin rinse in addition to hot water treatment*

Exp. No.	Cream in vat	Boiling water (50 gal.) in closed churn P. M. & A. M.			Formalin rinse (1 qt. in 30 gal. water) P. M. and A. M. after hot water	
		Plate count—col. per ml.			Plate count—col. per ml.	
		Cold A. M. rinse		Butter	Cream in churn after 10 min. run	Butter
	Y. & M.	Bacteria	Y. & M.	Y. & M.	Y. & M.	Y. & M.
28	0	1,590	40	670	220	210
29	3	.....	.....	470	61	50
30	9	980	240	380	99	210
Ave.	4	1,285	140	506	126	156

rel. The formalin rinse both night and morning was not effective to the desired degree.

USE OF STEAM

It was recognized that in most of the above trials in which hot water was used as a "check" treatment the quantity used was insufficient to produce any great destruction of microorganisms. As indicated in table 2, best results with hot water were secured with 150 gallons of boiling water in a closed churn. Since steam had been used by several investigators, it seemed desirable to determine what could be accomplished with it in the Simplex churn. While its use would not be expected to offer any advantage in economy as compared with the use of adequate quantities of hot water for each churn, the use of steam would be time-saving, especially if it could be used after the alkali rinse without a rinse of clear hot water. In the first trials one churn was closed up and steamed for 15 minutes each night by means of a hose inserted in the churn-gate. This was done after the churn received the usual boiling water rinse of 50 gallons, run closed for 15 minutes. In the morning the hot water rinse was repeated, followed by a cold rinse. Yeast and mold counts on the butter are given in table 7 and show that in 7 out of 10 trials the steamed churn yielded butter with a lower count than the churn which received the hot water treatment only, the butter again coming from the same cream. The average for the steam treatment was 526 yeasts and molds per ml. and for the hot water 706. In experiment 38 the yeast and mold count on the cream in the steam-treated churn after a 10-minute run was 58 per ml., the count on the cream in the vat having been 5 per ml.

In another series of trials a steaming period of 30 minutes or longer was employed, usually without the boiling water rinse at night, the churn

TABLE 7. *Effect of steaming in addition to hot water treatment*

Exp. No.	Boiling water (50 gal.) in closed churn P. M. & A. M.	Steam for 15 min. at night in addition to boiling water treatment
	Plate count—colonies per ml. Butter, Y. & M.	Plate count—colonies per ml. Butter, Y. & M.
38	1,050	500
39	870	300
40	460	210
41	750	390
42	760	410
43	1,200	420
44	620	1,550
45	240	420
46	390	160
47	720	900
Ave.	706	526

being flushed out with the hose after the alkali rinse and then steamed. The morning treatment was a 50-gallon rinse of boiling water, churn run closed for 10 minutes, followed by a cold rinse. The workers were scalded outside of the churn both night and morning. This treatment was compared with the "ordinary" hot water treatment received by another churn—a rinse of boiling water at night with the workers in—but supplemented by a hot rinse in the morning before the cold. The impression was growing that the workers were a serious source of contamination; in order to secure information regarding this, yeast and mold counts were made in some cases on the butter both before and after washing, as well as on the finished butter. Counts on the butter after washing would show the extent of contamination up to the entry of the workers, while the finished butter counts would include that from the workers also. Table 8 gives the data secured.

The yeast and mold counts on the cream in the churn after a 10-minute run show the extent of contamination from the churn barrel in the case of the steam treatment and demonstrate that the treatment was reasonably effective compared with previous treatments tried. The counts, together with the counts on the cream in the vat, are evidence that the large increases observed with previous treatments after a 10-minute run were not increases resulting merely from a breaking-up of clumps but, instead, definite increases brought about through contamination from the churn barrel. The steam treatment was so effective that the average yeast and mold count on the cream in the churn after 10 minutes was only 22 per ml. The average counts before and after washing were 15 and 13, respectively; thus, the butter ready for working was

TABLE 8. *Steam treatment compared with "ordinary" hot water treatment*
Plate counts—yeasts and molds per ml.

Exp. No.	Cream in vat	Ordinary hot water—boiling water P. M. & A. M.—workers in Finished butter	Churn steamed at night				
			Period of steaming	Cream in churn after 10-min. run	Butter granules Before washing	Butter granules After washing	Finished butter
52		570	30′				220
53		900	30′				370
54		800	45′	10			390
55	0	450	30′	17			520
56	5	460	30′	48			660
57	14	590	30′	21			590
58		1,050	30′	13			450
59		1,160	30′	51			1,150
60	1	300	60′	18	10	6	60
61		120	30′		9	13	26
62		1,080	60′		12	12	800
63	14	1,150	45′	11	35	9	510
64	5	680	45′	19			320
65		320	45′	13	12	10	320
66		480	30′		11	11	410
67		850	30′		14	9	440
68		480	30′		22	32	290
69		1,600	30′		13	16	360
Ave.	7	724		22	15	13	438

fairly low in yeast and mold content, yet the counts on the finished butter averaged 438 per ml., only two churnings out of 18 being below 200 and the balance ranging from 220 to 1,150 per ml.

This pointed definitely to heavy contamination from the workers. In experiment 69 the workers received a flush of scalding water both night and morning as well as a strong sodium hypochlorite solution in the morning, yet the yeast and mold count of the butter increased from 16 per ml. after washing to 360 in the finished butter. While this churning was being worked, a sample of the brine dripping from the shelves, before complete incorporation, was taken. This was plated, with a resulting count of 3,800 yeast and mold colonies per ml. With such contamination coming from the workers, the high yeast and mold counts secured in the finished butter throughout the entire series of trials are readily understandable. In the

series of 18 comparisons between the steam and "ordinary" hot water treatments shown in table 8, the cream in each comparison coming from the same vat, the steam treatment resulted in a lower yeast and mold count in 14 cases, a slightly higher one in 2, and the counts were the same in the other 2. For the steam treatment the average was 438 yeasts and molds per ml. in the finished butter, as compared with 724 for the hot water.

INFLUENCE OF TYPE OF CHURN

The influence of the workers in contaminating the product was shown in a final experiment in which a 60-pound portion of butter was removed from the Simplex churn after washing and worked up to the finished moisture content in a small laboratory churn of the combined churn-and-worker type. The latter had been kept in satisfactory condition by a boiling water treatment, and was given a rinse of boiling water followed by a cold one preparatory to the working of the butter. The Simplex churn had received the 30-minute steam treatment at night with a rinse of boiling water followed by cold water in the morning. The butter granules after washing gave a plate count of 19 yeasts and molds per ml.; the 60-pound portion of butter worked in the laboratory churn gave a final count of 31 per ml. The same butter worked in the Simplex churn gave a final count of 220 yeasts and molds per ml. (unsalted butter).

A sample of the water taken from the Simplex churn early in the working process gave a plate count of 3,900 yeasts and molds per ml., showing essentially the same condition as the brine sample in experiment 69. A sample of the water-buttermilk mixture which drained from the 60-pound portion of butter removed from the churn after washing gave a plate count of only 8 yeasts and molds per ml., showing the condition previous to the entry of the workers.

The work reported above was all done in one commercial creamery. In the replacement of old churns in a second creamery in another state the opportunity presented itself to secure a direct comparison between the Simplex churn and the combined churn-and-worker type on the yeast and mold content of butter. In these comparisons cream from the same vat was pumped into the two churns, which were both receiving a routine treatment of 150 gallons of boiling water at night (Simplex closed with workers scalded outside) and a boiling water rinse of 50 gallons in the morning before the cold rinse. Results are shown in table 9. Yeast and mold counts were made on potato dextrose agar adjusted to pH 3.5.

While the bacterial counts on the butter were higher for the Simplex churn in five cases out of seven, the yeast and mold counts showed the pronounced difference between the two types of churns, the heavy contamination in the Simplex undoubtedly resulting from the workers. The highest yeast and mold count on butter from the combined churn-and-worker was 57 per ml., while the counts on butter from the Simplex

TABLE 9. *Comparative plate counts on butter from two types of churns*

	Combined churn and worker type			Simplex churn		
Vat of cream	Lot No.	Bacteria col. per ml.	Yeasts & molds col. per ml.	Lot No.	Bacteria col. per ml.	Yeasts & molds col. per ml.
1	348	16,000	20	349	74,000	900
2	354	34,000	16	355	110,000	137
3	356	33,000	15	357	27,000	520
4	360	10,700	23	361	24,000	620
5	363	10,900	57	364	13,800	1,010
6	365	380,000	45	366	109,000	1,400
7	367	23,000	39	368	56,000	1,170
Ave.		72,514	31		59,114	822

churn varied from 137 to 1,400 per ml.; averages were 31 and 822, respectively.

Such differences are of definite significance in the manufacture of unsalted butter. That the contamination from the workers is of importance also from the standpoint of the bacterial content of the butter has been demonstrated by the bottle test in this laboratory in several instances in the examination of butter for development of surface taint.

The high yeast and mold counts obtained on the finished butter in the Simplex churns throughout the entire series of experiments demonstrate the inadequacy of all treatments in the attempted sterilization of the workers, and they prove the futility of intensive treatment of the churn barrel when the workers cannot be successfully treated. It is believed that a steam chest in which the entire workers could be subjected to prolonged steaming would furnish the only successful means of eliminating contamination from this important part of the Simplex churn, and then only if used daily from the time the churn was first placed into use. In the absence of such a sterilizing chamber the workers are a menace to the quality of butter; the impossibility of eliminating contamination from them through ordinary measures has been one of the factors responsible for the gradual withdrawal of the Simplex churn from creamery equipment.

SUMMARY

1. An attempt was made to study, by yeast and mold counts, the contamination of butter from Simplex churns and workers in regular operation in a commercial creamery.

2. Bacterial, yeast and mold counts were made on the churn rinse water; yeast and mold counts were made on the cream in the churn after a 10-minute run and on the finished butter.

3. A steam treatment of 30 minutes, when used daily, left the churn barrel in a reasonably satisfactory condition. A number of low yeast and mold counts were obtained on the finished butter when a boiling water treatment of 150 gallons was used in a closed churn.

4. The use of chlorine compounds usually resulted in a reduction in the yeast and mold content of butter as compared with the inadequate hot water treatment which had been in routine use, but the counts invariably remained excessive.

5. It was demonstrated that heavy contamination was coming from the workers of the Simplex churn.

6. None of the treatments used—hot water or rinses containing chlorine compounds—was effective on the workers.

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A Comparison of Media for Determining the Total Bacterial Count of Butter

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VARIOUS media have been used in bacteriological studies of butter according to the specific investigation under consideration and the types of organisms to be determined. The increasing interest in the microbiological content of butter as a means of sanitary control made it desirable to study different media used for bacteriological analysis with a view to the standardization of media, as well as other procedures, in order that the results obtained in different laboratories would be more comparable.

With this aim in view, the committee appointed by the American Dairy Science Association to study bacteriological methods of analyzing dairy products, through the subcommittee on butter, published a preliminary report (1) in September, 1930, on bacteriological methods of analyzing butter. In this report three media were suggested for making total bacterial counts of butter, namely: (1) Bacto peptonized-milk agar, (2) whey agar, and (3) beef-infusion agar containing 1 percent lactose.

REVIEW OF LITERATURE

Hammer (2) states that whey agar is satisfactory for plating fresh butter, especially when made with a starter, but that beef-infusion agar is likely to give higher counts on old butter in which many of the original organisms have died. He also concludes that beef extract agar gives a poor growth of butter organisms. Hammer and Hussong (3) used beef-infusion agar in studying changes in the bacterial content of butter during holding, while Derby and Hammer (4) used beef-infusion agar for determining total counts on surface taint butter.

Grimes (5) used whey agar for starter butter but beef-infusion agar when plating butter made from raw and pasteurized sweet cream because it gave higher counts. Grimes (6), in a study of the relation of the micro-

biological content of butter to the flavor score, used nutrient agar with the addition of 1 percent lactose. In commenting (7) on the report of the committee of the American Dairy Science Association, he recommends this medium for making total counts of butter.

Myers (8) states that Bacto nutrient caseinate agar was satisfactory for making total counts on butter; he found it comparable to peptonized milk agar for this purpose.

North and Reddish (9) in a bacteriological study of high grade experimental butter used whey agar for determining total bacterial counts, while Macy (10) used whey agar in determining the quantitative changes in the microflora of butter during storage.

Brown and Peiser (11) used litmus lactose agar (Standard nutrient agar + 1 percent lactose + .003 percent azolitmin) and casein agar for making total counts on butter and for the differentiation of bacteria. They found that litmus lactose agar gave slightly higher counts than casein agar when plating fresh butter made from cream ripened with a starter. After the butter was 30 days old, the counts were closer and the average counts for 12 samples showed very little difference for the two media.

Cullen (12) used nutrient agar and gelatin for making total counts on butter made from sweet cream in a milk receiving factory and found that in some cases the count on gelatin was higher than on the nutrient agar.

Sadler and Vollum (13) used several media for making quantitative and qualitative bacterial studies on butter. In nearly every case they found that the total count on beef-peptone-lactose agar was considerably higher than the total count on beef-peptone agar.

Hood and White (14) used Bacto nutrient agar for making total bacterial counts on surface taint butter and obtained high counts on most samples of the defective butter.

As no data could be found giving comparative counts on butter using the media suggested by the butter committee of the American Dairy Science Association, it appeared desirable to gather data on the total bacterial counts by employing the media suggested by the committee as well as the media recommended or suggested by other workers.

Since this study was completed, the subcommittee on butter has published a revised report (15) in which beef-infusion agar made from fresh beef or Bacto beef plus 1 percent lactose is the only medium recommended for determining total bacterial counts on butter.

METHODS

The experimental platings of butter were run in two series. In the first series comparative counts were obtained on Bacto peptonized-milk agar, Bacto whey agar, and two beef-infusion agars. One beef-infusion agar was prepared from fresh lean beef infused in tap water for 20 hours in a refrigerator at 40° F. with further procedures as recommended in the

report of the Butter Committee (1), and the other agar was made from dehydrated Bacto beef using 50 grams to 1,000 cc. of water. The latter medium is only about half strength of the former, but it is recommended by the manufacturer for making satisfactory culture medium. One percent of peptone, 0.5 percent of sodium chloride and 1 percent of lactose were added to both beef-infusion agars which were standardized to a pH of 6.8 by means of a Hellige comparator, standard model.

In the second series of samples, five media were compared, namely: Bacto peptonized-milk agar, Bacto whey agar, Bacto nutritive caseinate agar, Bacto nutrient agar ("Standard Methods" formula) + 1 percent lactose, and beef-infusion agar prepared from Bacto beef plus 1 percent lactose as in the first series.

The butter samples were plated using dilutions of 1:100 to 1:1,000,000. All dilutions were poured in duplicate with each medium, and the sets of plates were made from the same dilution blanks. The plates were prepared and poured in rotation to compensate for any difference in the time of holding the samples in the dilution water.

The plates were incubated for five days at 25° C. since a controlled temperature of 21° C., recommended in the report of the Butter Committee (1), was not available. Counting was done with the aid of a lens; the usual procedure followed was to count and mark all colonies visible to the naked eye and then to go over the plates with the lens. The results are expressed as the average count of the duplicate plates for each medium. As far as possible, plates of the same dilution were counted for each medium, but in a few cases this was impractical.

TYPES OF BUTTER ANALYZED

The samples of butter that were plated represented, for the most part, first and second grades according to Canadian standards of quality and were taken from both freshly made and stored butters two to three months old. Several of the samples were from high-scoring exhibition butters which had been in storage for six to eight weeks. Practically all lots of butter were manufactured from pasteurized cream and, as far as is known, all of the butter was made without starter. The counts obtained were not influenced, therefore, by the addition of starter organisms.

THE pH OF THE MEDIA

As all media were prepared from dehydrated products of the Digestive Ferments Company, no attempt was made to standardize the pH, with the exception of the Bacto beef-infusion agar, which was standardized before sterilization to 6.8 ± .2. Nevertheless, the pH of several lots of the various media were checked colorimetrically by means of a Hellige comparator. The pH of each medium was found to be as follows:

Medium	pH
Bacto whey agar	6.6
Bacto peptonized-milk agar	6.4
Bacto nutritive caseinate agar	6.7
Bacto nutrient agar + 1% lactose	6.8
Bacto beef-infusion agar + 1% lactose	6.8

EXPERIMENTAL RESULTS

RESULTS OF THE FIRST SERIES OF PLATINGS

The comparative data secured from plating 16 samples of butter on the media used in series I are given in table 1, the samples having been plated between April and August, 1931. All of the samples employed had higher counts on the beef-infusion agars than on either whey or peptonized-milk agars with the exception of sample 2, which had a slightly higher count on whey agar than on the beef-infusion agars, and sample 4, which showed a slightly higher count on both whey and peptonized-milk agars than on the Bacto beef-infusion agar. In 11 of the 16 samples plated, the highest counts obtained on beef-infusion agars were from 40 to over 100 percent greater than the lowest counts obtained on peptonized-milk or whey agars. In eight trials the highest counts were obtained on fresh beef-infusion agar, in six trials with Bacto beef-infusion, and in one trial with whey agar; one trial had the same high count on both beef-infusion agars.

TABLE 1. *Comparative total bacteria counts of butter on four different media*

Sample No.	Bacteria per cc. of butter on			
	Peptonized milk agar	Whey agar	Fresh beef-infusion agar + 1% lactose	Bacto beef-infusion agar + 1% lactose
1	13,250	13,650	19,500	20,350
2	10,000	18,000	17,500	17,500
3	5,850	5,500	6,850	6,850
4	46,500	45,000	67,000	43,500
5	46,000	49,000	60,500	57,500
6	405,000	385,000	505,000	540,000
7	410,000	445,000	900,000	845,000
8	144,000	161,500	242,000	265,500
9	253,500	277,000	337,000	305,000
10	16,200	19,850	25,700	26,000
11	270,500	300,000	346,500	339,000
12	61,000	61,000	78,000	73,500
13	647,500	689,000	793,000	777,000
14	5,400	5,400	8,800	6,700
15	4,150	5,350	9,600	9,700
16	37,000	49,500	60,000	67,000
Ave.	148,490	158,112	217,309	212,506

Although the differences in the counts on whey and peptonized-milk agar were not great, 11 samples had higher counts on whey agar, while 3 samples showed higher counts on peptonized milk, and 2 samples had the same count. In comparing the two beef-infusion agars the counts obtained show only slight differences except for sample 4, where the fresh beef-infusion agar gave a count over 50 percent greater than the Bacto beef-infusion agar.

The average counts for the different media given in table 1 show that the peptonized-milk agar regularly gave the lowest count, and the fresh beef-infusion agar gave the highest count. There was no significant difference in the average counts on peptonized-milk and whey agars, or in the average counts on the two beef-infusion agars. There was, however, an appreciable increase in the counts on the beef-infusion agars over those obtained on either peptonized-milk or whey agar.

RESULTS OF THE SECOND SERIES OF PLATINGS

After data had been secured on 16 samples of butter, copies of written comments by Myers (8) and Grimes (7) on the report of bacteriological analytical methods were received by the Division of Dairy Research at Ottawa, which made it advisable to include the media recommended by these workers, namely, Bacto nutritive caseinate agar and Standard nutrient agar plus 1 percent lactose. Since in the first series there was no significant difference in the results obtained when fresh lean beef or Bacto beef agars were used, only the Bacto beef-infusion medium was employed in the second series.

The data obtained from comparative platings of 55 samples of butter on 5 different media are presented in table 2. In this second series beef-infusion agar again showed the highest counts for the majority of samples and, in addition, had the highest average count, since in 32 trials the counts were highest on beef-infusion agar. Nutrient agar plus lactose gave the highest counts in 17 comparisons, whey agar in 5 and nutritive caseinate agar in 1. In no case did peptonized-milk agar have the highest count when the five media were compared; but it did show higher counts for some samples when compared with the other media separately, although the differences in the counts were not significant. In two comparisons the counts on peptonized-milk agar were higher than on beef-infusion agar and nutrient agar plus lactose, while in two other trials the counts were higher on the peptonized-milk agar than on nutritive caseinate or whey agar.

The lowest counts for the 55 trials on the different media were distributed as follows: peptonized-milk agar, 46; whey agar, 4; nutritive caseinate, 2; and beef-infusion agar and nutrient agar plus lactose, 1 each. In one comparison the low count was the same on peptonized-milk and whey media.

TABLE 2. *Comparative total bacteria counts of butter on five different media*

Sample No.	Bacteria per cc. of butter on				
	Peptonized milk agar	Whey agar	Nutritive caseinate agar	Nutrient agar + 1% lactose	Bacto beef-infusion agar + 1% lactose
1	28,500	32,500	50,500	87,500	116,500
2	1,500	3,650	5,100	6,500	7,200
3	8,750	15,350	13,650	16,950	67,000
4	13,150	169,500	178,500	188,500	219,000
5	26,300	151,000	162,000	179,000	174,500
6	33,750	162,500	139,500	180,500	198,500
7	13,900	44,000	58,500	66,000	65,000
8	340,000	2,750,000	4,400,000	3,960,000	4,275,000
9	24,000	207,000	51,000	131,000	690,000
10	3,400	4,550	6,050	6,750	5,900
11	67,500	76,000	85,500	87,500	94,500
12	76,000	106,500	142,000	168,000	188,000
13	9,550	19,300	17,500	17,700	18,400
14	815,000	1,015,000	1,270,000	1,460,000	1,465,000
15	64,000	88,500	259,000	720,000	815,000
16	1,655,000	1,660,000	2,280,000	5,675,000	8,595,000
17	95,000	115,000	125,000	145,000	335,000
18	83,000	172,000	142,500	237,500	288,000
19	7,750	8,200	9,700	14,350	15,500
20	13,750	17,500	20,750	22,700	23,150
21	7,900	9,700	11,300	12,950	12,600
22	11,500	13,200	19,100	20,650	19,600
23	3,400	4,350	6,800	10,500	10,250
24	333,000	397,000	407,000	463,500	388,000
25	77,500	78,500	96,000	119,000	109,500
26	80,000	100,500	90,500	144,500	155,000
27	11,700	14,850	18,450	20,050	19,850
28	34,000	31,150	32,800	34,750	34,350
29	27,500	31,000	50,000	64,000	79,500
30	11,300	13,950	13,700	17,000	19,650
31	439,000	386,000	532,500	648,000	655,000
32	156,000	167,500	175,000	223,500	228,500
33	523,000	524,000	752,500	844,000	941,500
34	1,740,000	3,790,000	2,770,000	3,070,000	5,370,000
35	276,500	317,500	280,500	364,500	357,000
37	365,000	470,000	1,425,000	1,280,000	1,855,000
38	2,490,000	2,470,000	2,795,000	2,860,000	3,205,000
39	11,375,000	13,650,000	13,600,000	12,950,000	13,680,000
40	18,450,000	23,100,000	20,300,000	17,400,000	16,250,000
41	168,500	211,000	258,500	260,500	265,500
42	1,090,000	1,595,000	1,695,000	1,860,000	1,840,000
43	2,805,000	2,785,000	3,210,000	3,880,000	4,475,000
44	4,970,000	5,820,000	5,395,000	4,710,000	4,395,000
45	11,450	12,900	13,800	14,050	16,500
46	11,750	13,350	14,050	20,700	16,800
47	12,900	14,150	17,350	25,100	22,100
48	11,000	13,850	16,750	32,750	25,300
49	70,000	125,000	85,000	110,000	120,000

TABLE 2. (Continued)

Sample No.	Bacteria per cc. of butter on				
	Peptonized milk agar	Whey agar	Nutritive caseinate agar	Nutrient agar + 1% lactose	Bacto beef-infusion agar + 1% lactose
50	8,135,000	8,370,000	8,630,000	9,205,000	10,090,000
51	9,300,000	10,200,000	10,150,000	9,450,000	10,450,000
52	2,150,000	2,600,000	5,100,000	6,000,000	15,700,000
53	7,100,000	8,750,000	5,950,000	8,700,000	7,700,000
54	290,000	290,000	405,000	540,000	530,000
55	56,000	52,000	44,500	61,500	63,000
56	232,000	289,000	330,000	374,500	374,000
Ave.	1,385,567	1,700,518	1,711,061	1,802,935	2,139,539

The average counts as given in table 2 show that beef-infusion agar gave the highest, which was over 54 percent greater than the lowest average count given by the peptonized-milk agar. Nutrient agar plus lactose gave the second highest average count, but this was only slightly higher than those obtained on nutritive caseinate and whey agars. The average counts on nutrient agar plus lactose, nutritive caseinate and whey agars were 30.1, 23.5 and 22.7 percent higher than on peptonized-milk agar, respectively.

Table 3 shows the distribution of the high and low counts of the 55 samples for the different media and indicates the relative standing of the various media for total bacterial counts of butter.

COMPARISON OF THE MEDIA FOR PLATING LOW- AND HIGH-COUNT BUTTER

In order to determine if there was a marked variation in the relative value of the different media for low- and high-count butter, the counts for

TABLE 3. *Analysis of highest and lowest counts*

Medium	Highest counts		Lowest counts	
	No. of samples	Percent samples	No. of samples	Percent samples
Peptonized-milk agar	0	0.0	46	85.2
Whey agar	5	9.1	4	7.4
Nutritive caseinate agar	1	1.8	2	3.7
Nutrient agar + 1% lactose	17	30.9	1	1.85
Beef-infusion agar + 1% lactose	32	58.2	1	1.85
	55	100.0	54[1]	100.00

[1] One sample had the same lowest count on whey and peptonized-milk agar.

TABLE 4. *A comparison of the bacterial counts on different media of low-count butter*

Sample No.	Bacteria per cc. of butter on				
	Peptonized milk agar	Whey agar	Nutritive caseinate agar	Nutrient agar + 1% lactose	Bacto beef-infusion agar + 1% lactose
2	1,500	3,650	5,100	6,500	7,200
3	8,750	15,350	13,650	16,950	67,000
7	13,900	44,000	58,500	66,000	65,000
10	3,400	4,500	6,050	6,750	5,900
11	67,500	76,000	85,500	87,500	94,500
13	9,550	19,300	17,500	17,700	18,400
19	7,750	8,200	9,700	14,350	15,500
20	13,750	17,500	20,750	22,700	23,150
21	7,900	9,700	11,300	12,950	12,600
22	11,500	13,200	19,100	20,650	19,600
23	3,400	4,350	6,800	10,500	10,250
27	11,700	14,850	18,450	20,050	19,350
28	34,000	31,150	32,800	34,750	34,350
29	27,500	31,000	50,000	64,000	79,500
30	11,300	13,950	13,700	17,000	19,650
45	11,450	12,900	13,800	14,050	16,500
46	11,750	13,350	14,050	20,700	16,800
47	12,900	14,150	17,350	25,100	22,100
48	11,000	13,850	16,750	32,750	25,300
55	56,000	52,000	44,500	61,500	63,000
Ave.	16,825	20,647	24,767	28,622	31,807

these two types of butter were grouped together. Any sample for which the maximum bacterial count on any medium was 100,000 or less was considered to be low-count butter, and all samples which had a minimum on any medium of 1,000,000 was considered to be high-count butter. The comparison of the counts obtained on different media for low-count butter is given in table 4, while the comparative counts for the high-count butter are given in table 5.

In the 20 comparisons of low-count butter, the average counts for the different media showed greater differences than the average counts of all samples, although the relative standing of the various media was not changed. Whereas the high average count for beef-infusion agar was only approximately 54 percent greater than the low average count for peptonized-milk agar with all samples, in the low-count butter the average count for beef-infusion agar was 89 percent greater than the average count for peptonized milk. With the low-count butter, the differences in the average counts on nutrient agar plus lactose and nutritive caseinate agar, as compared with those on peptonized milk were also considerably greater than for all samples. The difference in the average count for whey agar as compared with that for peptonized milk was practically the same for low-count butter as for all samples.

TABLE 5. *A comparison of the bacterial counts on different media of high-count butter*

Sample No.	Bacteria per cc. of butter on: Peptonized milk agar	Whey agar	Nutritive caseinate agar	Nutrient agar + 1% lactose	Bacto beef-infusion agar + 1% lactose
8	340,000	2,750,000	4,400,000	3,960,000	4,275,000
14	815,000	1,015,000	1,270,000	1,460,000	1,465,000
16	1,655,000	1,660,000	2,280,000	5,675,000	8,595,000
34	1,740,000	3,790,000	2,770,000	3,070,000	5,370,000
37	365,000	470,000	1,425,000	1,280,000	1,855,000
38	2,490,000	2,470,000	2,795,000	2,860,000	3,205,000
39	11,375,000	13,650,000	13,600,000	12,950,000	13,680,000
40	18,450,000	23,100,000	20,300,000	17,400,000	16,250,000
42	1,090,000	1,595,000	1,695,000	1,860,000	1,840,000
43	2,805,000	2,785,000	3,210,000	3,880,000	4,475,000
44	4,970,000	5,820,000	5,395,000	4,710,000	4,395,000
50	8,135,000	8,370,000	8,630,000	9,205,000	10,090,000
51	9,300,000	10,200,000	10,150,000	9,450,000	10,450,000
52	2,150,000	2,600,000	5,100,000	6,000,000	15,700,000
53	7,100,000	8,750,000	5,950,000	8,700,000	7,700,000
Ave.	4,852,000	5,935,000	5,931,333	6,164,000	7,290,333

In the 15 trials using high-count butter, the percentage differences in the average counts were slightly less than those obtained on all samples. Beef-infusion agar gave an average count that was approximately 50 percent greater than the average count on peptonized milk for high-count butter as compared with a difference of 54 percent for all samples. The differences in the average counts with the other media as compared with peptonized milk were also slightly less for the high-count butter than for all the samples. The only variation in the relative value of the different media as indicated by the average counts was for whey and nutritive caseinate agars. For all samples, the average count of the nutritive caseinate agar was higher than for whey agar, while for the high-count butter the average count on whey was very slightly higher than the average count on nutritive caseinate agar.

TABLE 6. *The comparative values of the average counts on different media for all samples and for low- and high-count butter*

Butter	No. of samples	Peptonized milk agar	Whey agar	Nutritive caseinate agar	Nutrient agar + 1% lactose	Bacto beef-infusion agar + 1% lactose
All samples	55	100	122.7	123.5	130.1	154.4
Low-count butter	20	100	122.7	147.2	170.1	189.0
High-count butter	15	100	122.3	122.2	127.0	150.3

The percentage differences in the average counts with the different media were much greater for the low-count butter than for the high-count butter when compared with the differences in the average counts of the various media for all samples. This is shown clearly in table 6, wherein the comparative values of the average counts for all samples and for both high- and low-count butter are tabulated. A value of 100 was given to the low average count obtained on peptonized milk, and the average counts for the other media were valued accordingly.

OBSERVATIONS ON THE SIZE AND TYPES OF COLONIES

In both series the colonies on the beef-infusion agars appeared to be slightly larger than on the other media employed, although in some cases the surface colonies on peptonized milk were as large as on the beef-infusion agars. Colonies on the other media were much the same size. Most of the colonies on all media were visible to the naked eye, but there were a few which could only be detected with the aid of a lens.

While no attempt was made to classify the types of colonies present, it was observed that chromogenic colonies were frequently present in considerable numbers on all media except peptonized-milk agar; the yellow colonies on the latter were generally few in number and often failed to show up at all, either being absent altogether or failing to produce color.

When spreading colonies appeared on the plates they seemed to grow profusely on the peptonized-milk agar and very little on the whey agar.

CONCLUSIONS

Comparative total bacterial counts on sweet cream butter made without starter, using whey, peptonized-milk, nutritive caseinate, nutrient plus 1 percent lactose and beef-infusion plus 1 percent lactose agars, showed that there was a better development of bacterial colonies both as to numbers and size on beef-infusion agar than on the other media. Comparative counts on beef-infusion agars made from fresh beef and from Bacto dehydrated beef did not show any significant differences.

The counts on peptonized-milk agar were commonly lower than those obtained on the other media employed. There were no significant differences among the counts obtained on whey, nutritive caseinate or nutrient lactose agars.

The percentage differences among the average counts on the various media, with the exception of whey agar, were comparatively higher for low-count butter than for high-count butter.

Chromogenic colonies of yellow organisms often failed to appear on peptonized-milk agar but they were present on other media in considerable numbers. Spreader colonies grew profusely on peptonized-milk agar.

While it is recognized that a medium giving the highest total counts is not necessarily the most suitable for making routine analyses and that

factors of cost, ease of preparation and availability must be considered, the use of beef-infusion agar plus 1 percent lactose as a medium for routine analyses of butter does not offer any serious disadvantages as to the cost or the preparation.

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Some Observations on the Yeast and Mold Count of Salted Butter Made from Sour Cream

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SINCE the work of Lund (1), Bouska and Brown (2), the yeast and mold count of butter has been used with varying degrees of success as an index to the sanitary conditions under which the butter has been produced. In butter made with butter culture, the total bacterial count cannot logically be used to measure the sanitary conditions of production, and attempts to use groups of microorganisms other than yeasts and molds have not been successful.

The influence of medium upon the yeast and mold count has been studied, and from the data obtained it appears that the most suitable medium is potato dextrose agar acidulated with tartaric acid. The work of White and Hood (3) stated that 21° C. for 5 days was the optimum temperature and time for incubation, though later studies have indicated that, with potato dextrose agar, the yeast and mold count secured at 3 days at 21° C. was equivalent to the 5-day count.

In the past the yeast and mold count on butter has been made by using 1 ml., 0.1 ml. and 0.01 ml. of the sample; the 1-ml. amount was plated directly with the melted butter, while the 0.1- and 0.01-ml. amounts were obtained by the ordinary dilution method. This practice is still followed in many laboratories. With this procedure it was not uncommon to find upon examination of the 1-ml. plate a poorer distribution of colonies than was obtained in the two higher dilutions and a wide disagreement in count between dilutions. This lack of distribution and lower count in the 1-ml. plate is believed to be caused by the difficulty in securing adequate distribution of the butter in the plate, due to the lack of agitation of the sample with subsequent failure to break up clumps of the microorganisms.

For the above reasons, as well as for convenience in the routine examination of butter for its yeast and mold count, the following method of preparing dilutions was used. Into a 90-ml. warm dilution bottle was introduced 10 ml. of the melted butter to be examined. This dilution blank was shaken, as in milk, and from this bottle dilutions were prepared. The three dilutions made were: 1 to 2, 1 to 10 and 1 to 100. The 1-to-2 dilution was prepared by adding 5 ml. of the 1-to-10 dilution to the Petri dish. This procedure is open to question and, in order to test it, data were collected on the yeast and mold counts of 100 samples of commercial butter and counts in the 1-to-2 dilution were compared with the counts obtained in the 1-to-10 dilution.

The average yeast and mold count of 100 samples of commercial salted butter made from sour cream was found to be 133.2 per ml. of butter when the 1-to-2 dilution was used; whereas the count was 148.9 when the 1-to-10 dilution was employed. The latter gave a ratio count 1.117 times greater than the former.

Higher counts were secured in 63 percent of the samples in the 1-to-10 dilution; in 32 percent of the samples higher counts were secured in the 1-to-2 dilution. In but 3 percent of the samples the difference between the counts secured on the two was greater than 100 percent, and in these samples the counts secured on the 1-to-10 dilution were greater than the counts on the 1-to-2 dilution.

From these data it would appear that an accurate enumeration of the number of yeasts and molds in a sample of butter is obtained by plating 5 ml. of the 1-to-10 dilution. The agreement secured between the 1-to-2 dilution and the 1-to-10 dilution is within the limits of error of the technique used.

Macy and Richie (4) in studying the yeast and mold count of butter found that the yeast and mold count did not serve as a reliable index of the keeping quality of individual samples of butter, but that samples of butter with low yeast and mold counts as a group showed a tendency toward slightly better keeping quality than those with higher counts. The butter examined by Macy and Richie had been stored at low temperatures and held for periods of one to five months. Inasmuch as in the industry samples of butter are submitted to temperatures of 15.6° to 20.0°C. for 10 days to measure their keeping quality, it seemed desirable to correlate the yeast and mold count and the keeping quality of the butter as measured by the flavor change after incubation for 10 days at 15.6°C.

During the summer of 1936, 221 samples of salted butter made from sour cream were examined as to their yeast and mold content and keeping quality. This butter was produced in some 60 different plants. The salt concentration of the butter studied varied from 1.7 to 2.7 percent, and within the samples studied no definite trend was found between the

salt concentration (within these salt limits) and the keeping quality of the butter.

The size of sample used to determine keeping quality was about one-fourth pound; this was stored in a sterile jar with a tight-fitting cap and the samples were held in an incubator thermostatically controlled to 15.6°C. The results obtained were as follows:

No. of samples	Decrease in score after 10 days at 15.6° C.	Percent of samples containing less than 100 yeasts & molds per ml.
56	No change	58.75
50	0.5 point	54.00
38	1.0 point	49.97
77	More than 1.0 point	46.36

The results secured in correlating the yeast and mold count of butter with the change in flavor score after incubation for 10 days at 15.6°C. are similar to the findings of Macy and Richie (4).

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The Influence of Filtration of Inoculated Wash Water on Bacterial Count and Keeping Qualities of Butter[1]

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FILTRATION of butter wash water usually involves only the elimination of extraneous material. The removal of bacteria has received little attention due, presumably, to the infrequent occurrence of defects in butter in which contaminated water is responsible. The tendency in recent years for increasing the production of low salted and of unsalted butter, in which the restraining action of the salt is partially or wholly eliminated, emphasizes the necessity for reducing bacterial contamination of butter. The object of the work herein reported was to study the efficiency of a filter designed to remove bacteria from water and to determine the influence of filtration of contaminated butter wash water on the keeping qualities of butter.

METHODS

The general procedure was as follows: Plate counts were made on the inoculated water before and after filtration; a portion of butter was washed with the unfiltered water and another portion washed with the filtered water. Plate counts were made on the fresh salted and unsalted butter from each lot, the butter then stored at various temperatures and examined periodically for flavor defects, and bacterial counts again made after storage.

For each trial a few gallons of water were inoculated with a broth culture of a test organism and half of the water filtered through a Seitz filter[2] that had been treated with flowing steam for about 30 minutes. Two

[1] Journal Paper No. J427 of the Iowa Agricultural Experiment Station, Ames, Iowa, Project 113-114.

[2] The filter was obtained through the courtesy of Dr. B. Lichtenberger, American Seitz Filter Corporation, 31 Union Square, New York, N. Y.

lots of butter churned from sweet cream without butter culture added were removed from a churn after draining the buttermilk and one was washed with the unfiltered water while the other was washed with the filtered water. The butter was worked in sterile granite pans with sterile paddles, a portion of each lot salted and the butter then packed in sterile glass jars each holding about one-third of a pound. Plate counts were made on the unfiltered and filtered water and on the fresh salted and unsalted butter from each lot, after which samples from each lot of butter were stored at 21° C. for 7 days, 15° C. for 14 days, 5° C. for 28 days and 0° C. for 56 days; the storage temperatures were approximate rather than actual. Examinations for flavor defects were made at intervals during the storage periods and plate counts were made on the samples after storage. For the plate counts the medium used was beef-infusion agar with 0.5 percent sterile skim milk added; the plates were incubated at approximately 21° C. for 4 days.

ORGANISMS USED

The test organisms used were from stock cultures and were known to be able to produce definite defects in unsalted butter. They were: *Serratia marcescens, Pseudomonas fluorescens, Achromobacter lipolyticum, Alcaligenes viscosus,* and *Pseudomonas fragi,* which produce primarily rancidity, and *Achromobacter putrefaciens,* which produces surface taint.

RESULTS

Data were secured in which each of the six test organisms was used in two trials. The counts on the unfiltered and the filtered water and on the fresh salted and unsalted butter are presented in table 1.

EFFICIENCY OF FILTRATION

The counts on the unfiltered and the filtered butter wash water given in the table indicate a high degree of efficiency for the filter employed. The treatment of the filter with flowing steam would not be expected to destroy all the organisms, and this may account for the few bacteria detected in the filtered water; it must also be recognized that air contamination probably contributed a few organisms. When a test organism developing distinctive colonies, such as *Serratia marcescens,* was used, no colonies of the inoculated organism appeared on the plates poured from the filtered water.

BACTERIAL COUNTS ON THE FRESH BUTTER

Salted butter. The results in the table show that the counts on the fresh salted butter washed with the unfiltered water were, in general, slightly higher than the counts on the butter washed with the filtered water but there was no great difference between the counts in any trial regardless of the counts on the wash water used. The butter washed with

TABLE 1. *Plate counts on the butter wash water and on the fresh butter*

Trial No.	Organism used	Organisms per ml. of					
		Butter wash water		Fresh salted butter washed with		Fresh unsalted butter washed with	
		Unfiltered	Filtered	Unfiltered water	Filtered water	Unfiltered water	Filtered water
1	*S. marcescens*	510,000	2	210,000	240,000	490,000	355,000
2	*Ps. fluorescens*	185,000	4	188,000	194,000	295,000	283,000
3	*Ach. lipolyticum*	370,000	12	268,000	174,000	360,000	180,000
4	*Ach. putrefaciens*	448,000	8	330,000	310,000	400,000	420,000
5	*Alc. viscosus*	283,000	1	370,000	360,000	490,000	510,000
6	*Ps. fragi*	1,020,000	1	284,000	245,000	450,000	420,000
7	*S. marcescens*	1,800,000	11	940,000	690,000	1,170,000	1,220,000
8	*Ps. fluorescens*	1,720,000	8	1,000,000	820,000	1,820,000	1,690,000
9	*Ach. lipolyticum*	7,300,000	4	1,210,000	1,150,000	4,100,000	2,030,000
10	*Ach. putrefaciens*	1,760,000	12	4,500,000	4,300,000	4,000,000	4,300,000
11	*Alc. viscosus*	275,000	19	5,600,000	6,000,000	5,400,000	5,300,000
12	*Ps. fragi*	250,000	35	4,300,000	5,200,000	5,700,000	6,200,000

the unfiltered water had the higher count in seven trials, whereas the butter washed with the filtered water had the higher count in five.

The salt content of the butter washed with the unfiltered water ranged from 1.53 to 4.08 and averaged 2.48 percent; the salt content of the butter washed with the filtered water ranged from 1.57 to 4.68 and averaged 2.67 percent.

Unsalted butter. The data given in the table show that the counts on the unsalted butter washed with the unfiltered water were, in general, slightly higher than the counts on the butter washed with the filtered water; but in only two comparisons (trials 3 and 9) were the differences significant. In one of these trials (trial 3) the count on the unfiltered water was similar to that in many of the trials, but in the other (trial 9) the count was by far the highest of all the trials. The butter washed with the unfiltered water had the higher count in seven trials, whereas the butter washed with the filtered water had the higher count in five.

In general, the counts on the unsalted butter were considerably higher than those on the salted butter and the influence of filtration of the wash water was more conspicuous in the counts on the unsalted product. It might be emphasized that the numbers of bacteria in the wash water appeared to have no significant effect on the bacterial content of the fresh butter.

KEEPING QUALITIES OF THE BUTTER AT VARIOUS TEMPERATURES

Salted butter. In the salted butter stored at 21° C. for seven days, extensive changes in flavor were not common, but in four trials (trials 5, 7, 8 and 9) the butter washed with the unfiltered water became rancid; none of the samples of butter washed with the filtered water developed defects due to bacterial action. After storage the counts on the butter washed with the unfiltered water ranged from 70,000 to 105,000,000 per ml., whereas those on the butter washed with the filtered water ranged from 25,000 to 57,000,000 per ml.; the butter washed with the unfiltered water had the higher count in five trials and the butter washed with the filtered water had the higher count in seven. In both lots of butter the numbers of bacteria increased during storage in eight trials and decreased in four.

In general, the salted butter stored at 15° C. for 14 days kept well, but in two trials (trials 7 and 9) the butter washed with the unfiltered water became rancid and in one trial (trial 12) cheesy; none of the butter washed with the filtered water showed defects due to bacterial action. After storage the counts on the butter washed with the unfiltered water ranged from 40,000 to 20,400,000 per ml., whereas those on the butter washed with the filtered water ranged from 30,000 to 32,000,000 per ml.; the butter washed with the unfiltered water had the higher count in four trials, and the butter washed with the filtered water had the higher count in eight. The counts on the butter washed with the unfiltered water increased during storage in six trials and decreased in six, whereas the

counts on the butter washed with the filtered water increased in seven and decreased in five.

In the salted butter stored at 5° C. for 28 days no definite defects due to bacterial action occurred in either the butter washed with the unfiltered or with the filtered water. After storage the counts on the butter washed with the unfiltered water ranged from 45,000 to 11,500,000 per ml., whereas those on the butter washed with the filtered water ranged from 57,000 to 6,100,000 per ml.; the butter washed with the unfiltered water had the higher count in five trials and the butter washed with the filtered water had the higher count in seven. During storage the counts on the butter washed with the unfiltered water increased in four trials and decreased in eight and those on the butter washed with the filtered water increased in five and decreased in seven.

During storage at 0° C. for 56 days the salted butter washed with the unfiltered water became rancid in two trials (trials 9 and 12) but none of the samples of butter washed with the filtered water developed defects. After storage the counts on the butter washed with the unfiltered water ranged from 44,000 to 12,500,000 per ml. and those on the butter washed with the filtered water ranged from 38,000 to 4,200,000 per ml.; the butter washed with the unfiltered water had the higher count in seven trials and the butter washed with the filtered water had the higher count in five. During storage the counts on the butter washed with the unfiltered water increased in three trials and decreased in nine, whereas those on the butter washed with the filtered water increased in four and decreased in eight.

Unsalted butter. The unsalted butter stored at 21° C. for seven days commonly showed pronounced deterioration but, in general, the butter washed with the filtered water was distinctly superior in keeping quality to the butter washed with the unfiltered water. In nine trials (trials 1, 2, 3, 5, 6, 7, 8, 9 and 11) the butter washed with the unfiltered water became rancid, yet none of the samples of butter washed with the filtered water showed this defect. In two trials (trials 4 and 10) the defect characterized as surface taint developed in the butter washed with the unfiltered water while in the butter washed with the filtered water the slight cheesiness that appeared was not characteristic of the organism *(Ach. putrefaciens)* which had been used in these trials. In several trials, cheesiness developed in both the butter washed with the unfiltered and with the filtered water; in fact, this defect appeared in nearly all of the butter in the last six trials in which the bacterial counts on the fresh butter were rather high. After storage the counts on the unsalted butter washed with the unfiltered water ranged from 8,100,000 to 180,000,000 per ml.; the counts on the butter washed with the filtered water ranged from 28,500,000 to 520,000,000 per ml. The butter washed with the unfiltered water had the higher count in two comparisons, and the butter washed with the filtered water had the higher count in 10. All the

samples of butter showed large increases in bacterial content during storage.

The butter stored at 15° C. for 14 days showed essentially the same changes that occurred in the butter stored at 21° C. for seven days. The butter washed with the unfiltered water became rancid in nine trials (trials 1, 2, 3, 5, 6, 7, 8, 9 and 11) but none of the samples of butter washed with the filtered water showed this defect. In two trials (trials 4 and 10) surface taint appeared in the butter washed with the unfiltered water but not in the butter washed with the filtered water. After storage, counts on the butter washed with the unfiltered water ranged from 14,000,000 to 190,000,000 per ml., whereas those on the butter washed with the filtered water ranged from 26,000,000 to 120,000,000 per ml.; the butter washed with the unfiltered water had the higher count in two trials while the butter washed with the filtered water had the higher count in ten. Large increases in bacterial count were noted in all the samples during storage.

The unsalted butter stored at 5° C. for 28 days showed less deterioration than the butter stored at the higher temperatures. The butter washed with the unfiltered water became rancid in five trials (trials 1, 2, 3, 8 and 12) and developed surface taint in two trials (trials 4 and 10); none of the samples of butter washed with the filtered water developed definite defects. After storage the counts on the butter washed with the unfiltered water ranged from 6,000,000 to 137,000,000 per ml., whereas the counts on the butter washed with the filtered water ranged from 8,500,000 to 141,000,000 per ml.; the butter washed with the unfiltered water had the higher count in three trials and the butter washed with the filtered water had the higher count in nine. Large increases in bacterial count were noted in all the samples during storage.

The unsalted butter stored at 0° C. for 56 days showed no conspicuous deterioration after 28 days' storage, but by the end of the holding period definite defects were evident in a few of the samples of butter washed with the unfiltered water. The butter washed with the unfiltered water developed rancidity in four trials (trials 1, 2, 3 and 9), surface taint in two (trials 4 and 10), and pronounced cheesiness in one (trial 12), but none of the rest of the samples developed defects beyond a slight cheesy or slight unclean flavor. After storage the counts on the butter washed with the unfiltered water ranged from 7,600,000 to 227,000,000 per ml., while the counts on the butter washed with the filtered water ranged from 18,000,000 to 310,000,000 per ml.; the butter washed with the unfiltered water had the higher count in three trials, whereas the butter washed with the filtered water had the higher count in nine. Large increases in bacterial content occurred in all the samples during storage.

DISCUSSION AND SUMMARY

Results were obtained in 12 trials in each of which a portion of inoculated water was filtered through a Seitz filter and the unfiltered and filtered water each used to wash a small lot of freshly churned butter; bacterial counts were made on the unfiltered and the filtered water and on the fresh salted and unsalted butter from each lot and samples of the butter then stored at various temperatures to determine their keeping qualities and changes in bacterial content.

The counts on the unfiltered and the filtered water indicate very efficient filtration and the few organisms detected in the filtered water apparently resulted from contamination rather than from passage through the filter.

The bacterial counts on both the fresh salted and fresh unsalted butter washed with the unfiltered water were, in general, slightly higher than the counts on the butter washed with the filtered water and the influence of filtration was more conspicuous with the unsalted than with the salted butter. In this connection it should be emphasized that the bacterial content of the butter wash water did not greatly influence the number of bacteria in the fresh butter. In only two comparisons were there significant differences between the count on the butter washed with the unfiltered water and on the butter washed with the filtered water even though in many trials the unfiltered water contained enormous numbers of organisms while the filtered water contained very few.

The results obtained with the salted butter indicate that pronounced deterioration was not common under the storage conditions employed but that when deterioration occurred the defects involved the butter washed with the unfiltered water. In general, the bacterial content of the salted butter increased during storage at 21° C. for 7 days or at 15° C. for 14 days but decreased during storage at 5° C. for 28 days or at 0° C. for 56 days. Unfortunately, the conditions for working and salting the butter did not permit accurate composition control and, consequently, the salt content varied considerably. The inhibitory influence of the salt was roughly proportional to the concentration but in some instances conspicuous increases in bacterial content and definite deterioration were noted in samples having fairly high salt content.

The results obtained with the unsalted butter indicate that deterioration occurred more frequently and more extensively than with the salted butter and that the butter washed with the filtered water was distinctly superior in keeping quality to the butter washed with the unfiltered water. The defects that involved the butter washed with the unfiltered water were usually the ones expected from the organisms used, while the defects that appeared in the butter washed with the filtered water were usually not. Deterioration was more common in the butter stored at 21° C. for 7 days or at 15° C. for 14 days than in the butter stored at 5° C. for 28 days or at 0° C. for 56 days. Enormous increases in bacterial content

were common in all the samples of unsalted butter during storage at the various temperatures and, in general, the butter washed with the unfiltered water had lower counts at the end of the storage period than did the butter washed with the filtered water. In this connection it was noted that the samples having pronounced defects often had low counts due, presumably, to the toxic effect of the products formed and that the samples of butter washed with the filtered water often had high counts but showed little or no deterioration.

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A Method for the Microscopic Examination of Butter

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THE microscopic examination of butter is made difficult by the presence of a relatively high percentage of fat which must be removed before the organisms can be stained. Hammer and Nelson (1) have described a method for separating the serum from the fat by centrifuging the butter in a separatory funnel, after which the serum is drawn off, smeared on a slide and stained. Fay (2) has described a method for staining butter in which Mayer's egg-glycerine mixture is placed on the slide. The butter is then mixed with it and the fat dissolved in xylene.

The above methods require either considerable equipment or special reagents; therefore, a need was felt for a rapid method which would eliminate centrifuging or the use of fixatives. It seemed that if a solvent could be found which would remove both the water and the fat from the smears that the preparation of smears would be more rapid. The reagent selected was acetone because it was miscible with both water and fat. The method used is described below.

METHOD

A small piece of butter is placed on a clean glass slide and is gently melted over a low flame or on a hot plate. A few drops of acetone are placed on the slide and the melted butter and acetone thoroughly mixed by gently moving the slide or by stirring with a sterile needle. When the mixture is complete, the acetone-fat-water mixture is gently poured from the slide. The casein and other milk solids will remain on the slide. A second extraction with acetone removes the remaining fat, after which the smear is allowed to dry. The curd will adhere to the glass and staining may be carried out readily.

The total time required to make a satisfactory smear by this proce-

dure is very short and very little equipment is necessary. The method is intended for the gross microscopic examination of butter rather than for making microscopic counts.

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The Influence of Starter on the Flavor of Butter

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RESEARCH workers in the major butter-producing countries in the world have, for many years, studied the influence of starter on the flavor of butter. The use of cultures of selected bacteria in the commercial manufacture of butter dates back about 50 years. The Danish bacteriologist, Storch, in 1884, began investigations on the causes of defects in butter. He developed starters containing bacteria which produced a desirable flavor and aroma when grown in milk under favorable conditions. The first of his starters was used commercially in the manufacture of butter in 1888. The results of Storch's early work were published by the Royal Danish Agricultural and Veterinary College in 1890 (1). The aim of the early research work was to discover whether the use of starter, containing selected bacteria, in pasteurized cream would result in butter having fine flavor and aroma and good keeping quality. Storch demonstrated conclusively that the butter obtained from pasteurized cream that had been ripened by the use of a high quality starter had as fine a flavor and aroma as the best butter made when either buttermilk from a previous churning or naturally soured milk was used as starter.

The practice of using starter to ripen the cream for buttermaking has been adopted universally by the creameries in Denmark and is considered an important factor in making butter of a uniform quality. Danish butter is sold in England in competition with butter, made from sweet, unripened cream, from other countries. The English consumers like Danish butter and generally pay more for it (2) than for butter made from sweet, unripened cream, even when this butter comes from British colonies that are favored with much better grazing conditions than those available in Denmark.

It has been estimated that only about 25 percent of the butter manufactured in the United States is made from cream to which starter has been added prior to churning. Starter is used very little for the butter made in the 11 western states. In some of these states starter is not used at all because it is claimed that the consumers prefer butter made without starter. In Oregon about 25 percent of the butter manufactured is made from cream to which starter has been added. Ripening of the cream is not usually practiced.

The reasons for not using starter more extensively in buttermaking have been the following: (a) Lack of suitable equipment, (b) additional work and expense, (c) no higher price obtained for the butter, (d) lack of appreciation by the consumers of the flavor and aroma imparted to the butter by starter.

The wholesale butter merchants have generally advised the buttermakers in western United States to refrain from using starter. The most common reasons for giving this advice have been: (a) The buttermakers generally have used poor starter; (b) the flavor of the butter has shown too great variation; (c) an undesirable flavor often developed in the butter during storage.

At the Iowa Agricultural Experiment Station (3) it has been demonstrated that when a good-flavored starter was used the butter had a more desirable flavor than that made without starter. It was also shown that the keeping quality of the butter would not be impaired if the acidity of the cream at the time of churning was not allowed to go beyond a certain limit.

The extensive work by Hammer and his associates during the past quarter of a century has added much to our knowledge regarding the preparation and use of starter. The *Streptococcus lactis* organisms have been classified, and the aroma-producing citric-acid-fermenting streptococci, common in good starter, have been identified. Much valuable data have been obtained regarding the growth characteristics of the bacteria in starter, the changes they cause and the products that are formed in milk by them.

There is no doubt in the minds of many experienced creamerymen and butter dealers that the proper use of fine flavored starter in first-grade, neutralized sour cream and in sweet cream results in butter that scores higher in flavor than that made without starter. Since confidence had been lacking in the western buttermakers' ability to make good starter and to manufacture butter that showed uniformity in flavor from day to day and also possessed keeping quality, the Oregon Agricultural Experiment Station in 1930 outlined a research project on the subject of preparing and using starter in the manufacture of butter. It was recognized that if the objections to butter made with starter were to be overcome, it would be necessary for the buttermakers to make starter under controlled condi-

tions and to employ proper methods in using it with the cream so that the butter would have a superior flavor and aroma and a good keeping quality.

Very few of the creameries that were using starter in 1930 had adequate facilities for preparing mother starter or satisfactory equipment for proper temperature control of large lots of starter. Only the older buttermakers had experience in making starter. The work by Hammer and his associates had shown what conditions are essential in preparing good starter. It was necessary, therefore, to provide these conditions in the Oregon creameries if success in making starter was to be attained. Accordingly, simple, inexpensive equipment for making starter was designed and constructed, and an electric heater, equipped with a three-heat switch, was made for maintaining a uniform temperature of inoculated milk kept in starter cans.

The equipment designed for mother starter included the following: (a) A tank for pasteurizing and cooling jars of milk to be used for mother starter, (b) a simple sterilizer for sterilizing transfer tubes, (c) a water-jacketed incubator in which a temperature of 68° to 72° F. could be maintained during all seasons, and (d) an insulated box for cooling jars of starter in ice water. This equipment is fully described in a bulletin published by the Oregon Agricultural Experiment Station (4). Directions for preparing mother starter and large lots of starter are given in the bulletin. The equipment has been in daily use at the State College Creamery for five years. Three mother cultures always have been carried. The equipment has been found very satisfactory. The mother starters and the large lots of starter have without fail shown good coagulation every morning. Similar equipment is now used in several large creameries in the state.

Simultaneously with the development of equipment for the preparation of starter and the standardization of the methods of making starter in the Oregon creameries, experimental churnings were made to determine whether starter was of value in improving the flavor and aroma of butter. The starter culture used for these churnings was No. 122 developed by Hammer at the Iowa Agricultural Experiment Station. Through the courtesy of the Iowa State College Department of Dairy Industry a replacement culture was obtained once a month.

This work involved the manufacture of butter from a large number of lots of sweet cream over a period of nearly two years. A total of approximately 20,000 pounds of butter was made in 157 churnings.

In one series of churnings (5) butter was made from 93 different lots of cream; with 59 of these starter was used, while with 34 no starter was used. The butter was judged by creamerymen and by representatives of the United States Department of Agriculture and the Oregon State Department of Agriculture. A summary of the scores of the butter from the different churnings is shown in table 1.

In general, the butter made with starter scored higher than the butter made without starter. Even after storing the butter for one

TABLE 1. *Average scores and range of scores on fresh and stored butter made with and without starter*

59 churnings of butter made with starter
34 churnings of butter made without starter

	Fresh butter		After one month at 35° to 45° F.			After six months at 0° to 10° F.		
	Range in scores	Average score	Range in scores	Average score	Average decrease in score	Range in scores	Average score	Average decrease in score
Butter made with starter	90.50-95.00	93.03	89.00-94.00	92.07	0.96	90.00-94.00	92.40	0.63
Butter made without starter	90.50-93.50	91.96	89.00-94.00	91.60	0.36	89.66-93.50	91.63	0.33
Difference in favor of butter made with starter		1.07		0.47			0.77	

month at from 35° to 45° F. and for six months at from 0° to 10° F., the average score of the butter made with starter was higher than that of butter made without starter. The chief comments of the judges on the butter made with starter were: "creamy flavor," "sweet and clean," "fine aroma," "fine flavor." Those on the butter made with no starter were: "flat," "lacking in character," "insipid," "tallowy." A frequency distribution of the scores of the fresh butter showed that 93.23 percent of the churnings made with starter scored 92 or above, whereas only 61.77 percent of those made without starter scored 92 or above.

In another series of churnings (6) 16 different lots of sweet cream were used. Each lot of cream was divided into four equal parts for the purpose of treating each differently before churning. All the cream was pasteurized at 150° F. for 30 minutes. One part was cooled and held cold until the time of churning, but to the other three starter was added. Eight percent starter was added to each lot. With one lot the starter was added after the pasteurized cream had been cooled to 70° F. It was held at this temperature until an acidity of 0.28 percent (serum acidity 0.42 percent) had been developed. The cream then was cooled quickly to below 40° F. and held at this temperature over night until churning the following morning. Starter was added to another lot after the cream had been cooled below 40° F. This cream was also held cold over night. The remaining lot of cream was cooled to below 40° F. and held cold over night without adding starter. Immediately before churning 8 percent starter was added to this cream. The butter was scored by the same judges who scored the previous 93 churnings. Table 2 shows a summary of the scores obtained.

The data confirmed the previously obtained results. Starter increased the score of the butter with each of the methods used. The improvement in quality was most pronounced when the cream was either ripened and then held cold over night or held cold for 16 hours after the addition of the starter without ripening. It was less pronounced when the starter was added immediately before churning. With the two first-mentioned methods the average score of the fresh butter was increased nearly one point.

There was no marked decrease in the scores of the butter made with or without starter during a one-month holding period at from 35° to 45° F. A decrease was observed, however, in the average score of the butter made by each of the four methods when the butter was stored for six months at from 0° to 10° F. The average decrease in score of the control samples was 0.57 and the average decreases for the butter made with starter were: 1.15 when the cream was ripened, 0.74 when starter was added and the cream held cold 16 hours, and 0.95 when starter was added immediately before churning. The average scores of the butter made with starter, at the end of six months, were 0.30, 0.66, and — 0.06 points in favor of the butter made with starter, in the order just given, than the average score of the butter made without starter.

TABLE 2. *Influence of various methods of using starter on the scores of fresh and stored butter*

Average of 16 trials

	Control No starter added			Starter added; cream ripened to 0.42% serum acidity		
		Stored			Stored	
	Fresh	1 month 35°-45°F.	6 months 0°-10°F.	Fresh	1 month 35°-45°F.	6 months 0°-10°F.
Average score	92.41	92.53	91.84	93.29	92.99	92.14
Average decrease after storage		+0.12	0.57		0.30	1.15
Difference in favor of butter made with starter				0.88	0.46	0.30

	Starter added and cream held cold 16 hours			Starter added to cream immediately before churning		
		Stored			Stored	
	Fresh	1 month 35°-45°F.	6 months 0°-10°F.	Fresh	1 month 35°-45°F.	6 months 0°-10°F.
Average score	93.24	93.18	92.50	92.73	92.55	91.78
Average decrease after storage		0.06	0.74		0.18	0.95
Difference in favor of butter made with starter	0.83	0.65	0.66	0.32	0.02	—0.06

Approximately 100,000 pounds of butter are made annually in the State College Creamery. Starter always is added to the cream, and the cream is ripened to a serum acidity of 0.42 to 0.44 percent. About 15,000 to 20,000 pounds of butter are stored during the summer months for consumption during the fall months. No objectionable flavor has ever developed in the butter made during the past six years when starter has been used. The demand for the butter always has been good.

It is well known that when butter is sent to a contest, starter is used with the majority of the churnings. The scores of butter made with starter usually average higher than those made without starter. The following data conclusively show this:

Cold Storage Contest, National Creamery Buttermakers' Association, St. Paul, Minnesota, 1924 (7)

	Into storage Average score	Out of storage (4 months) Average score
68 churnings made with starter	93.44	92.90
48 churnings made without starter	92.51	92.03
Difference in favor of butter made with starter	0.93	0.87

Cold Storage Contest, National Creamery Buttermakers' Association, Cleveland, Ohio, 1928 (8)

	Into storage Average score	Out of storage (4 months) Average score
165 churnings made with starter	92.95	92.82
50 churnings made without starter	92.30	92.21
Difference in favor of butter with starter	0.65	0.61

Pacific International Dairy Products Show, Portland, Oregon, 1931 (9)

	Average score
28 churnings made with starter	93.54
16 churnings made without starter	92.14
Difference in favor of butter made with starter	1.40

Oregon Butter and Ice Cream Makers' Annual Convention, Corvallis, Oregon, 1932 (10)

	Average score
29 churnings made with starter	93.13
11 churnings made without starter	92.46
Difference in favor of butter made with starter	0.67

Pacific International Dairy Products Show, Portland, Oregon, 1933 (11)

	Average score
44 churnings made with starter	92.80
11 churnings made without starter	91.00
Difference in favor of butter made with starter	1.80

Pacific International Dairy Products Show, Portland, Oregon, 1935 (12)

	Average score
70 churnings made with starter	93.20
10 churnings made without starter	92.40
Difference in favor of butter made with starter	0.80

A total of nearly two billion pounds of creamery butter is consumed annually in the United States. Butter is sold in competition with various fats of both animal and vegetable origin, some in the form of butter substitutes. The annual per capita consumption of butter, which is known as a food of high energy and vitamin value, has increased during the past few

years. Butter should have a desirable flavor and aroma, however, for the consumer to relish it, and should have a waxy body and a pleasing color. As buyers are becoming more and more critical, low-grade butter in the future will be difficult to sell. The responsibility of the American buttermakers lies in the manufacture of the finest quality of butter possible from the grade of cream available. They necessarily will have to make butter of the quality the market demands. Will the trend show a demand for butter of an insipid flavor or will it be for butter of the degree of flavor preferred by the connoisseurs at the various scoring contests?

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The Influence of Various Methods of Neutralizing Cream on the Quality of Fresh and Stored Butter

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THE practice of partially neutralizing with lime and soda neutralizers the acidity of sour cream intended for buttermaking has been a well-recognized commercial procedure for years. Early investigations comparing butter made from sour, partially neutralized cream with butter made from sour, untreated cream have definitely demonstrated the beneficial effects which may be derived from the intelligent application of certain neutralizers. Recently the influence of different types of neutralizers and the methods of applying them have received considerable attention. The results indicate a lack of agreement as to the type of neutralizer and the method of neutralization that should be employed for the most satisfactory results. Most of the butter manufactured in North Dakota creameries is made from sour cream; therefore, further investigational work to determine the influence of various neutralizers and different methods of applying them seems desirable.

The studies herein reported were for the purpose of determining (1) the chemical composition of the neutralizers commonly employed in the neutralization of cream for buttermaking; (2) the relationship between the type of neutralizer used and the quality of the butter from the standpoint of the flavor score; and (3) the effect of the method of neutralization upon the quality of butter.

HISTORICAL

In a study of the neutralization of sour cream for buttermaking, Ramsey (4) mentions the possibility of objectionable, volatile substances being removed from cream during the pasteurization process when bicarbonate of soda is employed as the neutralizing agent. Jackson (3), Davel (1) and Hunziker (2) believe that lime gives the best neutralizing

results. Stiritz and Ruehe (5) reported that no one of the neutralizers tried in their experiments could be said to produce better results than another in fresh butter. Bicarbonate of soda tended to reduce the score of the fresh butter slightly, since a perceptible bicarbonate flavor was present. Walts and Libbert (6) concluded from their examinations that, of the neutralizers studied, all had about the same effect on the flavor score of butter.

Double neutralization involves the reduction of the acidity of cream in two stages, either the same or different neutralizers being employed for each stage. The results of the experiments conducted by Stiritz and Ruehe (5) indicate that double neutralization shows no beneficial effects as compared with neutralizers used singly.

METHODS

Chemical analyses and alkalinity determinations were made on the neutralizers. The alkalinities of hydrated lime, quick lime, calcium and magnesia limes were determined by boiling a one-gram sample with an excess of N/2 hydrochloric acid and back titrating with standardized N/10 sodium hydroxide, using phenolphthalein as the indicator. The alkalinities of the soda neutralizers were determined by double titration, first with phenolphthalein and then with methyl orange.

In most of the trials cream containing more than 0.20 percent acidity, calculated as lactic acid, was reduced to approximately 0.20 percent by the addition of 10 percent aqueous solutions or suspensions of one of the following neutralizers: Hydrated lime ($Ca(OH)_2$), magnesia lime ($MgO \cdot Ca(OH)_2$), bicarbonate of soda ($NaHCO_3$), soda ash (Na_2CO_3) and sodium sesquicarbonate ($Na_2CO_3NaHCO_3$). Combinations of these were used for the comparisons involving double neutralization. The following method of adding the neutralizing materials was used: The temperature of the cream was raised to approximately 90° F. and the acidity determined. The cream was then divided into separate lots in tinned metal containers and the calculated amount of neutralizing mix was added by means of a small garden sprinkler. The cream was pasteurized at 145° F. for 30 minutes, cooled to 40° F. and held in a refrigerator over night. The churning was done the following morning.

When the cream was neutralized to various acidities with the same neutralizing mix, the acidity in each lot of cream was reduced by adding different amounts of the neutralizer and was then pasteurized by the method already described.

Churnings were made in a combined churn. The butter was washed by revolving it 8 to 10 revolutions in wash water equal to the amount of buttermilk drained from the churn. Salt was added at the rate of two ond one-half percent of the calculated amount of butter. The butter was packed in 10-pound tubs lined with butter circles and liners which had been boiled in a saturated brine solution. After being held in a refriger-

ator at 40° F. from two to six days, the butter was shipped to a cold storage plant, where it was scored while fresh by two commercial butter judges. The butter was then placed in cold storage at 0° F. for periods ranging from three to four months.

The titratable acidity was determined in duplicate by titrating 20-gram portions of cream with N/10 sodium hydroxide.

RESULTS

Table 1 gives the composition and neutralizing strength of the neutralizers used in the trials.

For the sake of convenience, in the following discussion each butter will be specified by a symbol indicative of the neutralizer employed: Bicarbonate of soda (B.S.); hydrated lime (H.L.); soda ash (S.A.); sodium sesquicarbonate (S.S.C.); hydrated calcium and magnesium (H.C.M.).

The flavor score based upon 45 points as the perfect score was employed in all trials. Six trials, involving a total of 33 comparisons of various neutralizers, were made and the results are tabulated in table 2.

COMPARISONS OF BICARBONATE OF SODA (B.S.) WITH HYDRATED LIME (H.L.)

The data show that B.S. and H.L. produced slight but significant differences in the quality and keeping quality of the butter. Fresh B.S. butter appeared to be slightly superior to fresh H.L. butter since it ranked high in four comparisons, while in no comparison did the H.L. butter rank high. The average score of fresh B.S. butter was 0.50 points higher than that of the H.L. butter, but at the end of three months the difference in favor of B.S. butter was reduced to 0.07 points. Each type of butter exhibited a noticeable increase in score during the first month of storage, after which there was a gradual decrease. At the end of the three months the average score of the B.S. butter was 0.37 points lower than that of fresh B.S. butter, whereas the score of H.L. butter was 0.06 higher than that of the same butter when fresh.

COMPARISONS OF SODA ASH (S.A.) WITH HYDRATED CALCIUM AND MAGNESIUM (H.C.M.)

It is evident that there was no significant difference in the quality and keeping quality of the butters made with H.C.M. and S.A. When fresh, the S.A. butter scored high in one comparison, the H.C.M. butter in two, while the scores were the same in two. After one month the S.A. butter scored high in one comparison, the H.C.M. butter in three, while in one the scores were the same. After three months the S.A. butter ranked high in one comparison, the H.C.M. in two, and the scores were the same in two.

COMPARISONS OF HYDRATED LIME (H.L.) WITH SODIUM SESQUICARBONATE (S.S.C.)

The results indicate no apparent difference in the quality and keeping quality of the butter made with H.L. and S.S.C. neutralizers. The average

TABLE 1. *Composition and neutralizing strength of various neutralizers*

Cream neutralizer classification	Name of neutralizer	Pounds dry neutralizer required to neutralize one pound of lactic acid	CO_2 %	Fe %	SiO_2 %	SO_3 %
I. Hydrates and carbonates of calcium	1. Ash Grove	0.552	9.35	0.52	0.36	T+[1]
	2. Peerless	0.540	12.46	0.155	0.72	T+
II. Hydrates and carbonates of magnesium	1. K. and M. Technical	0.309	4.58	T	1.58	T
III. Oxides, hydrates and carbonates of calcium and magnesium	1. Kelley Island	0.454	13.70	0.129	0.28	T+
	2. K. and M.	0.660	36.22	T	0.47	T+
	3. Neutralac	0.447	6.47	0.11	0.35	T+
	4. Perfection	0.445	10.54	0.078	0.20	T+
IV. Oxides and hydrates of calcium and magnesium	1. Allwood	0.325	T+	0.418	0.35	T
V. Sodium bicarbonate	1. Bicarbonate of soda (U.S.P.)	1.110	51.42	none	0.02	T
VI. Sodium carbonate	1. Soda Ash (C.P.)	0.824	.....	.....	.....	.....
VII. Sodium sesquicarbonate	1. National Neutralizer	0.894	40.33	none	0.08	T
	2. Neutralene	0.920	40.82	none	0.04	T
	3. Wyandotte C.A.S.	0.956	39.98	none	0.06	T
	4. J. I. Holcomb Neutralizer	1.016	.....	.....	.....	.....

[1] T+ indicates slightly more than a trace; T indicates trace.

TABLE 2. *Influence of different types of neutralizers on the flavor score of butter*

No. of comparisons	Neutralizer used	Average acidity of the cream			Average scores on butter			Ave. change in butter scores		Relative placings of butter					
										Fresh		One mo.		Three mos.	
		Raw	Past.	churning	Fresh	One mo.	Three mos.	One mo.	Three mos.	high	same	high	same	high	same
8	B. S.[1]	0.54	0.25	0.21	34.56	34.88	34.19	+0.32	−0.37	4	4	3	4	4	1
	H. L.[1]		0.22	0.20	34.06	34.63	34.12	+0.57	+0.06	0	4	1	4	3	1
5	S. A.[1]	0.50	0.21	0.21	34.80	33.10	34.40	−1.70	−0.40	1	2	1	1	1	2
	H. C. M.[1]		0.20	0.20	35.00	33.90	34.60	−1.10	−0.40	2	2	3	1	2	2
4	H. L.[1]	0.62	0.18	0.18	33.63	33.50	33.38	−0.13	−0.25	1	1	3	0	3	0
	S. S. C.[1]		0.23	0.21	33.75	33.25	33.25	−0.50	−0.50	2	1	1	0	1	0
					fresh	two mos.	four mos.	two mos.	four mos.	fresh		two mos.		four mos.	
										high	same	high	same	high	same
6	S. A.[1]	0.49	0.19	0.19	34.58	33.33	34.25	−1.25	−0.33	2	3	3	1	3	1
	H. L.[1]		0.20	0.20	34.42	34.17	33.67	−0.25	−0.75	1	3	2	1	2	1
4	S. A.[1]	0.67	0.21	0.19	32.88	33.00	32.63	+0.12	−0.25	1	0	1	0	1	0
	B. S.[1]		0.23	0.20	33.25	33.38	33.28	+0.13	+0.13	3	0	3	0	3	0
6	H. L.[1]	0.63	0.23	0.21	33.63	33.50	33.38	−0.13	−0.25	4	0	3	1	3	1
	H. C. M.[1]		0.19	0.17	33.75	33.25	33.25	−0.50	−0.50	2	0	2	1	2	1

[1] See "Results" for explanation of abbreviations.

flavor scores of the H.L. butter when fresh and after two and four months were 33.63, 33.50 and 33.38, respectively, compared with 33.75, 33.25 and 33.25 for the S.S.C. butter. The slight differences in the scores were too small to be of significance. The decrease in the scores during storage was about the same for the two types of butter.

COMPARISONS OF SODA ASH (S.A.) WITH HYDRATED LIME (H.L.)

The data show that there was no significant difference in the quality and keeping quality of the two types of butter. The fresh S.A. butter and H.L. butter were fairly evenly divided as to the number of times each scored high in both the fresh and the stored products. The average flavor scores of the S.A. butter when fresh and after two and four months were 34.58, 33.33 and 34.25, respectively, compared with the average scores of 34.42, 34.17 and 33.67 for the H.L. butter. The large average decrease of 0.75 points in the H.L. butter after four months was attributed to a four point decrease in one comparison. With the exception of this comparison, the rate of decrease in score was practically the same for both types of butter.

COMPARISONS OF SODA ASH (S.A.) WITH BICARBONATE OF SODA (B.S.)

Although there were only four trials using S.A. and B.S. neutralizers, the data show relatively high scores for the B.S. butter since it ranked first in three of the four comparisons on both fresh and stored samples. A relatively large decrease in score during storage was shown by the S.A. butter, whereas the score of the B.S. butter increased during storage.

COMPARISONS OF HYDRATED LIME (H.L.) WITH HYDRATED CALCIUM AND MAGNESIUM (H.C.M.)

The data presented show no significant difference in the quality and keeping quality of the two types of butter. Although the fresh H.L. butter scored high in four trials compared with two for the H.C.M. butter, the samples were fairly evenly divided as to the number of times each ranked high or low in the stored products. There was a slight difference of 0.12 points in the average score in favor of the fresh H.C.M. butter; but after two and four months there was a difference of 0.25 and 0.13 points, respectively, in favor of the H.L. butter. These differences were not enough to be of any significance.

SINGLE VERSUS DOUBLE NEUTRALIZATION

Table 3 presents the results obtained on the 10 comparisons made to determine the difference in the quality and keeping quality of butter made from sour cream neutralized by single and double methods. When the double method was employed, the acidity of the sour cream was reduced to approximately 0.40 percent with hydrated lime; then the final acidity was reached with a different type of soda neutralizer. The cream was pasteurized at 145° F. for 30 minutes.

TABLE 3. *The influence of different neutralizers on the flavor of butter*
Single vs. double neutralization

Lot No.	Kind of neutralizer used	Percent acidity			Butter scores			Changes in the score from the fresh butter	
		Raw cream	Pasteurized cream	Cream at churning	Fresh	Two months	Four months	Two months	Four months
5	Hydrated lime (H.L.)	0.80	0.16	0.16	36.0	34.0	33.0	−2.00	−3.00
	Hydrated lime (H.L.) to 0.40 Bicarbonate of soda (B.S.) to 0.27		0.27	0.27	35.5	35.0	34.0	−0.50	−1.50
13	Hydrated lime (H.L.)	0.43	0.25	0.25	34.5	32.0	32.0	−2.50	−2.50
	Hydrated lime (H.L.) to 0.30 Sodium sesquicarbonate (S.S.C.) to 0.19		0.23	0.23	35.0	34.0	34.0	−1.00	−1.00
18	Hydrated lime (H.L.)	0.63	0.21	0.21	34.0	33.0	33.0	−1.00	−1.00
	Hydrated lime (H.L.) to 0.40 Bicarbonate of soda (B.S.) to 0.21		0.21	0.21	35.0	34.0	33.0	−1.00	−2.00
20	Hydrated lime (H.L.)	0.64	0.21	0.18	33.0	33.5	33.0	+0.50	0.00
	Hydrated lime (H.L.) to 0.40 Bicarbonate of soda (B.S.) to 0.13		0.13	0.14	34.0	33.5	33.5	−0.50	−0.50
21	Hydrated lime (H.L.)	0.66	0.25	0.23	34.0	34.5	34.5	+0.50	+0.50
	Hydrated lime (H.L.) to 0.40 Sodium sesquicarbonate (S.S.C.) to 0.25		0.26	0.25	33.0	33.0	33.0	0.00	0.00
22	Hydrated lime (H.L.)	0.76	0.24	0.25	33.0	32.0	32.0	−1.00	−1.00
	Hydrated lime (H.L.) to 0.40 Soda ash (S.A.) to 0.21		0.21	0.21	33.0	32.0	32.0	−1.00	−1.00
18	Bicarbonate of soda (B.S.)	0.63	0.23	0.23	33.5	33.5	34.0	0.00	+0.50
	Hydrated lime (H.L.) to 0.40 Bicarbonate of soda (B.S.) to 0.21		0.21	0.21	33.5	34.0	33.0	+0.50	−0.50
20	Bicarbonate of soda (B.S.)	0.64	0.22	0.19	33.0	33.0	33.0	0.00	0.00
	Hydrated lime (H.L.) to 0.40 Bicarbonate of soda to 0.14		0.13	0.14	34.0	33.5	33.5	−0.50	−0.50
21	Sodium sesquicarbonate (S.S.C.)	0.66	0.23	0.23	34.0	34.0	34.0	0.00	0.00
	Hydrated lime (H.L.) to 0.40 Sodium sesquicarbonate (S.S.C.) to 0.25		0.26	0.25	33.0	33.0	33.0	0.00	0.00
22	Soda ash (S.A.)	0.76	0.16	0.16	32.0	32.0	31.0	0.00	−1.00
	Hydrated lime (H.L.) to 0.40 Soda ash (S.A.) to 0.21		0.21	0.21	33.0	32.0	32.0	−1.00	−1.00
Average scores:	S. butter				33.70	33.15	32.95		
	D. butter				33.90	33.40	33.10		
Differences in the average scores					0.20	0.25	0.15		

For convenience, the butter made with a single method of neutralization will be designated as S. butter and that made with the double method as D. butter.

The fresh S. butter scored high in three comparisons, the D. butter in five, while in two comparisons the scores were the same. When the S. butter scored high, the maximum difference in the score of the two samples in a comparison was 1.0, the minimum difference was 0.5 and the average difference was 0.83. When the fresh D. butter ranked high, the maximum, minimum and average differences in the scores of the two lots in a comparison were 1.0, 0.5 and 0.9, respectively. After two months the S. butter scored high in three comparisons, the D. butter in four, and in two the scores were the same. When the stored S. butter ranked high, the maximum difference in the scores of the two samples was 1.5, the minimum 0.5 and the average 1.0. When the D. butter was high, the maximum, minimum and average differences in the scores in a comparison were 2.0, 0.5 and 1.0, respectively. After four months the S. butter scored high in three comparisons, the D. butter in five, while in two the scores were the same. When the S. butter ranked high, the maximum difference in the score of the two samples was 1.5, the minimum 1.0 and the average 1.2. When the D. butter was high, the maximum, minimum and average differences in the scores of the two samples were 2.0, 0.5 and 1.0, respectively.

The fresh S. butter had an average score of 33.70 compared with 33.90 for the fresh D. butter, or a difference of 0.20 points favoring the D. butter. After two months the average score of the S. butter was 33.15 as against 33.40 for the D. butter, or a difference of 0.25 points in favor of the D. butter. After four months the S. butter scored 32.95 compared with 33.10 for the D. butter, or a difference of 0.15 points in favor of the D. butter.

DISCUSSION

The types of neutralizers included in these studies would not be expected to produce large differences in the quality and keeping quality of the butter made from sour cream, because of their comparatively mild action on the cream. The high rate of solubility of the milder soda compounds, together with rapid chemical action, offsets largely the comparatively slow chemical changes produced by the more concentrated but less soluble calcium and magnesium neutralizers. The technique employed in the application of the neutralizers is undoubtedly of much more importance than the type of neutralizer used insofar as the quality of the resulting butter is concerned.

Considerable foaming was observed when bicarbonate of soda was employed, especially when the cream was relatively high in acid. Foaming of the cream may be a factor of some importance from the standpoint of pasteurization efficiency.

The data indicate that the scores of the butter commonly increased

during the first month of storage. This may be attributed to the disappearance, during the storage, of heated or neutralizer flavors.

CONCLUSIONS

1. Butter made from cream neutralized with bicarbonate of soda was commonly of better flavor when fresh than butter made from cream neutralized with hydrated lime or soda ash; after a three month storage period the scores were about the same.

2. The flavor scores of butter made from cream neutralized with bicarbonate of soda showed a comparatively rapid decrease during storage.

3. Only slight differences were found in the flavor scores of fresh and stored butter made from cream neutralized with soda ash, hydrated lime, sodium sesquicarbonate and hydrated calcium and magnesium.

4. Whether single or double neutralization was employed, the flavor and keeping qualities of the butter were not significantly affected.

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A Comparative Study of Mississippi and Minnesota Butter From the Standpoints of Certain Fat Constants and Heat Resistance

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MUCH of the butter made in the cotton belt has characteristics somewhat different from those of butter made in the northern states. Southern butter commonly has a different texture, described as firm, hard or gummy, and a flavor that is flat, oily and slow to "clear up." The butter does not melt completely and quickly with the production of the cooling sensation and the flavor of northern butter.

The character of southern butter is of economic importance to the southern dairyman and butter manufacturer, since the butter is discriminated against when placed on the northern markets, receives a lower score and, therefore, a lower price than butter made from cream of equal quality in the northern states. The Cooperative Creamery, State College, Miss., rarely receives a score above 91, although 90 percent of the butter is made from sweet cream, with good equipment and by skilled workmen.

In view of the apparent differences between southern and northern butter, a comparative study of certain fat constants of the two seemed desirable.

The comparative study was made systematically over a period of one year beginning Macrh, 1931, using butter from Minnesota and Mississippi. Two samples of butter scoring 93 or above were secured on the first and fifteenth of each month from the Land O' Lakes Creameries at Minneapolis, Minn.; the samples were selected from two creameries dependent on local cream. Samples of butter were obtained on the same dates from the Cooperative Creamery located at State College, Miss.

DETERMINATION OF FAT CONSTANTS AND HEAT RESISTANCE

The various fat constants were determined upon arrival of the samples. The butter for analysis was melted and filtered in an insulated box warmed by an electric light which kept the fat in a liquid condition.

The melting points were determined by the Wiley method (1). At least four determinations of the melting point were made on each sample and the results were averaged.

The Zeiss butyro-refractometer was used in running the refractive indices (1). These tests were made at 40° C. and the readings only were recorded. The Reichert-Meissl numbers were obtained in duplicate by the Leffman and Beam method (1). The Iodine Absorption numbers were determined in duplicate by the Hanus method (1), five-tenths being considered the maximum variation permissible. The ability of the butter to withstand heat was determined by the method of Eckles and Palmer (2).

RESULTS AND DISCUSSION

The data showing the fat constants obtained on Mississippi and Minnesota butter over a period of one year are given in table 1.

A comparative study of the melting points show that the Mississippi butter reached the highest value of 41.1° C. in December and the lowest value of 33.9° C. in June, whereas the Minnesota butter gave a maximum melting point of 34.0° C. in December and a minimum of 31.5° C. during June. There was a variation of 7.2° C. in the melting points of the butter from Mississippi as compared with only 2.5° C. in the Minnesota butter, which indicates a comparatively low variation in the melting points of the northern butter. The lowest melting point of the southern butter was practically the same as the highest point of the northern butter. In the southern butter the minimum melting point was 2.4° C. higher than the minimum shown by the northern butter, while the maximum point reached by the former was 7.1° C. higher than that of the latter.

Usually the lowest melting points of butter are expected in the spring and early summer when fresh, green grass makes up a large part of the cows' ration. The effect of the earlier arrival of grass in Mississippi was shown by a decided lowering of the melting points beginning in March and continuing to decrease until a minimum value of 34.0° C. was reached the first of May, whereas the minimum point of the Minnesota butter was reached about one month later. The southern butter appeared to establish two distinct melting point levels; from March until October a comparatively low melting point was maintained, followed by an abrupt increase in October, with the higher level prevailing until the following March. The melting point of the southern butter produced in March, 1932, was 2.2° C. higher than that produced in March, 1931, and was due,

TABLE 1. *Fat constants of Mississippi and Minnesota butter over a period of one year*

Date	Mississippi butter				Minnesota butter			
	Melting point °C.	Refractive index values[2]	Reichert-Meissl No.	Iodine No.	Melting point °C.[1]	Refractive index[1]	Reichert-Meissl No.[1]	Iodine No.[1]
3-15-31	36.8	42.5	28.7	35.6	33.7	41.3	30.1	32.5
4- 1-31	36.4	42.6	30.2	36.2	33.4	41.3	29.2	32.0
4-15-31	35.5	42.6	29.5	35.5	33.6	41.8	30.7	33.3
5- 1-31	34.0	42.0	31.0	34.7	32.1	42.0	28.9	33.5
5-15-31	34.3	42.0	30.7	36.8	32.4	42.5	28.9	35.3
6- 1-31	33.9	42.2	30.1	36.0	31.5	43.3	29.2	38.5
6-15-31	34.9	42.3	31.0	32.9	31.8	43.3	28.8	37.3
7- 1-31	35.4	42.3	28.6	33.9	32.3	44.2	27.0	41.5
7-15-31	34.1	42.2	27.6	34.4	33.0	43.6	24.3	42.8
8- 1-31	34.2	42.5	29.1	34.3	33.0	44.2	24.1	41.5
8-15-31	34.6	42.4	27.1	34.6	33.0	43.8	24.2	41.0
9- 1-31	34.3	42.5	28.3	33.6	33.0	44.1	25.7	41.3
9-15-31	35.5	42.4	27.7	34.0	33.8	43.3	26.5	39.0
10- 1-31	35.7	41.5	25.8	33.0	33.6	43.0	26.6	38.0
10-15-31	39.3	42.7	25.5	33.9	33.2	43.0	26.8	37.0
11- 1-31	38.7	40.7	26.7	31.8	32.9	43.3	27.6	36.0
11-15-31	39.2	42.4	26.3	32.1	33.1	42.9	30.0	36.0
12- 1-31	41.0	42.4	25.7	31.5	34.0	41.7	29.5	32.4
12-15-31	41.1	42.2	25.7	32.5	33.7	41.0	31.0	30.1
1- 1-32	39.1	42.2	26.8	34.1	33.0	41.0	30.8	31.6
1-15-32	40.1	42.5	27.0	33.1	33.6	41.3	31.0	31.5
2- 1-32	40.0	42.1	27.7	32.9	33.1	41.1	30.1	31.6
2-15-32	40.2	42.3	27.5	33.9	33.1	41.3	29.9	31.5
3- 1-32	39.8	42.8	26.9	34.9	33.0	40.7	30.9	30.8
3-15-32	39.0	42.6	27.5	32.7	33.2	41.9	29.6	33.1

[1] Values are averages of two samples.

[2] Values obtained with Zeiss butyro-refractometer.

presumably, to the very late spring which retarded the growth of pasture grasses.

The refractive value of the Mississippi butter reached its highest point of 42.8 in March and its lowest point of 40.7 in November. With the Minnesota butter the maximum was 44.2 in July and August; then the values declined to about 41.0 during December, January, February and March. With the exception of two tests in October and November, the refractive values of the Mississippi butter showed less than a one point variation, whereas a variation of 3.5 points was recorded for the butter received from Minnesota. Butter from both sources showed a minimum value of 40.7. The highest value (42.7) found with the Mississippi butter was 1.5 points below the highest point recorded for the Minnesota butter.

The Reichert-Meissl numbers of the Mississippi samples were highest in May and June, reaching 31.0 in both months, and declined to a low point of 25.5 in October. A value of 31.0 in December and January was the highest observed in the Minnesota butter, and the lowest value of 24.1 was reached in August. The southern butter showed a variation of 5.5 points in the Reichert-Meissl numbers during the year and the northern butter varied 6.9 points. Higher values from the Mississippi samples than from the Minnesota samples were obtained during May, June, July, August and September. There seemed to be no tendency for the Reichert-Meissl numbers of the southern butter to vary with the seasons, but with the northern butter there was an abrupt drop in June which persisted at a low level until September, followed by a progressive increase leading to a high level in December and January.

The iodine numbers of the Mississippi butter were highest in May when the maximum value of 36.8 was reached, and the lowest point of 31.5 occurred in December. With the Minnesota butter the highest number (42.8) was obtained in July and the lowest (30.1) in December. The southern samples showed a variation of 5.3 points between the maximum and minimum values, whereas a difference of 12.7 points was observed in the northern samples. The highest iodine number found in the southern butter was 6.0 points below the maximum for the northern butter; the lowest value for the former was 1.4 points higher than that for the latter. No seasonal trend in the iodine numbers was observed in the samples collected from Mississippi which was in marked contrast to the seasonal variations of the Minnesota butter. Beginning in April the iodine number of the northern butter increased abruptly to a high level in July which was partially maintained until September followed by an equally rapid decrease to a low point in December. This low level reached in December persisted until March, when the numbers again showed an increase.

As the samples of butter were received a portion of each was put in a cold storage room having an average temperature of — 17° C. At the end of the year one sample from Mississippi and one from Minnesota, representing each month, were removed, tempered at about 7° C. for several days, cut in blocks 1¼″ x 1¼″ x 1″ and these pieces placed in an insulated room. The temperature was gradually increased to 37° C. over an 8-hour period. The butter was then hardened and the samples, arranged in monthly sequence, were reviewed. A visual examination of the samples showed that the Mississippi butter produced during the months of January, February, March and December manifested great resistance to the heat applied since the blocks of butter representing these months were affected only slightly by the temperatures used. None of the samples of Minnesota butter, on the other hand, showed much resistance to the heat. The northern butter, however, melted with considerable

uniformity, which was not the case with the southern butter. Due, presumably, to the effect of early pasture in Mississippi, relatively soft butter was produced during April and May, whereas the northern butter which was least resistant to heat was produced in June and July.

From the data presented, as well as from general observation, it is evident that southern butter is commonly firmer and more resistant to heat than much of the northern butter. The production of this type of butter in the south has certain merits. While churning temperatures are generally higher than those used in the north, the butter is easily handled through the different manufacturing steps during the warm season. This type of butter is also desirable from the standpoint of the southern consumer and unquestionably gives the southern creameries advantages during the summer months. The flat, oily and gummy tendencies of southern butter, however, and the lack of uniform melting properties are considered very undesirable from the standpoint of marketing.

CONCLUSIONS

1. The melting point of Mississippi butter was higher than that of Minnesota butter; it followed rather definite seasonal trends and reached its high points during the fall and winter months.

2. The resistance of the Mississippi butter to heat followed the variation in the melting point. There was very little variation in the heat resistance of Minnesota butter throughout the year.

3. The values for the refractive indices, and the Reichert-Meissl and iodine numbers of the Mississippi butter did not follow the decided seasonal trends shown by the Minnesota butter.

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The Manufacture of High-Scoring Butter[1]

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HIGH-SCORING butter must be full and rich in flavor and aroma without showing any indication of uncleanness or sourness. The butter must be smooth and close in texture. A trier plug taken from the butter should show very little loose moisture and no tendency toward stickiness.

The selection of the cream for high-scoring butter is of primary importance. The buttermaker's chief aid in cream selection is his sense of taste. The acid test is of little value, since cream showing a noticeable degree of acid development is undesirable for the manufacture of high-scoring butter. The cream must be sweet and clean in flavor without even a trace of such defects as oily, metallic, rancid, tallowy or weedy flavors. Certain feed flavors, such as those due to grass and silage, are not heavily penalized by the judges; however, it is usually a good plan to use for fancy butter the cream which has the least grass flavor during the grass season and the cream with the least silage flavor during the season when silage is fed. Such feed flavors as those due to rye pasture, rape, musty hay or musty cornstalks are very undesirable. The cream should be at least one to two days old so the undesirable flavors have a chance to develop to such an extent that they are easily discernible. It is usually a good plan for at least two people to grade the cream, since certain flavors are sometimes recognized by one person and not by another. Cream testing 30 to 35 percent in fat is desirable from the standpoint of obtaining a good body and texture in the finished butter.

In the pasteurization of cream for fancy butter care should be taken not to overheat or scorch the cream. This is especially important when

[1] Journal Paper No. J422 of the Iowa Agricultural Experiment Station, Ames, Iowa, Project 127.

TABLE 1. *The manufacture of high-scoring butter*

Trial	Original acidity of the cream in %	Treatment as to neutralization	Kind of butter culture	Amount of butter culture in %	Method of using the butter culture
1	0.14	.....	Regular	8	Not ripened; held 6 hrs.
2	.15	.....	Regular	8	Not ripened; held 6 hrs.
3	.15	.....	Regular	9	Not ripened; held 6 hrs.
4	.14	.....	Regular	7	Not ripened; held 6 hrs.
5	.13	.....	Regular	8	Not ripened; held 6 hrs.
6	.15	.....	Regular	7	Ripened 1 hr.; held 12 hrs.
7	.14	.....	Modified[1]	8	Not ripened; held 12 hrs.
8	.18	.....	Modified	7	Not ripened; held 12 hrs.
9	.17	.....	Regular	7	Not ripened; held 12 hrs.
10	.16	.....	Regular	7	Not ripened; held 12 hrs.
11	.18	.....	Regular	7	Not ripened; held 12 hrs.
12	.16	.....	Modified	8	Not ripened; held 12 hrs.
13	.14	.....	Modified	8	Not ripened; held 12 hrs.
14	.14	.....	Modified	8	Not ripened; held 12 hrs.
15	.18	.....	Modified	8	Not ripened; held 12 hrs.
16	.18	.....	Regular	8	Not ripened; held 12 hrs.
17	.17	.....	Regular	8	Not ripened; held 12 hrs.
18	.18	.....	Regular	7	Not ripened; held 12 hrs.
19	.16	.....	Regular	7	Not ripened; held 12 hrs.
20	.13	.....	Regular + 0.15% citric acid	8	Not ripened; held 12 hrs.
21	.14	.....	Regular + 0.15% citric acid	8	Not ripened; held 12 hrs.
22	.18	.....	Regular + 0.15% citric acid	8	Not ripened; held 8 hrs.
23	.13	.....	Regular + 0.15% citric acid	12	Not ripened; held 8 hrs.

[1] Made from milk cultures of *S. paracitrovorus* acidulated with 0.30% sulphuric acid and 0.15% citric acid.

TABLE 1. (Continued)

Acidity at churning in %		Time of contest	Type of contest	Fresh butter score	Storage butter score	Criticism
Cream	Cream serum					
0.26	0.33	Dec. '31	State	94.00		
.25	.32	Jan. '32	State	94.00		
.27	.37	Feb. '32	National	93.16		Sl. coarse
.24	.33	March '32	State	94.00		
.26	.36	April '32	State	93.50		Sl. coarse
.27	.37	May '32	State	93.66		Sl. coarse
.25	.33	June '32	National	94.25	93.50	
.27	.37	July '32	State	92.00		Coarse
.26	.36	Aug. '32	National	93.00		Sl. coarse
.25	.36	Nov. '32	State	93.16		
.26	.35	Dec. '32	State	93.00		
.24	.33	Jan. '33	State	94.00		
.23	.30	Feb. '33	National	94.00		
.23	.31	March '33	State	94.00		
.25	.35	April '33	State	92.50		Coarse acid
.26	.37	May '33	State	92.00		Coarse acid
.26	.36	June '33	National	94.00	93.50	
.26	.36	July '33	State	92.00		Coarse acid
.25	.35	Dec. '33	State	93.00		Sl. coarse
.23	.31	Jan. '34	State	94.50		
.24	.33	Feb. '34	National	94.00		
.27	.36	March '34	State	93.00		Sl. coarse
.24	.33	April '34	State	94.50		

TABLE 1. (Continued)

Trial	Original acidity of the cream in %	Treatment as to neutralization	Kind of butter culture	Amount of butter culture in %	Method of using the butter culture
24	.15	.....	Regular + 0.15% citric acid	12	Not ripened; held 8 hrs.
25	.12	.....	Regular + 0.15% citric acid	10	Not ripened; held 8 hrs.
26	.18	.....	Regular + 0.15% citric acid	7	Not ripened; held 8 hrs.
27	.18	.....	Regular + 0.15% citric acid	7	Not ripened; held 8 hrs.
28	.19	Neutralized to .1% using $NaHCO_3$	Regular + 0.15% citric acid	8	Ripened 1 hr.; held 8 hrs.
29	.17	Neutralized to .1% using $NaHCO_3$	Regular + 0.15% citric acid	9	Ripened 1 hr.; held 8 hrs.
30	.16	Neutralized to .1% using $NaHCO_3$	Regular + 0.15% citric acid	7	Ripened 1 hr.; held 8 hrs.
31	.13	Neutralized to .08% using Sesqui.	Regular + 0.15% citric acid	9	Ripened 1 hr.; held 8 hrs.
32	.13	Neutralized to .08% using Sesqui.	Regular + 0.15% citric acid	10	Ripened 1 hr.; held 8 hrs.
33	.15	Neutralized to .08% using Sesqui.	Regular + 0.15% citric acid	10	Ripened 1 hr.; held 8 hrs.
34	.14	Neutralized to .08% using Sesqui.	Regular + 0.15% citric acid	12	Not ripened; held 8 hrs.
35	.15	Neutralized to .08% using Sesqui.	Regular + 0.15% citric acid	9	Ripened 1 hr.; held 8 hrs.
36	.14	Neutralized to .1% using Sesqui.	Regular + 0.15% citric acid	10	Ripened 2 hr.; held 8 hrs.
37	.17	Neutralized to .1% using Sesqui.	Regular + 0.15% citric acid	10	Ripened 1 hr.; held 8 hrs.
38	.16	Neutralized to .1% using Sesqui.	Regular + 0.15% citric acid	10	Ripened 2 hr.; held 8 hrs.
39	.14	Neutralized to .1% using Sesqui.	Regular + 0.1% citric acid	12	Not ripened; held 8 hrs.
40	.18	Neutralized to .1% using Sesqui.	Regular + 0.1% citric acid	12	Not ripened; held 8 hrs.
41	.19	Neutralized to .1% using Sesqui.	Regular + 0.1% citric acid	12	Not ripened; held 8 hrs.
42	.19	Neutralized to .1% using Sesqui.	Regular + 0.1% citric acid	12	Not ripened; held 8 hrs.
43	.17	Neutralized to .1% using NaOH	Regular + 0.1% citric acid	14	Not ripened; held 8 hrs.
44	.17	Neutralized to .1% using NaOH	Regular + 0.1% citric acid	13	Not ripened; held 8 hrs.
45	.16	Neutralized to .1% using NaOH	Regular + 0.1% citric acid	14	Not ripened; held 8 hrs.

TABLE 1. (Continued)

Acidity at churning in %		Time of contest	Type of contest	Fresh butter score	Storage butter score	Criticism
Cream	Cream serum					
.26	.35	May '34	State	93.00		Sl. coarse
.24	.33	June '34	National	94.25	94.00	
.27	.36	July '34	National	93.00		Sl. coarse
.27	.36	Aug. '34	State	92.50		Coarse
.24	.33	Sept. '34	National	95.25		
.25	.34	Oct. '34	National	94.25		
.25	.34	Oct. '34	State	93.50		
.24	.32	Nov. '34	State	95.00		
.24	.33	Dec. '34	State	95.00		
.23	.30	Jan. '35	State	93.00		Mild
.23	.29	Feb. '35	National	94.75		
.25	.34	March '35	State	93.00		
.24	.33	April '35	State	94.50		
.23	.30	May '35	State	93.00		Mild
.24	.32	June '35	State	94.00		
.22	.27	June '35	National	95.00	94.00	
.22	.26	July '35	State	94.50		
.23	.27	Aug. '35	National	95.00		
.23	.27	Aug. '35	State	95.00		
.24	.33	Sept. '35	National	96.00		
.24	.33	Oct. '35	National	95.00		
.24	.32	Nov. '35	National	95.25		

pasteurizing small lots of cream in large vats. If the water in the coil of the pasteurizer is over 35 to 40 degrees hotter than the cream (when the usual positive circulating systems are used), the coil is apt to become steam-logged and a scorched and heated flavor may result. When cream is heated in the ordinary vat pasteurizers, it is usually best not to go above 150° to 155° F.; otherwise a slight heated or cooked flavor, which is undesirable in high-scoring butter, may result.

The treatments used by the author in the manufacture of butter entered in state and national contests during the years 1932 to 1936 are presented in table 1. Various procedures were employed from the standpoint of the type of culture used, the method of using culture and the neutralization of the cream before adding the culture. These are summarized as follows:

1. Twelve churnings were made by adding 7 to 9 percent regular culture to the cream after cooling.

2. One churning was made by adding 7 percent regular culture to the cream, ripening for 1 hour and cooling.

3. In six churnings 7 to 8 percent of modified culture was added to the cream after cooling.

4. Sixteen churnings were made with 7 to 14 percent culture prepared by adding 0.15 percent citric acid to the milk; the culture was added to the cooled cream.

5. Ten churnings were made with 7 to 10 percent culture prepared by adding 0.15 percent citric acid to the milk; the culture was added to the cream at 70° F., and the cream was then ripened 1 hour and cooled.

6. In 18 churnings the acidity of the cream was neutralized to 0.08 percent after pasteurization through the use of various soda neutralizers. The cream was then treated with culture and cooled; it was usually held from 8 to 12 hours at a low temperature before churning.

In a general way, the data indicate that the addition of 0.15 percent citric acid to the milk intended for culture resulted in the manufacture of higher scoring butter than had previously been obtained. The butter appeared to have a "fuller" flavor than ordinary butter which was expected because of the importance of citric acid as a source of butter flavor and aroma materials. Still further improvement in the scores of the butter was noted as a result of neutralizing the acidity of the sweet cream to 0.08 to 0.10 percent after pasteurization. This practice made it possible to develop a high degree of flavor in the butter, either by the addition of more culture or by ripening the cream, without the danger of developing a sour or coarse flavor.

The churning, washing and working procedure used for high-scoring butter was not greatly different from that employed in ordinary commercial practice. In order to obtain a firm, close, smooth-boring body on the

butter it was necessary to churn the cream at as low a temperature as possible, to use a wash-water temperature that would keep the butter firm and to work the butter intermittently, draining carefully through a cracked door between each working period.

The Effect of Certain Penicillia on the Volatile Acidity and the Flavor of Iowa Blue Cheese (Roquefort Type)[1]

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IOWA blue cheese, a Roquefort type made from cows' milk, has been manufactured successfully for several years at the Department of Dairy Industry, Iowa State College. Several hundred thousand pounds of milk have been made into cheese and sold through various marketing channels.

In order to learn more concerning the numerous factors involved in the ripening of blue cheese, microbiological and chemical studies, of which this report is a part, are in progress at the Iowa Agricultural Experiment Station.

The amounts and types of volatile acids produced in Roquefort type cheeses appear to be of primary importance from the standpoint of the cheese flavor. Currie (1) has shown that the characteristic peppery flavor of Roquefort cheese is due, in large part, to the accumulation of certain products in the cheese during ripening—namely, caproic, caprylic and capric acids with their easily hydrolyzable salts. Presumably, these products result largely from the hydrolysis of some of the cheese fat by enzymes of the penicillia.

It is logical to assume that individual strains of penicillia would show variations with respect to their action on the cheese fat, in which case certain strains might be more suitable than others in assisting the normal ripening of the cheese. The work herein reported compares the effects of each of eight strains of penicillia on the volatile acidity and the flavor of blue cheese made with the various strains.

[1] Journal Paper No. J421 of the Iowa Agricultural Experiment Station, Ames, Iowa, Project 386.

GENERAL PROCEDURE

Of the eight strains of penicillia used in the comparisons, three were obtained from experiment station collections, while the remaining strains were isolated from various samples of blue-veined cheeses. The sources and the identities of the strains employed are presented in the following table.

Strain No.	Source	Identified as
1	Butterfly brand cheese	*P. roqueforti*
2	Neptune brand cheese	*P. oxalycum*
3	European brand cheese	*P. chrysogenum*
4	N. S. Golding, 16	*P. roqueforti*
5	N. S. Golding, 33	*P. roqueforti*
6	Grove City cheese	*P. roqueforti*
7	C. Thom	*P. roqueforti*
8	Zenith brand cheese	*P. roqueforti*

Several hundred grams of mold-spore powder were prepared from each strain of mold by the method suggested by Hussong and Hammer (3). The powder was placed in sterile containers and stored at a low temperature until the cheese was manufactured.

In the experiments four lots of blue cheese were made; each lot contained eight cheese weighing about five pounds each. The method of manufacture employed was that developed by Goss, Nielsen and Mortensen (2). At the customary period in the manufacturing procedure, the curd of each cheese in a lot was inoculated with one of the mold strains; usually 0.2 grams of mold powder were dusted onto the curd necessary for one cheese. Care was taken to control the moisture and salt contents of the cheese in a lot so as to insure uniformity of composition. Analyses of lots of cheese at several periods during the ripening showed that the variations in the moisture content of individual cheese did not exceed 2.0 percent, while the salt content did not vary more than 1.8 percent.

EXAMINATION AND CHEMICAL ANALYSES

Each lot of cheese was examined organoleptically, and the volatile acidities were studied at a period during the ripening; lot 1 was examined after 17 weeks, lot 2 after 23 weeks, lot 3 after 30 weeks and lot 4 after 37 weeks. The cheese were scored for flavor by several competent judges and comparisons of the mold growth and color of the cheese were also made.

The volatile acidities of the cheese were studied by distilling the cheese with steam after the addition of water and sulphuric acid. Usually 200 grams of finely divided cheese, 400 ml. of water and 25 ml. of sulphuric acid (25 percent by volume) were distilled with steam from a balloon

flask until one liter of distillate was obtained. One hundred ml. of the distillate were titrated with N/10 NaOH and the volatile acidity calculated as the ml. of N/10 NaOH required for the neutralization of the first liter of distillate obtained when a 200-gram portion of cheese was distilled with steam.

The method employed to determine, in a general way, the types of volatile acids present in the distillates was the estimation of the percent Ba in the Ba salt. The procedure used in determining the percent of Ba was to add a little less than the calculated amount of $Ba(OH)_2$ to the remaining 900 ml. of distillate (the 100 ml. portion, used previously for determining the total volatile acidity and to which indicator had been added, was discarded). The aqueous solution of the Ba salt was concentrated on a water bath to about 50 ml. and filtered. After evaporation to dryness on the water bath the salt was recrystallized, dried at 100° C. and the percent Ba determined as follows: Approximately ½ gram was weighed out, dissolved in from 75 to 100 ml. of hot water, heated to boiling and a slight excess of N/1 H_2SO_4 slowly added. After digesting from 8 to 10 hours on a hot plate the $BaSO_4$ was filtered off, ignited and weighed. From the weight of $BaSO_4$ and the weight of the original salt the percent Ba in the latter was calculated.

RESULTS OBTAINED

The results obtained on the effect of the individual strains of penicillia on the volatile acidity and the flavor of the cheese studied is shown in table 1. The total volatile acidities of all the cheese appeared to increase as the ripening progressed. There were, however, large variations in the amounts of volatile acids among the cheese in the same lot. In the relatively young cheese of lot 1 (aged 17 weeks), cheese made with strain 7 had a volatile acidity of only 15 as compared with 26 for the cheese made with strain 3. The same large variations in the amounts of volatile acids were also apparent in the well-ripened cheese. In the comparatively old cheese of lot 4 (aged 37 weeks), cheese made with strain 7 had a volatile acidity of 62 as compared with 100 for the cheese made with strain 1. These results indicate that the strain of mold employed may have considerable influence on the amounts of volatile acids produced in the cheese.

The barium values obtained on the salts prepared from the volatile acid distillates varied only slightly among the individual cheese in a lot. For example, in lot 1 the lowest value obtained was 46.87 on the cheese made with strain 2, whereas the highest value was 49.84 on the cheese made with strain 4, or a difference of only 2.97. Since the variations in the barium values of cheese in the same lot were relatively small, it appears that the strain of mold employed has little effect on the types of volatile acids produced. There were, however, relatively large differences in the barium values obtained among the four lots of cheese. Apparently the barium values decreased as the ripening periods increased. The

TABLE 1. *Effect of certain strains of penicillia on the volatile acidity and the flavor of Iowa blue cheese (Roquefort type)*

	Mold number							
	1	2	3	4	5	6	7	8
	Lot 1. Cheese aged 17 weeks							
Volatile acidity[1]	20	23	26	16	25	16	15	18
% Ba in barium salt	48.48	46.87	47.63	49.84	48.06	48.89	47.83	47.93
Placing on flavor	3	5	8	4	1	6	7	2
	Lot 2. Cheese aged 23 weeks							
Volatile acidity[1]	22	26	21	14	28	24	16	25
% Ba in barium salt	41.37	40.31	41.96	40.32	42.32	42.46	41.06	42.50
Placing on flavor	2	5	7	6	1	8	4	3
	Lot 3. Cheese aged 30 weeks							
Volatile acidity[1]	50	24	32	38	32	28	35	33
% Ba in barium salt	42.49	39.95	41.51	41.14	42.37	40.76	41.47	41.08
Placing on flavor	2	5	3	6	1	8	7	4
	Lot 4. Cheese aged 37 weeks							
Volatile acidity[1]	100	82	90	74	96	74	62	82
% Ba in barium salt	41.38	41.31	41.38	41.32	42.00	40.98	41.12	40.42
Placing on flavor	3	2	6	4	1	5	8	7

[1] No. ml. of N/10 normal NaOH required for the neutralization of the first liter of distillate obtained when a 200-gram portion of cheese was distilled with steam.

average of the barium values for the eight cheese of lot 1 (aged 17 weeks) was 48.19 as compared with 41.24 for those of lot 4 (aged 37 weeks). Presumably there was a greater percentage of volatile acids of relatively high molecular weight, such as caproic, caprylic and capric acids, in the old cheese than in the young cheese where acetic and propionic acids apparently predominated.

The strain of mold employed seemed to influence the flavor of the

cheese considerably. Certain strains regularly produced cheese having the characteristic peppery flavor of typical Roquefort in a relatively short time, while cheese made with other strains either lacked flavor and aroma or developed off flavors. Of the eight strains used, strains 5 or 1 appeared to be the most desirable from the standpoint of obtaining a desirable flavor in the cheese. The cheese made with strain 5 placed first in all four comparisons, whereas that made with strain 1 placed either second or third in all comparisons. Strain 7 appeared to be the least desirable of the eight strains employed, since the cheese made with this strain placed either seventh or eighth in three of the four comparisons. In general, the cheese containing comparatively large amounts of volatile acids regularly had considerable of the peppery flavor, whereas cheese containing relatively small amounts were commonly lacking in flavor.

CONCLUSIONS

1. Certain strains of penicillia appeared to bring about the production of considerably more volatile acids in blue cheese than other strains.

2. In general, the types of volatile acids produced in blue cheese by each of several strains of molds were about the same.

3. Apparently greater percentages of volatile acids of relatively high molecular weights were found in well-ripened blue cheese than in young cheese.

4. Certain strains of molds were regularly associated with blue cheese having a desirable flavor, while other strains were associated with cheese lacking in flavor or containing off flavors.

5. In general, cheese containing comparatively large amounts of volatile acids usually had much of the sharp, peppery flavor characteristic of Roquefort cheese, whereas cheese containing relatively small amounts were usually lacking in flavor.

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Methods Used to Increase Blue Mold Growth in Cheese[1]

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THE work of Thom and Currie (5) and several other investigators has shown the significance of oxygen for the growth of *Penicillium roqueforti*. Some of the possible methods of increasing the supply of oxygen to favor the growth of *P. roqueforti* in Wensleydale cheese are herein considered.

LITERATURE

The supply of oxygen has been shown to be low in Roquefort cheese (5). Undoubtedly this condition is produced largely by the high content of CO_2, which is formed during the ripening of the cheese (6) through the action of the *Streptococcus lactis* group (2) (6) and *P. roqueforti* (5) (1).

Although it is recognized that *P. roqueforti* will grow in an atmosphere of 5 percent oxygen (5), a large number of what are supposed to be blue-veined cheeses remain white because the oxygen supply is considerably below the above percentage (5). In addition, several authorities (3) (4) report the practice of skewering and scraping cheese to produce aerobic conditions which encourage the growth of *P. roqueforti*.

EXPERIMENT 1

DRAWING AIR INTO THE CHEESE

Wensleydale cheeses of from 5 to 10 months of age were fitted with glass catheter tubes made from one-half inch test tubes. The cheeses were bored in the center with a cheese trier and a corked catheter tube forced into the hole. The corks were removed and suction applied to two cheeses at a time from a suction water pump for 24 hours.

[1] The experiments herein reported were conducted at the University of British Columbia and directed chiefly by Dr. B. W. Hammer. The data were analyzed and prepared for publication at the University of Idaho.

The cheeses were examined about six weeks after the application of the suction. Out of the 14 cheeses treated, only 3 developed satisfactory mold. The remainder developed mold only in and near the hole produced by the trier. Air leaks down the sides of the tubes accounted for this growth. In general, drawing air into the cheese by suction does not point to a satisfactory way of increasing the growth of *P. roqueforti* in cheese.

EXPERIMENT 2

INJECTING OXYGEN INTO THE CHEESE

Cheeses similar to those in experiment 1 were fitted with catheter tubes by the method just described; and, in addition, hot wax was poured around the outside of the tube to seal the junction between cheese and tube. The oxygen was injected by connecting the catheter tube with an oxygen cylinder and allowing a slow flow of oxygen into each cheese for five minutes. Twelve five-minute injections were given each cheese over a period of 27 days.

Out of the 12 cheeses treated, only 4 developed satisfactory mold. In the other cheeses mold growth was uneven and occurred chiefly around the center hole. In general the method cannot be considered satisfactory because of uneven mold development.

EXPERIMENTS 3A AND 3B

Henry's law states: "The quantity of a gas (either weight or volume) at N.T.P. dissolved by a given volume of a given liquid at a given temperature is directly proportional to the pressure under which the absorption takes place." This principle was applied to remove CO_2 from cheese.

EXPERIMENT 3A

ALTERNATING REDUCED AND ATMOSPHERIC PRESSURE ON CHEESE IN AN IRON CYLINDER

Seven cheeses, six to eight weeks old, were placed in a specially constructed 10-inch steam pipe cylinder and subjected alternately to reduced and atmospheric pressure. Twenty-four applications of reduced pressure for seven hours each were given over a period of 28 days. The average reduced pressure was 144 mm., or 616 mm. vacuum.

Although it was proved that CO_2 was removed from the cheese to the extent of about 0.1 percent, little gain in the growth of mold in the cheese was noted. Three out of the seven cheeses treated showed slight mold growth, while the seven control cheeses showed no mold. The process of aeration did not materially reduce the weight of the treated cheese beyond that of the control.

Cultures of *P. roqueforti* grown on potato agar developed normally when subjected to the treatment.

EXPERIMENT 3B

A second experiment was conducted to determine the value of less frequent suction periods over an extended period, the permeability of the rind to gases and the significance of skewering the cheese.

Wensleydale cheeses, two to three months old, were selected and divided into four groups of seven cheeses each:

Group A, control.

Group B, subjected to reduced pressure twice a week for six weeks.

Group C, the bandages removed and cheese subjected to reduced pressure twice a week for six weeks.

Group D, the bandages removed and cheese skewered from one end (28 holes 1/16 of an inch) and then subjected to reduced pressure twice a week for six weeks.

The scores for mold growth for each cheese made directly after the experiment and 10 weeks later are given in table 1, in which two significant points are brought out:

1. The alternation of reduced and atmospheric pressure hastened the mold growth but did not permanently improve it.
2. Skewering produced a definite increase in mold growth for the cheese in Group D at both scorings.

EXPERIMENTS 4A AND 4B

SUBJECTING THE CHEESE TO VARIOUS PRESSURES

The door of the iron cylinder was strengthened with six extra bolts so that the cheese could be subjected to high pressure. In spite of these precautions high pressures could not be maintained but dropped about 50 percent in 24 hours.

EXPERIMENT 4A

SUBJECTING THE CHEESE TO FLUCTUATING BUT CONTINUOUS PRESSURE

Eight Wensleydale cheeses, from five to seven months old, were put in the iron cylinder and subjected for 21 days to a pressure which varied from 45 to 80 pounds per square inch.

These cheeses were examined directly after the process. Three cheeses only showed a slight improvement in mold growth over that of the control.

Cultures of *P. roqueforti* grown on potato agar showed a very abnormal and stunted growth when subjected to these pressures with the cheese. After removal from the pressure chamber, however, they assumed a more normal appearance in a few days.

EXPERIMENT 4B

ALTERNATE HIGH AND ATMOSPHERIC PRESSURE

Because of the abnormal growth of the cultures it was decided to subject the cheese to two-day periods of high pressure at intervals of

TABLE 1. *Score for mold growth of Wensleydale cheese subjected to semi-weekly applications of reduced pressure over a period of six weeks*

Group	1928 Date of first treatment	Jan. 7, 1929 Directly after completion of treatment. Mold growth score, maximum 25 pts.[1]	March 16, 1929 10 weeks after completion of the treatment. Mold growth score, maximum 25 pts.[1]
A	Aug. 21	0	23
Control	Aug. 23	0	15
(not treated to	Aug. 30	0	10
reduced pressure)	Sept. 6	0	0
	Sept. 12	0	15
	Sept. 14	0	18
	Sept. 19	0	21
Average		0	14.6
B	Aug. 21	23	23
Bandage on	Aug. 23	0	10
	Aug. 30	15	15
	Sept. 6	0	0
	Sept. 12	23	22.5
	Sept. 14	0	0
	Sept. 19	18	22
Average		11.3	13.2
C	Aug. 21	20	—
Bandage	Aug. 23	15	21
removed	Aug. 30	0	18
	Sept. 6	0	0
	Sept. 12	0	15
	Sept. 14	15	22
	Sept. 19	0	10
Average		7.1	14.3
D	Aug. 21	20	21
Bandage removed	Aug. 23	18	18
and cheese skew-	Aug. 30	15	18
ered	Sept. 6	0	10
	Sept. 12	21	20
	Sept. 14	18	22
	Sept. 19	15	23
Average		15.3	18.9

[1] For simplicity of recording, each cheese was scored on a basis of 25 points for mold growth by the following system, which has been used throughout these and other experiments: Scores from 0 to 15 points represent from no growth to slight growth; scores from 15 to 25 points represent from fair to excellent.

four days. Two pressure levels were used: One with a maximum of 50 pounds usually dropping to 20 pounds, and another of 100 pounds usually dropping to about 50 pounds. Cheeses from one to three months old were used in the experiment and examined at an age when they should have been mature—namely, seven months old.

At 50 pounds maximum pressure two out of six cheeses developed satisfactory mold growth and three others slight mold growth, while only one of the controls developed satisfactory mold and the remaining five no mold growth.

At 100 pounds maximum pressure four out of seven cheeses developed satisfactory mold and two others displayed some mold growth. None of the control cheeses developed mold.

Cultures of *P. roqueforti* grown on potato agar and subjected to either of the above conditions grew normally.

DISCUSSION

Sucking air and injecting oxygen into Wensleydale cheese must be considered an unsatisfactory means of attempting to increase mold growth. Neither method gave a substantial increase of mold growth in the cheese; moreover, the plug hole would materially reduce the sale value of the cheese.

Alternating reduced and atmospheric pressure hastened mold growth. This method in combination with skewering the cheese also increased the mold growth in the cheese. It is recognized, however, that skewering alone, without further treatment, would probably have increased the mold growth in the cheese.

Thus far the subjecting of cheese to intermittent, high pressure has displayed desired results and warrants further study. The most suitable age of the cheese to apply the pressure, the degree of pressure to apply and the time intervals should be determined before practical application can be attempted.

SUMMARY

1. Wensleydale cheeses which were bored in the center and equipped with a suction tube to admit air did not develop satisfactory mold growth.

2. Similar cheeses bored in the center and fitted with a tube for the injection of oxygen resulted in only a few of these cheeses developing satisfactory mold. In general the mold growth was uneven and chiefly around the center hole.

3. Subjecting Wensleydale cheeses to alternating reduced and atmospheric pressure in an iron cylinder hastened the growth of mold but did not permanently improve it. Skewering the cheeses in addition to this treatment produced a definite increase in mold growth.

4. Subjecting Wensleydale cheeses to high pressures in an iron cylinder during ripening to increase the dissolved oxygen was investigated. Both continuous and intermittent pressures were used. Although both methods showed some improvement, the intermittent method of applying the pressure gave the best results.

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The Effect of *Penicillium roqueforti* on Some Lower Fatty Acids[1]

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THE ABILITY of *P. roqueforti* to hydrolyze fat has long been known. In Roquefort cheese the flavor development is due, in part, to the breaking down of the fat through the action of this mold. Several investigators have concluded from their studies on *P. roqueforti* that the mold has the power not only to hydrolyze butterfat (1) but also to use some of the resulting fatty acids as a source of carbon (2) (3). Because of the general action of *P. roqueforti* on butterfat, an attempt was made to determine the effect of the mold on some of the lower fatty acids.

In preliminary experiments plates were poured, using a 1.5 percent agar to which mold spores and various amounts of butyric acid were added. The amounts of acid used per plate were none, 0.0001 cc., 0.001 cc. and 0.01 cc. The plates were incubated several days at room temperature and then observed for mold growth. The plates containing no butyric acid showed no growth. Those containing 0.0001 cc. showed sparse growth, those containing 0.001 cc. showed good growth and spore formation, while those containing 0.01 cc. showed no growth. The plates supporting mold growth were free from any odor of butyric acid; while the uninoculated checks, containing the various amounts of butyric acid, had a pronounced odor. These results suggest that butyric acid, up to a certain concentration, made possible the growth of *P. roqueforti* and, since the odor of butyric acid vanished with the appearance of mold growth, it probably was being used as a food constituent.

The effect of *P. roqueforti* on butyric, caproic, caprylic and capric acids was further studied by adding these acids to skim milk, which had been coagulated by *Streptococcus lactis,* and then inoculating the milk

[1] Journal Paper No J424 of the Iowa Agricultural Experiment Station, Ames, Iowa, Project 119.

with *P. roqueforti* spores. This medium was used in an attempt to approximate the conditions in Roquefort type cheese. The skim milk was placed in containers which gave a large surface area. In the first 3 trials 500 cc. of skim milk in 6-liter Erlenmeyer flasks were used, while in the remaining 6 trials 300 cc. of skim milk in 2-liter Erlenmeyer flasks were employed. The milk was sterilized in an autoclave and coagulated with a pure culture of *S. lactis.* The flasks were paired and each of the acids added to one pair. In the first 3 trials 0.1 percent of the acids was used. Except with butyric acid, this concentration proved toxic to the mold; therefore, after the third trial, the amounts of caproic, caprylic and capric acids were reduced to 0.05 percent. Mold spores were added to one flask in each pair and all flasks allowed to incubate at room temperature. The length of incubation varied with the rate of mold growth. After a thick felt of mold growth had formed and proteolysis of the curd had begun, the cultures with their control were slightly acidified with sulfuric acid and distilled with steam. The data obtained from these distillations are presented in table 1.

The most significant fact shown in the table is that the growth of *P. roqueforti* in every case caused an appreciable reduction in volatile acidity. This reduction was largest for butyric acid and gradually decreased as the molecular weights of the acids increased. The most complete reduction of the volatile acidity was found in trial 7, where 94.21 percent of the butyric acid was destroyed. The other extreme was found in trial 5, where only 27.68 percent of the capric acid disappeared. It must be kept in mind, however, that after the third trial the percentage of butyric acid used was double that of the other acids.

The amount of volatile acid produced in the control flasks was always low. This is to be expected since cultures of *S. lactis* never produce a high volatile acidity; however, even this small amount was markedly reduced by mold growth. The values for the control flasks were so consistently low that after the first three trials control flasks were not used.

The ability to reduce volatile acidity did not seem to vary with the different strains of *P. roqueforti* used. However, the appearance of the growth in the flasks suggested there was a difference in their ability to proteolyze the curd.

It was pointed out by Currie (1) that *P. roqueforti* hydrolyzes the fat in Roquefort cheese and that the resulting volatile acids are responsible, in a large part, for the flavor and peppery sensation so characteristic of Roquefort cheese. Thus, the typical flavor of a Roquefort type cheese does not appear until some time after the development of the mold. Since *P. roqueforti* was found to destroy certain of the lower fatty acids that were added to a medium on which the mold was grown, it might appear that the mold destroyed the flavor instead of producing it. However, there are other possibilities that should be considered. The fatty acids freed early in the ripening period may be used up by the mold and then later,

TABLE 1. *The effect of P. roqueforti on some lower fatty acids*

Cultures incubated at room temperature (about 21° C.).

Trial No.	Mold culture used	Acid added to milk culture of *S. lactis*		Days of incubation after inoc. of mold	cc. N/10 acid in 1,000 cc. distillate from 250 gm. culture		Percent acid destroyed
		Kind	Percent[1]		Without mold growth	With mold growth	
1	No. 6	none	.....	10	6.9	2.8	.....
		butyric	0.1	10	33.4	3.7	88.93
		caproic	0.1	31	27.8	3.7	86.70
2	No. 6	none	.....	10	6.0	2.1	.....
		butyric	0.1	10	39.2	2.6	93.40
		caproic	0.1	31	25.3	3.2	87.36
3	No. 6	none	.....	10	9.1	2.9	.....
		caproic	0.1	31	35.1	2.8	92.03
4	No. 6	butyric	0.1	12	31.1	2.3	92.61
		caproic	0.05	17	18.1	2.5	86.19
		caprylic	0.05	17	15.8	3.6	77.22
		capric	0.05	12	13.5	6.2	54.08
5	No. 6	butyric	0.1	12	34.1	2.7	92.09
		caproic	0.05	12	17.4	3.7	78.74
		caprylic	0.05	12	14.6	4.5	69.18
		capric	0.05	12	11.2	8.1	27.68
6	No. 6	butyric	0.1	12	33.2	3.6	89.16
		caproic	0.05	16	18.2	3.3	81.87
		caprylic	0.05	16	15.4	5.2	66.24
		capric	0.05	12	9.9	6.2	37.38
7	No. 3	butyric	0.1	21	32.8	1.9	94.21
		caproic	0.05	21	15.7	2.3	85.31
		caprylic	0.05	21	12.8	3.6	71.88
		capric	0.05	21	9.5	3.2	65.27
8	No. 8	butyric	0.1	16	29.8	3.6	87.92
		caproic	0.05	16	18.8	3.5	81.59
		caprylic	0.05	16	14.3	4.2	70.63
		capric	0.05	16	11.4	6.4	43.86
9	No. 9	butyric	0.1	21	32.8	2.3	92.99
		caproic	0.05	21	15.7	3.7	76.44
		caprylic	0.05	21	12.8	3.7	71.10
		capric	0.05	21	9.5	4.7	50.53

[1] Percent by volume.

when mold growth has practically ceased, the fatty acids could accumulate as a result of the activity of the mold lipase.

In a skim milk medium, coagulated by *S. lactis,* to which various fatty acids had been added, *P. roqueforti* caused an appreciable destruction of

the volatile fatty acids. This suggests that the mold used the volatile acids as food constituents.

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The Influence of Certain Bacteria on the Ripening of Cheddar Cheese Made From Pasteurized Milk[1]

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PASTEURIZATION of milk for cheddar cheese making is probably the most important contribution to the cheese industry in recent years. From a public health standpoint the pasteurization of all milk for cheese-making is of great significance since it minimizes the possibility of spreading infectious diseases through cheese. In the pasteurization of milk several changes occur which not only prevent the typical cheese flavor from developing but also materially increase the time necessary for proper ripening. If the increase in time required for the ripening of the cheese is due to the partial destruction of the natural bacterial flora of the milk by pasteurization, the addition of pure cultures of essential bacteria should tend to overcome this difficulty.

Evans, Hastings and Hart (2) concluded that organisms of the *Bacterium casei* group are responsible for the pungent taste that develops late in the ripening of both raw milk and pasteurized milk cheese. When milk cultures of *Bacterium casei* were added to pasteurized milk used for making cheese, however, a pronounced sour flavor developed in the cheese. Davies and associates (1) inoculated milk used for cheddar cheese with cultures of *Lactobacillus casei* or *Lactobacillus plantarum,* which had been isolated from ripened cheese. They found that the Lactobacillus cultures appeared to accelerate the protein degradation in the cheese during the early stages of ripening, but had no effect on the flavor or texture.

[1] Abstract of a thesis presented to the faculty of the Graduate College, Iowa State College, in partial fulfillment of the requirements for the degree of Doctor of Philosophy. Journal Paper No. J426 of the Iowa Agricultural Experiment Station, Ames, Iowa, Project 385.

Hucker and Marquardt (4) found that *Streptococcus paracitrovorus,* when added to pasteurized milk in addition to commercial starter, improved the flavor of the cheese; *S. citrovorus* had no effect on the flavor, while certain strains of proteolytic cocci produced a bitter flavor. Hansen, Bendixen and Theophilus (3) found that *S. paracitrovorus* and *S. citrovorus,* when used alone as starters, produced cheese with a bitter flavor and a weak body.

Lane (5) compared the effects of several organisms on the speed of ripening and the flavor of cheddar cheese made from pasteurized milk. Certain strains of *L. casei* produced a mild, buttery flavor in the cheese and hastened proteolysis; *Aerobacter oxytocum* produced an unclean flavor, while *S. liquefaciens* brought about a bitter flavor and abnormal proteolysis. *S. paracitrovorus* produced a typical mellow flavor during the early stages of ripening but had no effect on the hydrolysis of the protein. An unidentified Micrococcus slightly improved the flavor and ripening of the cheese.

The work herein reported is a study of the influence of additional pure cultures of bacteria on the nitrogenous decomposition and the flavor development in cheddar cheese made from pasteurized milk.

METHODS

MANUFACTURE AND SCORING OF CHEESE

For each lot of experimental cheese, 600 pounds of milk were used. The milk was pasteurized at 145° F. for 30 minutes, cooled and divided evenly among four small vats. Two percent of commercial cheese culture was used together with 0.5 percent of a pure culture of L4, a strain of *L. casei* (7). In addition to these cultures, each of three vats was inoculated with a milk culture of a test organism. The cheese manufacturing process employed was that described by Lane and Hammer (6, 7). The cheese were scored for flavor at regular intervals during the ripening by competent judges.

PREPARATION OF TEST CULTURES

The test cultures were cultivated in flasks of sterile milk and were incubated as follows: An unidentified Micrococcus, 70° F. for 7 days; *S. liquefaciens, Alcaligenes viscosus, Achromobacter lipolyticum, Pseudomonas fluorescens, Ps. fragi,* lipolytic acid-forming organism 12 and lipolytic non-acid forming organism 18, 70° F. for 4 days. When inoculated into the milk used for cheesemaking, 0.05 percent of each test culture was used except with the Micrococcus which was added at the rate of 0.5 percent.

CHEMICAL ANALYSIS OF CHEESE

NITROGENOUS DECOMPOSITION IN CHEESE

The methods used for the study of the nitrogenous decomposition in the experimental cheese were those suggested by Lane and Hammer (6).

Cheese serum was obtained at regular intervals during the ripening by submitting mixtures of finely divided cheese and sand to relatively high pressures in a hydraulic press. The serum was analyzed for total nitrogen, amino nitrogen and various fractions of protein and protein decomposition products which were soluble or insoluble in trichloracetic acid, ethyl alcohol and phosphotungstic acid. The values for the various forms of nitrogen were expressed as the milliliters of N/10 acid equivalent to the nitrogen of 1 milliliter of cheese serum.

ACID VALUE OF FAT

When the cheese and sand mixtures were submitted to pressure, considerable fat was obtained with the serum. This fat was used to determine the acid values. Twenty grams of fat were boiled with 50 ml. of neutral 95 percent ethyl alcohol and the mixture titrated with N/10 NaOH using phenolphthalein.

RESULTS

EFFECT OF S. LIQUEFACIENS, AN UNIDENTIFIED MICROCOCCUS OR BOTH

Table 1 presents the data on the nitrogenous decomposition and flavor development in one of the duplicate series of cheese in which *S. liquefaciens*, an unidentified Micrococcus or both were used in addition to the regular cultures.

Throughout the ripening little variation was shown in the amounts of total nitrogen in the serums of the four cheese in a series, although slightly more variation occurred at the end of the ripening than at the beginning. The amounts of nitrogen in the fractions soluble in the various reagents increased as the ripening progressed but showed very little variation. Even after 112 days of ripening the variations were small. Increases in the nitrogen content were also shown in the insoluble fractions, with the exception of the fraction insoluble in trichloracetic acid. This fraction increased during the first 28 days and after that gradually decreased so that after 112 days it was only slightly greater than after 3 days. Although the variations in the soluble fractions were small, the data indicate that the serum of the control cheese contained less soluble nitrogen when ethyl alcohol or phosphotungstic acid were used as precipitating agents than the cheese made with the test organisms.

Increases in the amounts of amino nitrogen in the serums were shown by all of the cheese as the ripening progressed, although the increases were generally less in the serums of the control cheese than in those of the cheese made with test organisms.

The data on the effect of the test organisms on the flavor of the cheese indicate that the control cheese regularly scored less than the cheese made with the test cultures. Very little variation was shown in the flavor scores of the cheese made with *S. liquefaciens*, the Micrococcus or both of these organisms.

Bacteriological examinations of the cheese serum at various intervals during the ripening indicated that considerable numbers of living test organisms were present.

EFFECT OF LIPOLYTIC ORGANISM 18, PS. FRAGI, OR LIPOLYTIC ORGANISM 12

The data on the nitrogenous decomposition and flavor development in one of the duplicate series of cheese in which lipolytic organism 18, *Ps. fragi,* or lipolytic organism 12 was used in addition to the regular cultures are given in table 2.

In the very young cheese there was considerable variation in the total nitrogen of the serums. With extended ripening the total nitrogen in the serums increased, and after 112 days it varied from 20.1 to 22.8 ml. of N/10 acid; the value for the control cheese was lowest in the series, and the cheese made with *Ps. fragi* was highest. In general, there was a steady increase in the various nitrogen fractions in the serums of all the cheese as the ripening progressed. Throughout the ripening there were no large variations in the fractions soluble in the various reagents although the fractions of the serum from the control cheese were commonly less than those of the other cheese in the series. The amounts of nitrogen in the fractions insoluble in the reagents regularly increased during the entire ripening except with the fraction insoluble in trichloracetic acid. This fraction did not appear to increase after the 28 days of ripening. Only very small variations were found in the amounts of insoluble nitrogen in the serums of the four cheese at the end of the ripening.

The serums of all of the cheese increased in amino nitrogen content as the ripening progressed. After 112 days the serums of the cheese made with the test organisms, especially that of the cheese made with *Ps. fragi,* were considerably higher in amino nitrogen than the serum of the control cheese.

The results obtained on the effect of the various test organisms on the flavor development in the cheese showed that, in general, the control cheese had a more desirable flavor than the cheese made with the test organisms after continued ripening. The cheese made with *Ps. fragi* or lipolytic organism 12 was criticized as being sour and bitter, respectively.

Bacteriological examinations of the cheese during the ripening indicated that considerable numbers of living test organisms were present.

EFFECT OF PS. FLUORESCENS, A. VISCOSUS OR A. LIPOLYTICUM

Table 3 shows the data obtained on the nitrogenous decomposition and flavor development in one of the duplicate series of cheese in which *Ps. fluorescens, A. viscosus* or *A. lipolyticum* was used in addition to the regular cultures.

In the very young cheese there were only slight variations in the amounts of total nitrogen in the serums. With continued ripening the total nitrogen of all of the serums increased and the differences among

TABLE 3. *Effect of Ps. fluorescens, A. viscosus or A. lipolyticum on the nitrogenous decomposition and flavor development in the cheese*

Series 5

Serial number of cheese	Age of cheese (days)	Test organisms used	Moisture (percent)	Ml. of N/10 acid equiv. to nitrogen in 1 ml. of cheese serum						Amino nitrogen (mgs. per ml. serum)	Flavor score of cheese	Remarks on cheese flavor
				Total nitrogen	Nitrogen fractionated into soluble and insoluble fractions with							
					Trichloracetic acid		Ethyl alcohol		Phosphotungstic acid			
					Sol.	Insol.	Sol.	Insol.	Sol.	Insol.		
5-1	3	None	38.6	6.4	4.6	1.8	2.0	4.3	1.5	4.8	1.02		
5-2	3	*Ps. fluorescens*	39.0	5.5	3.7	1.8	1.9	3.5	1.0	4.6	.97		
5-3	3	*A. viscosus*	38.4	5.7	3.4	2.2	2.0	3.7	1.0	4.8	1.07		
5-4	3	*A. lypolyticum*	37.8	5.2	3.6	1.6	1.9	3.2	1.0	4.2	1.07		
5-1	14	None	37.8	10.2	7.8	2.4	2.0	8.2	1.9	8.3	2.18	35.5	Bitter & sour
5-2	14	*Ps. fluorescens*	38.0	10.0	7.0	3.0	2.2	7.8	1.7	8.3	1.85	38.0	Lacks flavor
5-3	14	*A. viscosus*	37.5	10.3	7.6	2.7	2.0	8.3	1.7	8.6	1.74	36.0	Sour
5-4	14	*A. lipolyticum*	37.2	9.7	7.0	2.7	2.0	7.7	1.8	7.9	2.28	37.0	Sour
5-1	28	None	36.8	15.2	11.5	3.8	5.5	9.6	3.4	12.0	2.63	35.5	Bitter
5-2	28	*Ps. fluorescens*	37.2	16.4	12.5	3.9	5.8	10.6	3.5	13.0	3.18	37.5	Lacks flavor
5-3	28	*A. viscosus*	36.8	16.1	12.9	3.2	5.6	10.6	3.2	13.0	2.74	36.5	Sour
5-4	28	*A. lipolyticum*	36.7	16.5	13.9	2.6	4.2	12.4	3.7	12.8	2.78	37.0	Sl. sour
5-1	56	None	36.4	19.6	16.1	3.5	6.5	13.0	4.2	15.3	3.00	36.0	Bitter
5-2	56	*Ps. fluorescens*	36.2	18.0	14.4	3.6	6.0	12.0	3.9	14.0	3.42	37.5	Lacks flavor
5-3	56	*A. viscosus*	36.4	18.0	14.5	3.5	6.6	11.4	3.5	14.5	3.10	37.5	Sl. sour
5-4	56	*A. lipolyticum*	36.0	18.9	15.6	3.4	6.5	12.5	3.8	15.2	3.14	37.0	Sour
5-1	112	None	35.3	24.4	21.8	2.6	11.9	12.4	7.6	16.9	6.01	36.5	Sl. bitter
5-2	112	*Ps. fluorescens*	35.7	22.1	19.5	2.8	8.9	13.5	6.6	15.4	5.28	37.0	Lacks flavor
5-3	112	*A. viscosus*	35.5	23.1	21.2	1.9	10.7	12.6	7.2	16.2	6.37	37.0	Lacks flavor
5-4	112	*A. lipolyticum*	35.4	23.5	20.6	3.1	9.5	14.0	7.1	16.5	6.32	36.0	Sour

TABLE 1. *Effect of S. liquefaciens, an unidentified Micrococcus or both on the nitrogenous decomposition and flavor development in the cheese*

Series 1

Serial number of cheese	Age of cheese (days)	Test organisms used	Moisture (per cent)	Ml. of N/10 acid equiv. to nitrogen in 1 ml. of cheese serum						Amino nitrogen (mgs. per ml. serum)	Flavor score of cheese	Remarks on cheese flavor
				Total nitrogen	Nitrogen fractionated into soluble and insoluble fractions with							
					Trichloracetic acid		Ethyl alcohol		Phosphotungstic acid			
					Sol.	Insol.	Sol.	Insol.	Sol.	Insol.		
1-1	3	None	38.9	5.1	3.1	2.0	1.2	3.9	1.0	4.0	.68		
1-2	3	*S. liquefaciens*	38.6	5.0	3.0	1.9	1.1	3.8	1.1	3.9	.64		
1-3	3	Micrococcus	38.7	5.0	3.1	1.9	1.1	3.9	1.1	3.8	.72		
1-4	3	*S. liquefaciens* and Micrococcus	38.9	5.2	3.4	1.7	1.2	4.0	1.2	4.0	.68		
1-1	14	None	37.8	10.9	7.1	3.8	3.8	7.1	2.4	8.4	.90	37.0	Sour
1-2	14	*S. liquefaciens*	37.2	10.7	7.2	3.6	3.2	7.5	2.3	8.5	.86	38.5	Sl. fermented
1-3	14	Micrococcus	37.4	10.8	7.1	3.6	3.1	7.8	1.9	9.0	.94	39.5	Lacks flavor
1-4	14	*S. lique.* and Micro.	37.9	11.0	7.4	3.6	3.1	7.9	2.0	9.0	.93	38.5	Sl. fermented
1-1	28	None	37.2	14.0	10.6	3.3	4.6	9.5	3.4	10.6	1.73	38.0	Sl. sour
1-2	28	*S. liquefaciens*	37.0	14.1	10.7	3.5	4.1	10.1	2.4	11.9	1.73	39.5	Sl sour
1-3	28	Micrococcus	37.3	12.9	10.0	3.7	3.5	9.5	2.4	10.5	1.71	39.0	Sl. sour
1-4	28	*S. lique.* and Micro.	37.5	14.0	10.4	3.6	4.2	9.7	3.6	10.4	2.07	39.0	Sl. sour
1-1	56	None	37.0	16.9	14.6	2.4	5.8	11.1	4.0	13.0	3.90	36.5	Sour
1-2	56	*S. liquefaciens*	37.1	16.4	13.5	3.0	5.9	10.5	4.6	12.0	4.13	37.5	Sl. sour
1-3	56	Micrococcus	37.2	16.6	13.7	2.9	5.8	10.8	3.0	13.6	4.24	38.0	Sl. sour
1-4	56	*S. lique.* and Micro.	37.2	17.8	14.7	3.1	6.2	11.7	3.7	14.0	4.13	37.0	Sl. sour
1-1	112	None	36.4	19.6	17.2	2.5	9.6	9.9	6.8	12.7	5.12	36.5	Sour
1-2	112	*S. liquefaciens*	36.5	19.6	17.0	2.6	7.7	11.0	6.0	13.7	6.53	38.5	Sl. sour
1-3	112	Micrococcus	36.2	20.6	18.0	2.7	8.4	12.1	6.7	13.6	6.21	37.5	Sl. sour
1-4	112	*S. lique.* and Micro.	36.6	20.6	17.9	2.6	8.2	12.5	6.5	14.0	6.26	37.5	Sl. sour

TABLE 2. *Effect of lipolytic organism 18, Ps. fragi or lipolytic organism 12 on the nitrogenous decomposition and flavor development in the cheese*

Series 3

Serial number of cheese	Age of cheese (days)	Test organisms used	Moisture (percent)	Ml. of N/10 acid equiv. to nitrogen in 1 ml. of cheese serum						Amino nitrogen (mgs. per ml. serum)	Flavor score of cheese	Remarks on cheese flavor
				Total nitrogen	Nitrogen fractionated into soluble and insoluble fractions with							
					Trichloracetic acid		Ethyl alcohol		Phosphotungstic acid			
					Sol.	Insol.	Sol.	Insol.	Sol.	Insol.		
3-1	3	None	39.8	4.5	2.8	1.7	1.5	3.1	1.0	3.6	.82		
3-2	3	Lipolytic 18	39.2	5.1	3.3	1.8	1.9	3.2	1.0	4.1	.89		
3-3	3	*Ps. fragi*	39.3	4.8	3.3	1.7	1.6	3.4	0.9	3.4	1.26		
3-4	3	Lipolytic 12	39.3	6.6	4.6	2.0	1.6	3.9	1.1	5.5	1.21		
3-1	14	None	39.4	9.3	6.0	3.2	3.5	5.7	2.0	7.2	1.90	39.0	
3-2	14	Lipolytic 18	38.3	10.0	7.6	2.5	3.9	6.0	1.9	8.0	2.34	39.5	
3-3	14	*Ps. fragi*	38.2	9.8	7.3	2.5	3.6	6.2	1.5	8.3	1.90	37.0	Sour
3-4	14	Lipolytic 12	38.8	11.9	9.2	2.7	4.1	7.8	1.5	10.4	2.60	36.0	Bitter
3-1	28	None	39.0	13.1	10.3	2.8	3.7	9.4	2.0	11.1	2.21	38.5	
3-2	28	Lipolytic 18	38.0	13.6	9.8	3.8	3.8	9.8	2.2	11.4	2.85	38.0	
3-3	28	*Ps. fragi*	38.2	13.7	10.1	3.7	4.3	9.4	1.6	13.0	2.40	37.5	Sl. sour
3-4	28	Lipolytic 12	38.2	13.6	10.5	3.0	3.5	10.1	1.9	11.8	2.25	36.5	Sl. bitter
3-1	56	None	37.8	16.0	13.5	2.5	4.3	11.7	2.5	13.5	3.79	38.0	
3-2	56	Lipolytic 18	37.6	16.0	13.0	3.0	4.5	11.5	2.5	13.7	3.68	38.0	
3-3	56	*Ps. fragi*	37.7	16.7	13.9	2.8	4.8	11.9	2.0	14.7	3.57	37.0	Sl. sour
3-4	56	Lipolytic 12	37.9	16.5	14.2	2.3	4.2	12.3	2.3	14.2	3.84	37.0	Bitter
3-1	112	None	36.8	20.1	17.0	3.0	8.0	12.0	7.1	13.0	5.38	38.0	
3-2	112	Lipolytic 18	36.7	22.3	19.0	3.2	9.8	12.5	8.2	14.0	7.01	37.0	
3-3	112	*Ps. fragi*	36.7	22.8	19.7	2.9	9.7	12.8	6.2	16.5	8.25	36.0	Sour
3-4	112	Lipolytic 12	36.4	21.7	19.5	2.1	8.6	13.2	8.0	13.6	6.97	35.5	Bitter

them became slightly greater. After 112 days the total nitrogen varied from 22.1 in the serum of the cheese made with *Ps. fluorescens* to 24.4 in the serum of the control cheese. Although there was a steady increase in the various nitrogenous fractions in the serums of all the cheese during the curing, the variations among the fractions were relatively small. With the fraction insoluble in trichloracetic acid, however, no increase was apparent after 14 days; and after 56 days there was an actual decrease.

The serums of all the cheese showed about the same increases in amino nitrogen during the curing. No variations that could be attributed to the test organisms were apparent.

The data showing the effect of the test organisms on the flavor of the cheese showed that, in general, the cheese made with the test organisms scored higher than the control cheese.

Bacteriological examination of the cheese from time to time during the ripening indicated that relatively large numbers of living test organisms were present.

ACID VALUES ON FAT FROM CHEESE MADE WITH VARIOUS CULTURES

Acid values on the fat from the experimental cheese were obtained to determine changes in the values at various periods during the ripening. It is not unlikely that fat hydrolysis, in a relatively small degree, may benefit the flavor of cheddar cheese. The data showing the acid values of the fat from the cheese at different periods of curing are given in table 4; the values are expressed as the milliliters of N/10 NaOH required to neutralize 20 grams of fat.

TABLE 4. *Acid values on fat from cheese made with different cultures*

Series and number of cheese	Test culture used[1]	Ml. of N/10 NaOH required to neutralize 20 grams of fat after:				
		3 days	14 days	28 days	56 days	112 days
1-1	Control cheese				4.1	5.2
1-2	*S. liquefaciens*				4.2	5.3
1-3	Micrococcus				4.0	4.9
1-4	*S. liquefaciens* and Micrococcus				4.2	5.3
3-1	Control cheese	2.9	3.3	3.5	4.0	4.2
3-2	Lipolytic 18	3.0	3.5	4.1	4.5	4.7
3-3	*Ps. fragi*	3.0	3.8	4.8	4.9	4.9
3-4	Lipolytic 12	2.8	3.4	4.6	4.5	5.7
5-1	Control cheese	2.9	3.4	4.3	4.4	4.3
5-2	*Ps. fluorescens*	2.8	3.3	4.5	5.2	6.7
5-3	*A. viscosus*	2.8	3.3	4.1	4.4	6.7
5-4	*A. lipolyticum*	2.9	3.5	4.1	4.5	5.4

[1] Butter culture (122) and *L. casei* (L4) were used in all cheese.

A regular increase in the acid values on the fat from all the cheese was apparent as the ripening progressed. With the control cheese and the cheese made with organisms which were not lipolytic, the values were relatively low, whereas comparatively high values were obtained when lipolytic organisms were used in making the cheese. The data show, however, that none of the organisms employed hydrolyzed the cheese fat to any great extent. No rancid flavors were detected in any of the cheese even after 112 days of ripening.

SUMMARY AND CONCLUSIONS

The work reported involved a study of the effect of certain bacteria on the ripening of cheddar cheese made from pasteurized milk. All of the experimental cheese were compared with control cheese made by adding a pure culture of *L. casei* (L4) and a butter culture (122) to the pasteurized milk, because, according to the work of Lane and Hammer (7), the addition of certain strains of *L. casei* to pasteurized milk used for making cheddar cheese appeared to have a desirable effect on the nitrogenous decomposition, the flavor development and the uniformity of the resulting cheese. These cultures were also used in the milk inoculated with the various test organisms.

1. Within the limits of the study, as imposed by the numbers of cheese made and the scope of the chemical analysis, the inoculation of small amounts of milk cultures of the test organisms into pasteurized milk appeared to have the following effects on the cheese:

a. *S. liquefaciens* improved the flavor of the cheese but did not materially influence the nitrogenous decomposition.
b. An unidentified Micrococcus improved the flavor of the cheese but did not significantly influence the nitrogenous decomposition.
c. When both *S. liquefaciens* and the unidentified Micrococcus were added to the milk, the flavor of the cheese was improved and there was a small increase in the total nitrogen in the cheese serum.
d. Lipolytic organism 18 did not influence the flavor development of the cheese but increased the total nitrogen in the cheese serum.
e. *Ps. fragi* decreased the flavor score of the cheese but did not materially influence the nitrogenous decomposition.
f. Lipolytic acid-forming organism 12 decreased the flavor score of the cheese but had little effect on the nitrogenous decomposition.
g. *Ps. fluorescens* did not significantly affect the flavor score or the nitrogenous decomposition in the cheese.
h. *A. viscosus* did not appreciably affect the flavor score or the nitrogenous decomposition of the cheese.
i. *A. lipolyticum* had little effect on the flavor score of the cheese and the nitrogenous decomposition.

2. The acid values obtained on the fat from all of the cheese increased with continued ripening; relatively large increases occurred when *Ps. fluorescens*, *A. viscosus* and *A. lipolyticum* were employed as test organisms.

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Curing Small Units of American Cheese in Liquid Paraffin[1]

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INTEREST in a suitable method of curing and packaging American cheese in small retail size units has been gradually increasing in recent years, particularly following the advent of process cheese into the market just prior to the World War. Process cheese rather quickly gained a foothold in the market, probably for one reason because of the ease and success with which it could be placed in small retail-size units at the factory. This advantage of process cheese coincided with the trend toward retail packaging of other foods and was a feature which evidently appealed to both the retail grocer and his customer.

The success of the retail package of process cheese led to numerous attempts to place natural cheese also in a suitable retail form. The difficulties which have presented themselves in the solution of this problem have been such that no methods have been so successful and practical as to have attained the status of successful general application in the industry. Some cheese manufacturers make a small-style natural cheese which is marketed with a degree of success but which either is subject to excessive loss of weight during curing or becomes unsightly due to mold growth. Others have cured the cheese in large units and cut the cheese into suitable sizes and shapes after curing. This presents the problem of utilization of rind and waste material from cutting to weight. Further, it leaves a cheese surface which is frequently open and one to which it is difficult to apply successfully a coating or wrapper.

The method of curing cheese in oil permits the aging of the cheese in small units without excessive loss of weight and without the growth of

[1] U. S. Patent No. 1974847, entitled "Process for Curing Cheese," issued September 25, 1934, to E. F. Goss and B. W. Hammer and assigned to Iowa State College Alumni Patent Board. Journal Paper No. J417 of the Iowa Agricultural Experiment Station, Ames, Iowa, Project 385.

unsightly mold on the surface of the package. This method of curing cheese is suggested as having commercial possibilities for the curing of small-style retail units of natural cheese.

In 11 comparisons of American cheese in two-and-a-half-pound prints in a paraffin wax coating and cheese cured in mineral oil the flavor score of the latter was superior in four instances, inferior in two, and the same in five. There was a tendency for the flavor in the case of the oil-cured cheese to develop more rapidly; but at the same time the quality of the flavor seemed to undergo earlier deterioration, which suggests that the flavor results were more satisfactory on the short-cured product, especially in the case of high-moisture cheese. Earlier development of cheese flavor is likely caused by the greater bacterial and enzymatic activity in the presence of the greater percentage of moisture retained in the oil-cured cheese. This earlier development of flavor would permit a shorter curing period for the oil-cured cheese as compared with similar original cheese stored under such conditions as would permit considerable reduction of moisture content during aging. This shortening of the time required for curing would constitute a distinct commercial advantage.

In 11 comparisons of the body of paraffin-wax-cured cheese with oil-cured cheese the results indicated an improved score of the latter in seven instances and no difference in four. The oil-cured cheese exhibited a characteristic softness of body which was not present in the wax-cured cheese. This softness is frequently considered by the average consumer to be an indication of richness. In the case of oil-cured cheese this soft, mellow body is desirable or satisfactory in a mild or medium-cured cheese but also is conducive to earlier development of pastiness than in a normally dryer cheese or one which has become dryer through loss of moisture during the curing period.

Loss of weight during curing contributes a considerable amount to the cost of curing cheese. Not only does the drying of the cheese retard the curing agents so that a longer period of time is needed for body breakdown, but the loss of cheese weight is an added expense. Oil-cured cheese loses little weight during the time required to develop a cured cheese flavor. Moisture tests on oil-cured cheese in two-and-a-half-pound prints showed moisture loss during curing to be almost entirely eliminated. Moisture loss after one- and two-month periods was not sufficient to be definitely measurable, and cheese held for 12 months in an average of four trials in no case lost more than 1.6 percent moisture, while the two-and-a-half-pound paraffined prints held for the same period in the curing room lost an average of 10.3 percent.

Since small units of cheese cured in paraffin oil seem to possess certain desirable characteristics which are difficult to secure with conventional methods of curing, this method is suggested as being worthy of further study.

A Study of Some of the Physical Changes Involved in the Rennet Coagulation of Milk and the Subsequent Firming of the Curd

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COMPARATIVELY little is as yet known about the actual physical and physico-chemical changes taking place in milk during and immediately following rennet coagulation. In this study some observations were made on the changes in viscosity and electrical conductivity during coagulation, as well as some ultramicroscopic observations. Special attention was, furthermore, given to the effect of various factors on the increase and final degree of curd firmness following rennet coagulation.

VISCOSITY

The findings of various previous investigators vary regarding the changes in the viscosity of milk during rennet coagulation. While some of them noted a definite gradual rise in viscosity between the time of adding the rennet and visible coagulation, others could not observe any definite increase in the viscosity of the milk before coagulation had occurred.

To further investigate this point, viscosity measurements were made by means of a Lawaczeck viscosimeter at one- to two-minute intervals during rennet coagulation. This instrument seemed to lend itself admirably for this work. In general, it was possible to confirm the work of Gutzeit (1), who, by means of a Reischauer viscosimeter, found that the viscosity of the milk began to rise sooner and continued more gradually at the lower temperatures and with the greater rennet concentrations than it did when higher temperatures and less rennet were employed. Under the latter conditions the rise was almost imperceptible at first but

[1] Work carried on in the Dairy Physics Department of the German Dairy Research Institute, Kiel, Germany, under the direction of Prof. Dr. W. Mohr.

very sudden immediately before visible coagulation occurred. The results of our study seemed to indicate that the course of the viscosity curve is dependent upon the same factors which influence the firming of the curd after visible coagulation, so that it may be possible to anticipate the curd firming by an observation of the viscosity curve during coagulation.

The effect of aging the milk at room temperature on the viscosity increase was interesting. Skim milk was used in which the pH was kept nearly constant by the addition of mono-iodo-sodium acetate. A very stable, powdered rennet, carefully weighed each time on an analytical balance, was used and experiments were run in duplicate. Under these conditions aging definitely increased the coagulation time and delayed the rise in viscosity prior to curdling. Visible coagulation, however, always occurred at approximately the same viscosity, namely, 2.4 to 2.5 centipoises, so that the viscosity curves definitely flattened with each increase in the aging period of the milk. At the moment of visible coagulation there was always a slight reduction in viscosity which lasted only for an instant but was, nevertheless, very definite. This phenomenon might possibly be explained by the observation of Bleyer and Seidl (2) that the viscosity of calcium paracaseinate is lower than that of calcium caseinate.

The time necessary for the milk to reach a certain degree of viscosity was nearly proportional to the time required for coagulation. Thus, milk aged for 31 hours at room temperature required 39.5 minutes for coagulation compared with 20.5 minutes for the unaged milk, or about twice as long. The time required for the aged milk to reach a viscosity of 1.62 centipoises during coagulation was 24.5 minutes compared with 12.8 minutes for the unaged milk or, again, nearly twice as long. A viscosity of 2.26 centipoises was reached by the aged milk in 36 minutes and by the unaged milk in 18 minutes, again twice the time. The milk in these experiments was coagulated at 20° C.

ELECTRICAL CONDUCTIVITY

Few investigations are on record regarding the effect of rennet coagulation on the electrical conductivity of milk. Although no appreciable changes were recorded by any authors, fluctuations were noticed by Reichel and Spiro (3) during coagulation, and, as previously mentioned, Bleyer and Seidl (2) state that the electrical conductivity of calcium paracaseinate is greater than that of calcium caseinate.

The Kohlrausch apparatus with alternating current and telephone was used in this study together with a Wheatstone bridge. The resistance capacity of the Vessel (C) was ascertained by means of a saturated gypsum solution and found to be 0.322. This factor (C) divided by the resistance (R) found, gives the specific conductivity (K) of the milk ($K = C/R$). Results showed that the electrical conductivity of the milk was slightly raised by the addition of the rennet, due no doubt to the salt

content of the latter. No change, however, was noted after the addition of the rennet except when the acidity was increasing in the milk. The specific conductivity found in various kinds of sweet raw and heated samples of milk ran between 57×10^{-4} mho and 65×10^{-4} mho, but in a sample of souring raw milk it increased from 71×10^{-4} mho to 92×10^{-4} mho in 105 minutes.

ULTRAMICROSCOPIC OBSERVATIONS

A few ultramicroscopic observations of the rennet coagulation of diluted skim milk were made by means of a Zeiss cardioid ultramicroscope. The observations revealed comparatively little. Coagulation seemed to consist of a heaping up of the casein particles accompanied by a gradual slowing up of an initially very active Brownian movement of these particles.

FIRMING OF THE CURD

In order to be able to follow closely the development of firmness in the curd, an instrument was devised in the nature of a lever balance, using no springs, which, when lowered down into the curd, measures the resistance offered by the curd in grams. The instrument was lowered into the curd contained in vessels of uniform size to a constant depth and at a uniform speed by means of an electric motor; thus the human factor was completely removed and measurements could be made at one- to two-minute intervals. The temperature of the coagulating milk and of the firming curd was kept constant in a large water bath in which temperature was controlled to $\pm$ 0.2° C. Hydrogen ion concentration in the milk was kept nearly constant for the duration of each experiment, and electrometric determinations were made at the beginning and end of each run. The procedure gave check results and proved quite accurate until sooner or later, according to the conditions of temperature, acidity, amount of rennet, etc., accurate reading became vitiated by peptic decomposition induced by the pepsin in the rennet.

To maintain a constant pH in the milk throughout each one of the experiments, which extended over several hours and which frequently were conducted at temperatures favorable for acid formation by microorganisms, a preservative was sought which would prevent bacterial activity and which at the same time would not change the pH of the milk nor affect the action of the rennet enzyme on the milk as regards coagulation time and firmness of curd. Toluol, chloroform, perhydrol, a solution of iodoform in acetone, as well as several other preservatives, proved unsuitable for these experiments, as the smallest amounts needed to prevent acid development usually retarded rennet coagulation or produced other undesirable effects. The most suitable preservative found was a .02 to .06 percent concentration of mono-iodo-sodium acetate. Usually

15 cc. of a 6 percent solution of this preservative was used in 2,000 cc. of milk.

AMOUNT OF RENNET AND CURD FIRMNESS

According to the law of Storch and Segelcke, coagulation time is reversely proportional to the quantity of rennet added; or, in other words, the product of coagulation time and rennet quantity is a constant. Within the range of the amounts of rennet commonly used in cheese manufacture, this law was found to be fairly accurate. However, over a considerable range of variation in the quantity of rennet used, the law could not be confirmed. It was found that the product of the quantity of rennet and coagulation time rises with rising rennet concentrations and the curd firmness produced per unit of rennet in a definite time after coagulation drops with increasing rennet concentrations, and the more so, the later the firmness is measured. Thus, the curd firmness produced 30 minutes after coagulation per unit of the particular rennet powder used decreased in the ratio of 111:100:62:17:4 with the following increase in rennet powder concentrations: 1:100,000; 1:50,000; 1:25,000; 1:5,000 and 1:1,000 (i. e., one part rennet powder to 1,000 parts of milk). Thus, it is seen that the efficiency of the rennet (considering curd firmness produced) when added in the high concentration of 1:1,000 is only 4 percent of that of the same rennet when added in a concentration of 1:50,000; and, when the rennet was used in the concentration of 1:25,000, it was only 62 percent as efficient as when used 1:50,000. When curd firmness as measured two hours after coagulation instead of 30 minutes after coagulation was used as a criterion of rennet efficiency, the drop in efficiency with increasing concentration was still more pronounced, being in the ratio of 137:100:44:11:2 for the above mentioned rennet concentrations. Rennet is, therefore, most efficient when added in low concentrations, except at 50° C., when the weakening effect of the heat exerts itself most strongly on the low concentrations of the rennet enzyme.

TEMPERATURE AND CURD FIRMNESS

As the temperature of coagulation was lowered from 45° C. to 20° C., a successive lengthening of coagulation time and reduction of curd firmness occurred. This weakening effect on the enzyme action grew with each successive lowering of the temperature. The difference in coagulating speed and curd firmness at 45° C., 40° C. and 35° C. was small, while at 25° C., 20° C. and 15° C. the effect of each lowering in temperature was very marked and curd firmness was affected more severely than coagulation time. At 50° C., compared with 45° C., coagulation time was lengthened due to a partial destruction of the enzyme at the higher temperature. Since the pepsin in the rennet seems to be less severely affected by the heat than rennin, peptization of the curd markedly reduces curd firmness at the higher temperatures. When curd firmness was measured

from 25 to 40 minutes after coagulation, it actually decreased with each rise in temperature from 35° to 50° C. because of the continued influence of the pepsin action.

Maximum curd firmness was obtained at the lower coagulation temperatures; although, of course, the firming was very slow. Thus, the highest curd firmness (75 g.) was attained in a sample thermostatically held for 10 days at 10° C. Freezing the milk solid and holding it in a frozen state for three hours had no appreciable effect on the rennet coagulability of skim milk. In some experiments even a slight reduction of coagulation time was observed.

THE EFFECT OF AGING

Aging skim milk for 7, 26 and 77 hours at room temperature markedly retarded coagulation and the firming of the curd with each increase in holding time, although the pH value of the preserved milk dropped from 6.64 to 6.60. When the milk had been held 104 hours and the pH value had dropped to 6.57 the curd firmness curve, due to the lowered pH, ran slightly above that of the milk held 77 hours, but still considerably below the one of the milk held 26 hours. Aging affected in the same way the viscosity curves of the milk during coagulation.

THE EFFECT OF PASTEURIZATION OF THE MILK

Flash pasteurization of the milk at 85° C. retarded rennet coagulation and curd firming markedly; vat pasteurization at 62° to 63° C. for 30 minutes retarded it only slightly.

OTHER EFFECTS

Homogenization of the milk did not seem to reduce the rennet coagulability of milk, and homogenization of cold milk seemed even to improve it.

Shaking of milk and rennet for 20 seconds retarded coagulation, probably due to absorption of the enzyme in the foam. Churning skim milk for an hour both at 25° C. and at 40° C. retarded coagulation slightly and the firming of the curd markedly. Liquefied foam showed an improved coagulability in comparison with the milk from which it was obtained. The presence of foam in the coagulating milk of course reduces curd firmness.

Addition of gelatine to skim milk accelerated coagulation and curd firming, whereas *additions of albumin* had little effect.

Dilution of 10 percent with distilled water retarded coagulation and curd firming, while a 5 percent dilution was of only slight consequence.

Fat content within the range of normal variations in milk had no effect on coagulation and curd firming. *Increasing hydrogen ion concentrations* accelerated coagulation and curd firming; however, the kinds of acids, alkalies or salts used in changing the pH value was of great im-

portance. Thus, coagulability and curd firmness were higher in a milk to which calcium salts had been added than when HCl or $LaCl_3$ had been added in amounts to produce the same pH value. Calcium salts seemed to be of specific value in increasing the coagulability of milk and the use of a salt with a cation of greater valence, such as $LaCl_3$, seems to exert no added influence. NaOH reduced coagulability and curd firmness more than $Ca(OH)_2$ at the same pH.

TIME AFTER COAGULATION

At the lower temperatures curd firmness increased for a considerable time proportional to the time after coagulation, while at the higher temperatures the firming of the curd proceeded rapidly at first, but soon slowed up, especially with the higher concentrations of rennet, most likely because of the interference of pepsin action.

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Physical and Chemical Effects of Homogenization of Milk[1]

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THE homogenization of all or part of the milk supply has become a part of the regular procedure in the processing of milk in many market milk plants throughout the country. This is the natural outgrowth of a demand for a milk of uniform richness for use in children's homes, schools, restaurants and in many homes. Although the principle of homogenization is not new to the dairy industry, its application to the market milk industry has been somewhat limited. In Canada, however, and particularly in the Province of Ontario, plants offering homogenized milk for sale report that from 10 to 90 percent of the milk sold was homogenized, the amount depending largely upon the size of the city, competition and the extent to which the sale of homogenized milk has been stressed.

Although many data were available on the effects of the homogenization process on market cream and on ice cream, the data bearing upon the homogenization of average fat content milk were not extensive. Studies were undertaken at the Michigan Agricultural Experiment Station to determine the effects of homogenization on some of the physical and chemical properties of milk. Since the inauguration of the project two years ago hundreds of samples of average whole milk have been homogenized in an endeavor to secure a representative picture of the homogenized product in comparison with the original. The milk used in the major portion of the study was mixed milk, testing approximately 3.7 to 3.8 percent fat. In some phases of the study, milk from individual cows and from individual herds was used. The milk was homogenized before and after pasteurization at various temperatures and pressures in a viscolizer of 200

[1] Credit is due to Messrs. C. P. Halloran and Ira Gould, Jr., graduate assistants at the Michigan Agricultural Experiment Station, for much of the work connected with this study.

gallons capacity. The various properties of the milk were studied according to standard recognized procedures.

A summary of the various phases studied will be presented in the following separate units.

ELIMINATION OF THE CREAM LAYER

Perhaps the destruction of the creaming ability of the milk, the aim generally sought in the homogenization process, is the most noticeable change in milk as a result of proper homogenization. The fat globules, which in normal milk are sufficiently large to be buoyed up to the surface by the heavier serum surrounding them, are broken up into globules so fine that they remain uniformly dispersed throughout the serum.

In a visit to a number of plants in which homogenization of milk was practiced, it was observed that pressures ranging from 1,500 to 3,500 pounds per square inch were being used. Also, in some plants the homogenization process was carried out on the preheated milk previous to pasteurization. Our experiments were made to determine the minimum pressure which might be used to secure a stable homogeneous product. Both raw and pasteurized milk were homogenized at pressures of 500, 1,500 and 2,500 pounds per square inch. From the data secured it would appear that pressures of homogenization higher than 1,500 pounds per square inch were unnecessary in eliminating the formation of the cream layer irrespective of whether the process was accomplished before or after pasteurization. Even when this milk which was homogenized at 1,500 pounds pressure was held for 48 hours, a cream layer of measurable volume formed in only 1 of the 24 trials run.

SIZE OF FAT GLOBULES

The principal physical effect of homogenization of whole milk is the breaking up of the fat globules into many smaller ones. Several investigators—including Buttenberg (4), Weigner (15), Baldwin (1), Sommer (14), Doan and Minster (5)—have shown that in properly homogenized milk the fat globules average less than 2 microns in diameter. It has also been shown that the size of the globules varies inversely with the pressure.

The data secured in the present study are in agreement with these observations. It was found that the fat globules were broken down from an average diameter of 3.88 microns in unhomogenized milk to an average diameter of less than 1.5 microns in the milk homogenized at 2,500 pounds pressure. Apparently both the pressure and temperature govern the size of the fat globules. The fat globules in the milk homogenized at 90° F. were not as finely divided as those homogenized at higher temperatures even though the pressure of homogenization remained the same. Although the average diameter of the globules was greatly reduced, the size of the individual globules varied. The average for the largest fat globules in

unhomogenized milk was found to be 13.57 microns, while the largest for the milk homogenized at 2,500 pounds pressure was found to be between 3.4 and 4.0 microns in diameter.

FAT CLUMPING

Several investigators have studied the behavior of the fat globules in homogenized milk and cream. Doan and Minster (5) have shown that many factors affect the clumping of the fat in the homogenized product, and they found that fat clumping was more pronounced in higher fat-bearing products than in milk of normal richness. Since there appears to exist a relationship between fat clumping and instability of some proteins toward heat, clumping of fat in homogenized cream or high fat milk is of great importance. However, clumping of fat in homogenized milk of average fat content does not occur to the extent that it is a factor in the production of a high quality product. Clumping of the fat was observed at varying temperatures and pressures of processing but was not subjected to an intensive study.

SPECIFIC GRAVITY

Since the homogenization process changes the form and relationships of some of the constituents of milk rather than eliminates them, it would seem that the specific gravity of milk would not be influenced by proper homogenization procedure.

Weigner (15) is of the opinion that there is no measurable change in the density of milk after homogenization. Rahn (11) concluded that any decrease in the specific gravity of milk which occurs after passing through a separator, pasteurizer, pump or cooler was due to the incorporation of air during the process.

Although the homogenized lots in both the raw and pasteurized groups averaged a trifle lower specific gravity than did the unhomogenized lots, there was no change of importance due to homogenization. As a whole, the pasteurized groups showed a slightly higher specific gravity than the unpasteurized group. This might be expected since some evaporation of water occurs during pasteurization.

FOAMING

The foaming ability of milk was measured according to the method used by Sanmann and Ruehe (13). Homogenization affected the foaming of milk differently depending upon whether the milk homogenized was raw or pasteurized. Homogenization of the raw milk decreased the foaming ability, while homogenization of pasteurized milk increased the foaming ability. The character of the foams was very different. The foam on the homogenized raw milk contained large air cells which were glossy in appearance, while the homogenized pasteurized milk showed a very fine-textured, dull-appearing foam. Homogenization of raw milk decreased

the foam from 131 percent in the check sample to 75 percent in that homogenized at 1,500 pounds pressure. The pasteurized lots showed an increase in foam from 126 to 150 percent at the same pressures.

PROTEIN STABILITY

Homogenization of milk apparently decreased the stability of the proteins toward alcohol irrespective as to whether the milk was raw or pasteurized. Milk pasteurized before homogenization, however, was considerably more stable to alcohol than homogenized raw milk. As the pressure of homogenization increased the destabilizing effect became more pronounced, but this effect was far less marked in the pasteurized milk.

VISCOSITY

Twelve lots of raw milk preheated to 90° F. and 12 lots of the same milk pasteurized at 145° F. for 30 minutes were homogenized at pressures of 500, 1,500 and 2,500 pounds. The viscosity values were determined on the MacMichael viscosimeter immediately after homogenization and again after a 24-hour holding period. All viscosity determinations were made at 20° C. The results which were secured in the study of homogenization as it affects the viscosity of the product are not in perfect agreement with those reported in the literature. Our data show that homogenization of the raw milk preheated to 90° F. increased the viscosity slightly, while that homogenized at 2,500 pounds pressure after pasteurization decreased the viscosity from 2.142 in the check sample to 1.814 centipoises.

Aging the milk for 24 hours increased the viscosity both of the homogenized raw and of the homogenized pasteurized lots.

The decrease in viscosity of homogenized, pasteurized whole milk observed in these studies is in contradiction to practically all the work on viscosity as affected by homogenization. The results on homogenizing raw milk, however, are in close agreement. The milk on which these tests were made was mixed milk testing approximately 3.8 percent fat. Inasmuch as little fat clumping occurs in homogenized, normal fat-bearing milk, any increase in viscosity in homogenized milk could not be attributed to this factor as in the case of homogenized cream. The increase in viscosity of the raw milk due to homogenization as suggested by Bateman and Sharp (2) is undoubtedly due to adsorption of more of the casein to the increased surface area of the fat globules.

The results of the surface tension study indicate that the surface tension of unpasteurized milk preheated to 90° F. is always lowered by homogenization. Doan and Minster (5) have suggested that this holds true only for milk containing less than 5 to 7 percent fat. It would seem that the lowering of the surface tension of homogenized raw milk is, in part at least, the result of the activity of the lipase in bringing about the hydroly-

TABLE 1. *Effect of homogenization on the viscosity of raw and pasteurized milk immediately after processing. The viscosity is expressed in centipoises at 20° C.*

Lot No.	Raw (90° F.) Pounds pressure of homogenization				Pasteurized (145° F.) Pounds pressure of homogenization			
	0	500	1500	2500	0	500	1500	2500
1.	1.58	1.73	1.88	2.15	2.18	1.85	1.82	1.85
2.	2.12	2.09	2.24	2.24	2.06	2.03	1.87	1.81
4.	2.18	2.21	2.33	2.51	2.27	2.18	2.00	1.64
5.	2.24	2.18	2.12	2.33	2.24	1.82	1.79	1.82
6.	2.12	2.00	2.09	2.06	2.00	1.79	1.79	1.67
7.	2.13	2.26	2.26	2.23	1.97	1.90	1.83	1.70
8.	2.30	2.20	2.13	2.00	2.37	1.80	1.77	1.77
9.	2.27	2.30	2.37	2.43	2.27	2.03	1.90	2.00
10.	2.33	2.40	2.57	2.57	2.20	2.17	1.93	1.87
11.	2.17	2.33	2.40	2.47	2.23	2.13	2.13	2.13
12.	2.23	2.26	2.26	2.47	1.83	1.80	1.73	1.70
Ave.	2.152	2.178	2.241	2.315	2.142	1.954	1.869	1.814

sis of the fat. When the milk was pasteurized before homogenization, the surface tension was slightly increased.

CURD TENSION

Much information is availale in the literature showing the reduction in curd tension of whole milk as a result of homogenization. The data secured in this study substantiate those findings. They indicate that the curd tension of both raw and pasteurized milk is lowered by homogenization in proportion to the pressure used. The extent of change in the toughness of the curd obtained from different samples seemed to be dependent upon the fat content of the milk. Homogenization of skim milk showed no change in curd tension.

SEDIMENT IN HOMOGENIZED MILK

Those practicing homogenization of the milk supply have observed from time to time a defect in the bottled product which was often so evident as to merit serious complaints from customers. This defect appeared as a smudgy, dirty deposit in the bottom of the bottle after the milk had been held at a low temperature for 24 to 48 hours. The amount of this deposit varied greatly. Sometimes it manifested itself as a very fine, hair-like ring at the base and side of the bottle from 1/16 to 1/8 of an inch from the bottom. Occasionally it was so serious as to give the bottom of the bottle a dark appearance. In such cases, the discolored material may occur in several forms. It may be evenly distributed over the entire surface, may be flocculent or may be chunky. The distribution of this material appears to depend to some extent upon the position of the bottle

during storage. This material readily remixes with the milk upon slight shaking of the bottle, after which it does not resettle for some time.

When sediment discs were secured from pint samples of milk which had shown a deposit and which had been remixed, the sediment discs were clean and were in no way indicative of the nature of the milk from which the discs were taken. The sediment was so fine that it passed readily through the disc. By syphoning the liquid above the sediment and concentrating the material from 90 quarts of milk in which the defect was quite apparent, some of the material was obtained, dried and examined under the microscope. Before it was dried the material appeared as very dirty separator slime. After it was dry it was quite hard and tenacious. Under the microscope it appeared to be composed of extremely fine silt, which was possibly associated to some extent with the proteins of the milk. These dirt particles ranged from 2 to 10 microns in size and were irregularly shaped.

A total of 28.9 grams were obtained from the 90 one-quart samples, or, approximately, one-third of a gram of sediment per quart. Although the deposits varied somewhat in intensity of color, two distinct types of color appeared, grayish-black and yellow. The dark precipitate appeared to contain foreign particles, detritus, while the light precipitate appeared creamy and cheesy as if it were high in fat and casein. Generally, when the yellow color predominated, the precipitate was small in quantity. The composition of the sediment is presented in table 2.

TABLE 2. *The composition of sediment in homogenized milk as determined by the Mojonnier method*

Color of sediment	Water percentage	Fat percentage	Solids—not fat percentage	Total solids percentage
Dark gray to black	73.97	9.27	16.76	26.03
Yellow	69.88	12.14	17.98	30.12

In all cases where this defect appeared the check, unhomogenized sample showed a clear white bottom in the bottle. If any sediment specks did appear, they were coarse and of sufficient size to be filtered out.

Trials were run to determine whether the pressure of homogenization had any influence upon the amount of sediment settling out of the milk. Pressures of 1,500, 2,500 and 3,500 pounds were used at 145° F. with no consistent difference showing between any two pressures.

The sediment test proved to be of little value in the selection of milk for homogenization insofar as its relation to the degree of sedimentation was concerned. Even when milk was filtered through two thicknesses of filter cloth and then homogenized, the defect was very evident after 24

hours. It appeared practically as intense as that in the unfiltered homogenized sample.

Power clarification before homogenization was sufficient to overcome the defect.

TITRATABLE ACIDITY

An interesting phenomenon observed in the studies on homogenization was the effect of homogenization upon the increase in the percentage of titratable acidity. When raw milk was homogenized, an increase in the titratable acidity occurred in every case. The greater the pressure of homogenization, the greater was the increase.

Subsequent pasteurization did not reduce the acidity. When the milk was pasteurized previous to homogenization, there was no change in the percentage of acidity due to homogenization irrespective of the pressure employed. These observations are in agreement with those made by Dorner and Widner (7) and have been further substantiated by Ramsey and Tracy (12) and Doan and Minster (5). This increase in acidity may be the result of hydrolysis of the fat by the activity of the lipase brought about, in part at least, by the increase of the surface area of the fat globules as a result of homogenization. Pasteurization exposures inactivated the enzyme.

DEVELOPMENT OF RANCIDITY

Homogenization affected the development of rancidity in the milk to an extent depending upon the heat treatment to which the milk had been subjected previous to homogenization. Dorner and Widner (7) have shown that homogenized raw milk develops rancidity within 15 minutes to 4 hours. These findings have been substantiated by recent work of Halloran and Trout (8), Ramsey and Tracy (12) and Doan and Minster (5). The development of rancidity is associated with an increase in the titratable acidity. As the pressure of homogenization increases, the development of rancidity is accelerated. The agent of rancidity seems to be an enzyme, lipase, which hydrolyzes the milk fat, the increase in surface area of the fat globules apparently accelerating the hydrolysis.

In our studies on rancidity several hundred samples of milk, both mixed and from individual cows, have been homogenized at the preheating temperature of 90° F. In every case, rancidity developed to the extent that the milk was very repulsive to the taste. Usually the rancidity was pronounced after two hours; however, some milk upon homogenization showed rancidity 15 minutes after homogenization. Trials were run to ascertain if pasteurization immediately after homogenization would yield a desirable flavored milk. Fifty gallons of raw milk were heated to 90° F., homogenized at 2,500 pounds pressure and pasteurized at 145° F. for 30 minutes. Although the resulting product did not become as pronouncedly rancid as the unpasteurized samples, the flavor was distinctly

off. It would seem that, where vat pasteurization was employed, homogenization of the milk previous to pasteurization would be undesirable unless the milk were preheated to a temperature sufficiently high to inactivate the enzyme. Dorner and Widner (7) and Ramsey and Tracy (12) have shown that heating milk to 130° or 131° F. materially reduces the activity of the agent producing rancidity.

BABCOCK FAT TEST OF HOMOGENIZED MILK

As a result of some disagreement among investigators on the subject, the Babcock fat test was made the object of special study.

Hudson (10) and Hollingsworth (9) stated that dealers have come to realize that a 3.6 percent pasteurized milk will not yield a 3.6 percent homogenized milk by the Babcock test. The reason given for this discrepancy is that the fat globules are so finely divided that the smaller ones cannot rise with the fat column in the Babcock test bottle. Doan and Swope (6) disagreed with the majority of investigators and stated that homogenization of whole milk or cream, even at high pressures, exerted but little influence on the Babcock test.

If the size of the fat globule directly influenced the Babcock fat test, it would be expected that the butterfat content as shown by the Babcock test would be lowered as the pressure increased.

Even at the higher pressures of homogenization, no difficulty was experienced in forcing up the finely divided globules into the fat column.

TABLE 3. *The effect of various strengths of sulphuric acid upon the elimination of charred material from the Babcock fat test of homogenized milk*[1]

Lot No.	Sp. gr. H_2SO_4	H_2SO_4 purity percentage	Observation
I.	1.815	89.0	Greyish plug at the base of most fat columns although the fat columns were easily readable. The plug was not very heavy, being from 1/32 to 1/64 of an inch in thickness.
II.	1.820	90.0	About the same as in lot I. If any difference, it was in favor of the 1.820 acid. Plug thin but darker in appearance.
III.	1.825	91.0	Plug or scum at base of practically all fat columns. Fairly heavy. Dark.
IV.	1.830	92.0	Plug in all fat columns. Worse than that in any of the above lots. Black and charred.

[1] The Minnesota test reagent was used in making 24 fat tests of milk homogenized at 2,500 pounds pressure at 145° F. Although not all of the fat columns were free from the plug, most of them were. Those which showed the presence of the plug compared favorably in appearance with the best results obtained by the Babcock test.

The Babcock test seemed to be as effective with the raw homogenized milk as with the pasteurized. In only one case was there a variation in the fat percentage as great as two-tenths of 1 percent. This occurred at the beginning of the study when considerable difficulty was experienced at the time in securing clear tests, because of the use of too strong acid. After standardizing the sulphuric acid to a specific gravity of 1.82 to 1.825 such variations were eliminated.

A plug of foreign material was observed at the base of the fat columns in practically all the Babcock tests made on homogenized milk. This defect was subjected to a more intensive study. The results are presented in tables 3 and 4.

The nature and composition of the plug is difficult to explain. Its specific gravity was slightly greater than that of butterfat but less than the serum-water-acid mixture in the test bottle. Its appearance resembled, to a remarkable degree, that of curd which is sometimes observed in the fat columns of tests made from unhomogenized milk, when the Babcock test was improperly made. There appeared, however, to be a fineness of texture in the former which was lacking in the latter. The plug was tenacious and compressed. As the fat column receded upon cooling, the plug adhered to one side of the glass, where it appeared as a thin disc.

CENTRIFUGAL SEPARATION OF HOMOGENIZED MILK

Since the size of the fat globules varied with the pressure and since some creaming was observed at the lower homogenization pressures, the centrifugal separation of homogenized milk was made the subject of special investigation. Six lots of milk, consisting of 50 gallons each, were pas-

TABLE 4. *Effect of various temperatures of acid and milk upon the elimination of charred material from the Babcock fat test of homogenized milk*

Lot No.	Sp. gr. H_2SO_4	Temp. of acid (°F.)	Temp. of milk (°F.)	Observation
I.	1.815	65-70	65-70	Grayish plug at the base of most fat columns, although the fat columns were easily readable. The plug was not very heavy, being from 1/32 to 1/64 of an inch in thickness.
II.	1.820	50	50	Grayish plug.
III.	1.820	75	75	About the same as testing with acid having specific gravity of 1.815.
IV.	1.820	50	75	About the same as testing with acid having specific gravity of 1.815.
V.	1.820	50	100-105	Samples badly charred. Very poor.

TABLE 5. *The efficiency of centrifugal separation of milk homogenized at various pressures*

(The milk was pasteurized at 145° F. and was separated at 120° F.)

Lot No.	Fat test of milk (%)	Fat test of cream (%) Pressure of homogenization (lbs. per square inch)					Fat test of skim milk (%) Pressure of homogenization (lbs. per square inch)				
		0	500	1,500	2,500	3,500	0	500	1,500	2,500	3,500
I.	3.80	33.0	32.0	32.0	21.5	23.5	.02	.05	1.0	1.8	2.5
II.	3.60	32.5	29.0	22.0	18.0	.....	.01	.06	1.0	1.7	....
III.	3.75	35.0	32.0	25.0	20.0	15.0	.01	.08	1.1	2.0	2.4
IV.	3.70	41.5	41.5	30.0	27.0	18.0	.01	.09	1.2	1.8	2.4
V.	3.20	37.0	33.5	26.5	20.5	16.0	.01	.05	1.0	1.7	2.2
VI.	3.65	40.5	38.0	32.0	30.0	21.0	.01	.07	....	1.1	2.1

teurized at 145° F. for 30 minutes, homogenized at various pressures and then separated at 120° F. Ten gallons of milk from each lot were homogenized at a given pressure. The results are tabulated in table 5.

Since the milk was homogenized immediately after the pasteurization exposure and separated as quickly as the milk could be lowered to 120° F., the efficiency of separation was probably greater than if the temperature of the milk had been raised from below 100° F. to the separating temperature. According to these data, 98.2 percent of the fat was recovered in separating homogenized milk when the milk was homogenized at 500 pounds pressure; 70.7 percent at 1,500 pounds; 63.5 percent at 2,500 pounds, and 36.1 percent at 3,500 pounds as compared with 99.7 percent recovered when the milk was unhomogenized. From these figures it would seem that, when milk was homogenized at pressures not exceeding 1,500 pounds, the fat loss in skim milk during centrifugal separation should not exceed 30 percent.

CHURNING OF HOMOGENIZED CREAM

Since the separation of milk homogenized at 1,500 pounds pressure recovered 70 percent of the fat, several trials were run to determine the churnability and the exhaustiveness of churning homogenized cream. Sufficient quantities of sweet cream for churning in the 200-pound laboratory churn were secured and pasteurized at 145° F. for 30 minutes.

Following pasteurization, this cream was divided into three lots of 20 gallons each. Lot I was unripened and unhomogenized; lot II was unripened and homogenized at a pressure of 900 pounds per square inch; lot III was homogenized the same as lot II and ripened to a cream acidity of approximately sixty one-hundredths of 1 percent calculated as lactic acid. Homogenization pressures of 900 pounds were used since a survey of dairy practices showed that table cream homogenization was usually

TABLE 6. *Showing the churnability of ripened and unripened cream homogenized at 900 pounds pressure at 145° F.*

Lot No.	Treatment of cream	Weight (lbs.)	Fat test (%)	Acidity at time of churning (%)	Churning temp. (°F.)	Time to churn (min.)	Fat test of buttermilk (%)
			Trial I				
I.	(Check) Unhomogenized	155	33.0	0.15	50	27	0.17
II.	Homogenized Unripened	155	33.0	0.15	49	51	5.50
III.	Homogenized Ripened	155	33.0	0.55	49	59	3.30
			Trial II				
I.	(Check) Unhomogenized	160	32.5	0.12	51	33	0.30
II.	Homogenized Unripened	160	32.5	0.12	51	35	7.10
III.	Homogenized Ripened	160	32.5	0.60	52	25	2.60
			Trial III				
I.	(Check) Unhomogenized	160	33.0	0.12	50	21	0.35
II.	Homogenized Unripened	160	33.0	0.12	50	55	6.40
III.	Homogenized Ripened	160	33.0	0.62	50	37	4.40

carried out at pressures ranging from 800 to 1,000 pounds. The results are presented in table 6. Ripening of the homogenized cream resulted in more exhaustive churning than was obtained in similar cream unripened. The buttermilk obtained from the churning of the homogenized cream, both the ripened and the unripened, was of excellent quality, possessing a heavy, viscid body, but was relatively high in butterfat content as compared with that from unhomogenized milk.

The butter made from homogenized cream showed no inclination toward defects during the working process, but yielded a firm, waxy body, similar to that from unhomogenized cream.

Since the utilization of surplus homogenized milk and cream is a problem of great importance to those homogenizing milk and cream, the

more exhaustive churning as a result of ripening should be of special commercial interest.

Brown (3) has shown that when homogenized cream was mixed with unhomogenized cream in the proportion of one to eight, and then churned, excessive fat losses in the buttermilk did not occur.

MORE RESEARCH NEEDED ON HOMOGENIZED MILK

Although several phases of the problem have been studied, there appears to be a need for more research upon the homogenization of market milk. Some questions have suggested themselves in this study. Just where does the homogenizer best fit into the processing of market milk, before or after pasteurization? Does homogenized milk upon souring show an abnormal fermentation which would lead the consumer to look upon the product with suspicion? Does homogenized milk seem richer to the average person than similar milk unhomogenized? Can experienced judges of milk recognize homogenized milk by the "feel" of the sample as it passes over the tongue? Are there any advantages of high pressure homogenization when low pressure homogenization is sufficient to render the product homogeneous? Can the temperature of the milk be raised after homogenization rapidly enough under practical plant conditions to inactivate the enzyme responsible for rancidity so that an off flavor does not result? Are there any advantages to the homogenization of milk at higher temperatures than those of the ordinary pasteurization exposure? These and other questions have arisen in connection with the work herein reported.

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Distribution of Bacteria in a Quart Bottle of Whole Milk Held at 0° C.[1]

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IT IS a well-known fact that it is necessary to shake a bottle of milk thoroughly before a sample is removed for bacteriological study if the sample is to be representative of all of the milk. This study was made in an effort to determine the relative numbers of organisms at various locations in a standard-shaped, quart bottle of whole milk held for a known period at 0° C. and also to obtain information on the factors influencing the distribution of the organisms.

EFFECT OF HOLDING A STANDARD QUART BOTTLE OF WHOLE MILK AT 0° C. ON THE DISTRIBUTION OF THE BACTERIA

A two-quart sample of whole milk was mixed thoroughly and divided between two sterile quart milk bottles of standard shape. Immediately after filling and before capping with a sterile cap, 1 cc. was removed from each bottle and placed in two 99-cc. water blanks. The quart samples were then placed in ice water at about 0° C. Standard plate counts were made from each of the two water blanks using standard nutrient agar and the plates were incubated at 37° C. for 48 hours. All bacterial counts were made in three dilutions and the plates were poured in duplicate. The results of the counts represented the bacteria per cc. in the original milk.

After the samples had been held at 0° C. for 6 hours, one quart bottle was removed and, without shaking, four 5-cc. samples were taken from different locations in the bottle by means of a slender pipette. The samples were removed from the top of the cream layer, the center of the cream layer, the bottom of the cream layer and the center of the milk below the cream layer. Each of these samples was plated in the same manner as the

[1] Journal Paper No. J423 of the Iowa Agricultural Experiment Station, Ames, Iowa, Project 119.

normal sample taken at the time the milk was placed at 0° C. After the other quart had been held at 0° C. for 30 hours, samples were taken from the same locations as in the sample held for 6 hours and counts were made in the same manner as before.

Thirteen runs were made, 10 using raw milk as it was delivered to the Iowa State College Dairy Department and 3 using pasteurized milk from the College dairy. The data secured are given in table 1.

TABLE 1. *Effect of holding a quart bottle of whole milk at 0° C. on the distribution of the bacteria*

Trial number	Bacterial count on mixed milk	Bacterial count on samples held 6 hours at 0° C.			
		Top of cream	Center of cream	Bottom of cream	Milk below cream
1	285,000	2,330,000	1,875,000	175,000	54,500
2	13,000	201,000	70,000	49,500	1,300
3	475,000	5,800,000	890,000	207,000	10,000
4*	28,500	200,000	172,500	101,500	900
5	98,000	595,000	1,170,000	460,000	7,300
6*	19,000	166,000	32,000	18,000	700
7*	7,700	105,000	69,500	20,300	850
8	1,065,000	14,500,000	6,250,000	1,235,000	203,000
9	130,000	840,000	1,055,000	515,000	53,000
10	93,500	700,000	430,000	199,500	25,500
11	1,570,000	13,000,000	9,800,000	4,525,000	147,500
12	320,000	1,255,000	820,000	117,000	64,500
13	370,000	12,000,000	1,645,000	48,000	19,600
Log. ave.	124,000	1,195,000	500,000	169,800	12,350

TABLE 1—Continued

Trial number	Bacterial count on mixed milk	Bacterial count on samples held 30 hours at 0° C.			
		Top of cream	Center of cream	Bottom of cream	Milk below cream
1	465,000	1,545,000	860,000	74,000	26,500
2	11,650	241,500	115,000	16,000	6,500
3	254,500	4,895,000	3,390,000	248,000	10,150
4*	24,500	205,000	84,000	3,500	880
5	110,500	672,000	1,140,000	96,500	13,400
6*	21,000	105,000	46,500	6,000	350
7*	7,850	97,000	70,000	16,650	4,950
8	1,310,000	12,900,000	8,350,000	925,000	158,500
9	129,000	900,000	1,180,000	101,500	26,000
10	25,000	620,000	370,000	275,000	3,350
11	1,570,000	30,400,000	20,000,000	490,000	85,000
12	206,000	1,470,000	1,275,000	565,000	74,500
13	580,000	1,260,000	660,000	58,000	36,000
Log. ave.	112,000	1,005,000	680,000	78,500	12,050

* Pasteurized milk.

A study of table 1 shows that, in the milk held 6 and 30 hours, the top of the cream had higher bacterial counts than any other portion of the milk and that the counts obtained from the top of the cream were commonly several times as large as the original counts on the milk. The logarithmic average of all of the runs made shows that there were about 10 times as many bacteria per cc. on the top of the cream as in the original milk, sampled normally. In all of the runs except runs 5 and 9 the cream at the center of the cream layer showed lower counts than the cream on the surface; and, in general, the counts from the center were only about one-half those of the surface. In the milk held both 6 and 30 hours a number of samples obtained from the bottom of the cream layer showed lower counts than those obtained from the original milk.

The counts on the milk below the cream layer were always less than the counts on the original milk sampled normally, as shown by the logarithmic averages. The averages of the counts on the milk below the cream layer were 12,350 and 12,050 when held 6 and 30 hours, respectively, as compared with 124,200 and 112,000 for the counts on the fresh milk, sampled normally.

Whether the sample was held 6 or 30 hours did not seem to affect the counts obtained from the surface of the cream layer to any great extent, although slight variations in counts were noted from other portions of the cream layer. Counts made from the center of the cream layer were commonly lower when the milk was held 6 hours rather than 30 hours, while those obtained from the bottom of the cream layer were usually higher after 6 hours than after 30 hours.

FACTORS AFFECTING THE DISTRIBUTION OF BACTERIA IN A QUART BOTTLE OF WHOLE MILK HELD AT 0° C.

After the distribution of bacteria was found to vary considerably at various locations in the bottles of milk, the question arose as to what caused the distribution found. Were the organisms carried up mechanically by the rising fat globules, or did the motile organisms tend to go to the surface where there was a more abundant supply of oxygen? If the distribution of the organisms was due to the fact that they were carried up mechanically by the rising cream, the larger organisms should be more easily carried up than the smaller ones, leaving a relatively low percentage of the latter left in the milk below the cream layer. Yeasts, therefore, were chosen as the test organisms to determine whether or not size was a factor in determining the distribution. The experiments in which yeasts were employed were conducted in the same manner as those made to determine the distribution of the organisms normally present except that the milk was inoculated with a water suspension of pink yeast before it was divided into the two quart bottles, and the counts were made with Difco malt agar (pH of 3.5) instead of standard nutrient agar; the plates

were incubated at 21° C. for 96 hours. In making the counts only the pink colonies were counted, and the results are expressed as the number of pink yeasts per cc. of milk.

The results of six runs using pink yeast are given in table 2. A study of the table shows that about the same general distribution of yeasts existed in the samples held at 0° C. as was observed in the previous experiments based on bacterial counts. It is significant to note, however, that the milk below the cream line contained a smaller percentage of the original number of yeasts than was observed with the bacteria, which suggests that there may have been a greater tendency for the yeasts to rise than for the bacteria. With the exception of trial 1, the yeast counts on the milk below the cream line were lower in the samples held 30 hours than in those held 6 hours. This fact suggests that the yeasts may have continued to move upward in the milk below the cream layer after the cream layer had formed.

TABLE 2. *Effect of holding a quart bottle of whole milk at 0° C. on the distribution of yeast cells*

Trial number	Yeast count on mixed milk	Yeast count on samples held 6 hours at 0° C.			
		Top of cream	Center of cream	Bottom of cream	Milk below cream
1	12,850	250,000	101,500	27,500	275
2	12,250	133,500	71,000	26,500	555
3	30,500	188,000	149,000	75,000	3,500
4	20,700	146,500	100,000	78,000	1,500
5	16,850	135,000	91,000	7,500	1,350
6	6,500	255,000	36,000	1,650	580
Log. ave.	15,200	177,500	83,800	19,370	895

TABLE 2—Continued

Trial number	Yeast count on mixed milk	Yeast count on samples held 30 hours at 0° C.			
		Top of cream	Center of cream	Bottom of cream	Milk below cream
1	12,450	180,000	105,000	17,000	1,450
2	14,850	180,000	145,000	19,850	385
3	33,500	280,000	202,000	127,500	790
4	19,350	236,000	188,000	15,000	490
5	16,350	136,000	121,500	94,000	500
6	6,950	125,000	52,000	1,100	135
Log. ave.	15,200	177,500	83,800	19,370	895

Studies to determine the effect of motility on the distribution of organisms were carried out in the same manner as the previous experiments except that the milk was inoculated with motile organisms. Standard nutrient agar was employed and the plates were incubated at 21° C. for 96 hours. Two trials were made using *Pseudomonas fluorescens,* and two using *Serratia marcescens.* Only the greenish colonies were counted in the experiments in which *Ps. fluorescens* was used, while the red or pink colonies were counted when *S. marcescens* was employed. The data on these experiments are given in table 3 and the results are recorded as the number of *Ps. fluorescens* or *S. marcescens* organisms per cc. of milk.

TABLE 3. *Effect of holding a quart bottle of whole milk at 0° C. on the distribution of Pseudomonas fluorescens and Serratia marcescens*

Trial number	Count on mixed milk	Count on samples held 6 hours at 0° C.			
		Top of cream	Center of cream	Bottom of cream	Milk below cream
Ps. fluorescens					
1	14,600	68,000	29,500	11,600	1,090
2	55,000	215,000	195,000	146,000	5,300
S. marcescens					
3	103,000	494,000	380,000	260,000	15,500
4	72,000	347,500	225,000	170,000	9,900

TABLE 3—Continued

Trial number	Count on mixed milk	Count on samples held 30 hours at 0° C.			
		Top of cream	Center of cream	Bottom of cream	Milk below cream
Ps. fluorescens					
1	13,250	71,000	35,000	11,300	1,230
2	58,000	450,000	260,000	68,000	5,400
S. marcescens					
3	154,500	560,000	420,000	124,000	4,900
4	62,500	1,000,000	790,000	410,000	6,850

The four trials using the test organisms yielded about the same results as were reported in table 1. The top of the cream contained several times as many organisms per cc. as the original mixed milk. In both runs the center of the cream layer regularly showed lower counts than the top of the cream, and the counts from the bottom of the cream were, in all cases, lower than those from the center. The milk below the cream contained, in general, only about one-tenth as many organisms as the freshly inoculated milk, sampled normally. In the trials where *Ps. fluorescens* was used (trials 1 and 2) the counts obtained on the milk below the cream layer were about the same after 30 hours as they were after 6 hours, whereas in the trials where *S. marcescens* was employed (trials 3 and 4), the counts after 30 hours were considerably lower than those after 6 hours. These results suggest that *Ps. fluorescens* did not continue to rise toward the cream layer during extended holding, while with *S. marcescens* there was apparently a tendency for the organisms to move upward. These data, however, are not sufficient to warrant specific conclusions.

CONCLUSIONS

1. Bacterial counts obtained from the cream layer of standard quart bottles of milk held at 0° C. for 6 and 30 hours were several times larger than counts from the milk below the cream layer.

2. Counts from the extreme top of the cream layer were higher than those found at other locations, and the counts appeared to decrease progressively at greater depths in the bottle.

3. Apparently there was a tendency for the organisms to move upward in the milk after extended holding.

4. The distribution of the organisms appeared to be caused largely by the mechanical filtering action of the fat as it rose in the milk. This view is supported by the observation that yeast cells showed a greater tendency to accumulate at the top of the bottles than did bacterial cells.

5. The presence of motile bacteria may have some effect on the distribution of the organisms.

Influence of Growth Temperature on the Thermal Resistance of Some Aerobic, Spore-forming Bacteria From Evaporated Milk[1]

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THE HEAT treatment employed in manufacturing evaporated milk ordinarily is satisfactory for the destruction of the microorganisms present. Occasionally, spoilage occurs even though there has been no intentional or discernible modification of the heat treatment used with the milk. The spoilage outbreaks are frequently spasmodic but during their brief existence may cause considerable financial loss to the manufacturer.

In connection with various outbreaks of spoilage in evaporated milk, it has been observed at the Iowa Agricultural Experiment Station, and elsewhere, that the outbreaks often occurred during warm weather. It is entirely possible that the outbreaks were caused by an extensive contamination of the milk, with the causal organisms on the farm or in the plant since the warm weather may have been more favorable for the growth and development of the organisms. During warm weather there is also a greater opportunity for the contamination of milk because of the ease with which dry particles of soil, dirt, etc., containing organisms or spores, can be carried in the air. An extensive contamination increases the difficulty of sterilization because of the large numbers of organisms present. Another explanation may be that, if the causal organisms are thermophilic, they would not grow and develop at ordinary temperatures, even if present, but would grow at summer heat, thereby causing spoilage primarily at that season. From the suggestions advanced the importance of growth temperatures on the organisms responsible for spoilage in evaporated milk is apparent.

[1] Part of a thesis presented to the faculty of the Graduate College, Iowa State College, in partial fulfillment of the requirements for the degree of Doctor of Philosophy. Journal Paper No. J425 of the Iowa Agricultural Experiment Station, Ames, Iowa, Project 119.

With the importance of growth temperature in mind, the hypothesis has been advanced that certain strains of bacteria causing spoilage in evaporated milk may be capable of withstanding more severe heat treatment when grown at relatively high temperatures. This possibility, together with the frequency with which outbreaks of spoilage have occurred during hot weather, makes important the evaluation of the influence of increased growth temperature on the thermal resistance of the bacteria most frequently present in evaporated milk. The microorganisms commonly found responsible for spoilage in evaporated milk are aerobic, spore-forming bacteria as reported by Hammer (5, 6), Hammer and Hussong (7), Hussong and Hammer (8), Kelly (9), Morrison and Rettger (11), Spitzer and Epple (12) and others.

The primary interest of the problem is centered around the question: What influence does growth temperature have on the heat-resisting ability of aerobic, spore-forming bacteria found in evaporated milk? Data on this question may furnish at least a partial explanation for the heat-resisting ability of bacteria which frequently cause serious spoilage losses in evaporated milk.

METHODS

SOURCES OF ORGANISMS

The organisms used in the study were secured from three sources:

A. Isolated directly from spoiled evaporated milk.

B. Isolated directly from nonspoiled evaporated milk.

C. Isolated by a commercial laboratory from nonspoiled evaporated milk.

Most of the preliminary trials and all the major portion of the study were conducted in a DeKhotinsky constant temperature bath equipped with a high speed turbine agitator. Light mineral oil was used as the heating medium area; temperatures were maintained within the limits of $\pm$ 0.2° C.

Except for a series of comparative trials with evaporated milk, sterile skim milk was always used as the spore suspension medium.

Sterile agglutination tubes were used as the containers for the spore suspensions in all thermal resistance trials. The tubes were approximately 10 mm. in outside diameter by 75 mm. long, with about 1 mm. thickness of wall.

In order to reduce the factor of heat penetration to a minimum, small samples were used in all heating trials. The sterilized agglutination tubes were partially filled with 2.0 cc. samples of the spore suspension used and then sealed in a blast lamp flame.

Various culture media were used with the different cultures in order to secure the best possible growth and spore production. Nutrient agar proved very satisfactory for growth and development of spores with cul-

tures 1, 2, 3, 4, 7, 8, 9, 13, 15 and 16. It was necessary to resort to beef-infusion agar for satisfactory growth and development of spores with cultures 5, 10, 11, 12 and 17. Litmus milk was used as the medium to determine sterility with cultures 1 and 5, while with all other cultures dextrose broth containing bromocresol purple was employed.

Using suitable culture media for the various cultures, as given above, an effort was made to secure growth of each organism at 10°, 21°, 37°, 45° and 55° C. No growth was secured at 10° C., and none of the organisms had a growth range from 21° to 55° C.

In the preparation of spore suspensions nutrient or beef-infusion agar slants were inoculated with a pure culture of the organism to be tested and grown at one of the temperatures which preliminary work indicated would permit growth and development of spores. The periods of incubation at the different growth temperatures were varied in order to permit as large a production of spores as possible and yet secure comparisons of spores of approximately the same age. The production of spores was slower at low-growth temperatures than at higher temperatures, which necessitated a longer period of incubation at the low temperatures in order to secure approximately the same spore development as at the higher temperatures. The maximum periods of incubation at the higher temperatures were in turn limited by the injurious effect of the higher temperatures on the spores. Long periods of incubation at high temperatures decreased the number of viable spores on the agar slants. A portion of each growth was transferred to 60 cc. of sterile skim milk and the skim milk then agitated vigorously for several minutes. The spore content per cubic centimeter of skim milk in comparative thermal resistance trials was relatively constant due to the care with which definite quantities of growth were transferred. Thus, the growth temperature was the only variable factor. Each of eight sterilized agglutination tubes was then partially filled with 2.0 cc. of the spore suspension and sealed in a blast lamp flame. The tubes were immediately heated in the oil bath, and the time between removal of the spores from the incubator and immersion in the oil bath never exceeded 20 minutes. Although eight tubes were prepared for each trial, only seven exposures were used; and the eighth tube was needed only when a tube was accidentally broken. The skim milk remaining after the agglutination tubes were filled was heated at 80° C. for 10 minutes, and plated for spore content on the assumption that the spores but not the vegetative cells would survive this exposure.

In each trial on the comparative heat resistance of spores of different ages, every effort was made to secure spore suspensions containing approximately the same number of spores per cubic centimeter. In some instances it was necessary to make several runs before satisfactory spore counts were secured in comparative trials. By this means the only variable factor influencing heat resistance was the growth temperature.

The spores were exposed to heat by immersing the sealed tubes containing the spore suspension in the oil bath. Before the tubes were subjected to the desired temperature, they were exposed to the same temperature for 15 seconds in a preliminary oil bath. The exposure temperatures used, except for slight variations with specific cultures, were: 104° C., 108° C., 112° C., 116° C. and 120° C. The series of eight agglutination tubes, each containing 2.0 cc. of the spore suspension, was exposed to the desired temperature for definite periods of time.

With each culture the exposure periods used in the preliminary trial were 3, 5, 7, 10, 15, 20 and 25 minutes. After the first trial, the exposure periods were usually varied in order to have about the same number of periods above and below the period last showing growth. For example, if the preliminary trial showed the last survival at 7 minutes, the exposure periods were then changed to 3, 4, 5, 7, 9, 12 and 15 minutes. In practically all trials other than the preliminary ones the differences between exposures were 1 minute from 0 to 5 minutes, 2 minutes from 5 to 9 minutes, 3 minutes from 9 to 15 minutes and 5 minutes from 15 to 40 minutes. At the end of each exposure period a tube was removed, immediately placed in a bath of cold water and tested for sterility within 10 minutes.

Sterility of a spore suspension after exposure to heat was determined by inoculating a tube of litmus milk or dextrose broth containing bromocresol purple with a 1 cc. portion of the contents of the heated tube and incubating for 7 days at the optimum growth temperature for the culture. As a check, several loops of the heated spore suspension were streaked on a standard nutrient or beef-infusion agar slant and the slant incubated at the optimum growth temperature for 7 days.

All hydrogen ion determinations were made electrometrically, using quinhydrone, and calculated to the nearest 0.1.

RESULTS

COMPARISON OF HEAT RESISTANCE OF SPORES SUSPENDED IN SKIM MILK AND IN EVAPORATED MILK

Various investigators, notably Ayers and Johnson (1), Barthel and Stenström (2), and Brown and Peiser (3), have demonstrated that milk, when used as the suspension medium for the vegetative cells or spores of bacteria, aids them in resisting destruction by heat. Therefore, in determining the thermal resistance of any organism in milk, due consideration should be given to the influence of the protective action of the kind of milk used.

In studying the influence of growth temperature on the thermal resistance of some aerobic, spore-forming bacteria from evaporated milk, it was planned to use either sterilized skim milk or evaporated milk as the suspension medium instead of sterile water or broth; thus the conditions of heating would be more nearly comparable to those existing during the

TABLE 1. *Comparison of heat resistance of spores suspended in skim milk and in evaporated milk*

(Temperature of exposure 116° C.)

Culture used to secure spores			Milk used for suspension	Spores per cc. of milk heated	Period of heating (minutes)	Survival (minutes)
No.	Growth temperature °C.	Age (days)				
1	37	5	skim	17,400	2, 3, 4, 5, 7, 9, 11	7.0
			evap.	18,000	2, 3, 4, 5, 7, 9, 11	7.0
2	37	5	skim	3,500	16, 18, 20, 22, 24, 26, 28	20.0
			evap.	4,500	16, 18, 20, 22, 24, 26, 28	18.0
4	37	5	skim	250,000	16, 18, 20, 22, 24, 26, 28	20.0
			evap.	290,000	16, 18, 20, 22, 24, 26, 28	18.0
5	55	3	skim	450	26, 28, 30, 32, 34, 36, 38	30.0
			evap.	500	26, 28, 30, 32, 34, 36, 38	30.0
9	45	3	skim	40,000	1, 2, 3, 4, 5, 7, 9	2.0
			evap.	45,000	1, 2, 3, 4, 5, 7, 9	2.0

process of sterilizing evaporated milk. In order to determine the difference, if any, between the protective action of sterilized skim milk and evaporated milk, a series of comparative trials was run with five representative cultures.

Every effort was made to have the conditions as nearly identical as possible, except for the type of suspension medium. In each comparison the spore content per cubic centimeter of skim milk and evaporated milk varied only a little, and since the spores were from one source the growth temperature and age were the same. The evaporated milk used was not resterilized but was transferred aseptically from commercial cans of evaporated milk. It had a pH of 6.5, while the pH of the skim milk was 6.4.

Results of the trials are presented in table 1. In 3 of the 5 comparisons there was no difference in the protective action of the two suspension media. With cultures 2 and 4, however, the skim milk apparently had a slightly greater protective action than did the evaporated milk. In each instance the difference was only two minutes at the temperature used and does not appear to be significant, especially since the results of the three other comparisons showed no difference between the two media.

From the data presented it appears that results of thermal resistance trials using sterilized skim milk as the spore suspension medium are comparable to the results secured with evaporated milk as the suspension medium.

When it was found that there was little difference between the protective action of skim milk and of evaporated milk, it was decided to use

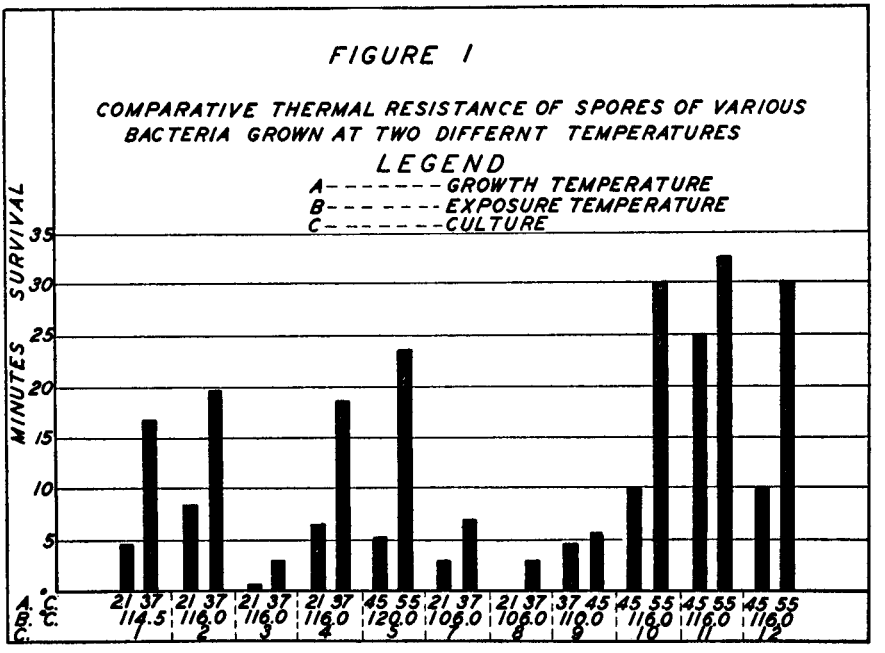
skim milk as the suspension medium for the spores in the thermal resistance trials. The decision was also based upon two other reasons: First, evaporated milk is difficult to handle in large quantities without contamination; and, second, it was considered inadvisable to resterilize evaporated milk since there is a possibility of changing its composition and consequently modifying its protective action on the suspended spores.

COMPARATIVE THERMAL RESISTANCE OF SPORES OF VARIOUS BACTERIA GROWN AT TWO DIFFERENT TEMPERATURES

In an effort to secure information on the influence of growth temperature on the thermal resistance of bacterial spores, all of the cultures examined which showed any appreciable resistance to heat were grown at two different temperatures and the spores tested for heat resistance. The higher of the two growth temperatures was, in all instances, the optimum or at least the temperatures which gave the most luxuriant growth of the culture being studied. The optimum growth temperatures employed were 37°, 45° and 55° C., while the lower growth temperatures used in comparison with these temperatures were 21°, 37° and 45° C., respectively. Before being used in comparative trials, each culture was carried through at least three transfers at the respective growth temperatures.

The results of the comparative trials with the various cultures are presented in fig. 1, which clearly shows the differences in average thermal resistance of the spores when grown at two temperatures. The figure shows that every culture possessed a lower average thermal resistance when grown below the optimum. The greater thermal resistance exhibited at the optimum growth temperature was, except for culture 9, very significant. With culture 9 the greater thermal resistance caused by growth at its optimum growth temperature was comparatively small, being only one minute. It is well to note that there was no instance of spores from a culture grown at a temperature below the optimum exhibiting a thermal resistance greater than that of spores from a culture grown at the optimum temperature. The spore content, per cubic centimeter of skim milk, varied somewhat in the comparative trials; but no significant difference in thermal resistance could be attached to any definite difference in the spore content. Within the limits of the spore contents used in the individual trials, there did not appear to be any correlation between the number of spores present per cubic centimeter of skim milk and the time of survival of the spores. The data definitely demonstrate that, with the cultures studied, growth at a temperature below the optimum decreased the thermal resistance.

The increases in thermal resistance of the cultures when grown at the optimum growth temperatures were particularly striking with cultures 1, 2, 4, 5, 10, 11 and 12, being 12.3, 11.4, 12.5, 17.5, 20.0, 7.5 and 20.0 minutes, respectively. These cultures were exposed to temperatures approximating the sterilization temperature used for evaporated milk. Cul-



ture 1 was exposed to 114.5° C., cultures 2, 4, 10, 11 and 12 to 116.0° C., and culture 5 to 120.0° C. At these temperatures the spores, when grown at the optimum growth temperature, survived longer than the normal holding period used in sterilizing evaporated milk, which is from 15 to 17 minutes. The average survival was 16.9, 19.9, 18.8, 23.6, 30.0, 32.5 and 30.0 minutes for cultures 1, 2, 4, 5, 10, 11 and 12, respectively. Outstanding in resistance to heat was culture 5, which, when grown at 55° C., survived a temperature of 120° C. for 23.6 minutes. This thermal resistance is sufficient to enable the organism to survive the ordinary autoclaving procedure used in laboratory work which demands a temperature of 120° C. for 20 minutes.

These observations indicate that spores of some of the organisms found in evaporated milk can survive, when grown at their optimum temperature and present in large numbers, the sterilization process normally used in the manufacture of evaporated milk.

COMPARATIVE THERMAL RESISTANCE OF SPORES OF VARIOUS BACTERIA GROWN AT THREE DIFFERENT TEMPERATURES

Having established the fact that a growth temperature below the optimum lowered the thermal resistance of the spores of the cultures studied, it was thought advisable to try other growth temperatures. Cultures 2, 4, 7, 8 and 9 were the only cultures which, with the temperatures

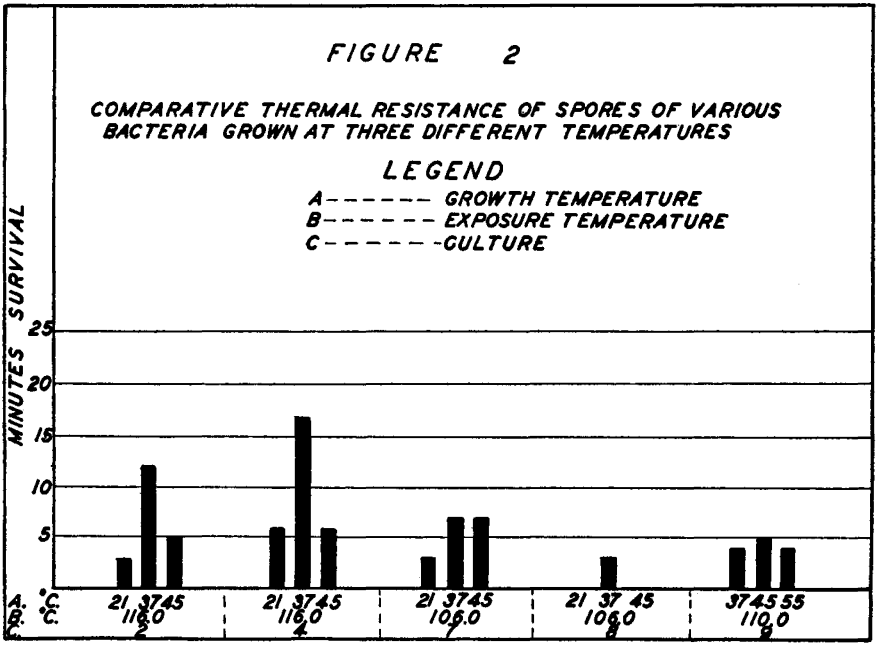
used, showed growth at a temperature above the optimum and also at a temperature below the optimum. These cultures were carried through at least three transfers at the respective growth temperatures before being used in comparative thermal resistance trials. The pH of the skim milk was 6.3 in all cases.

Figure 2 clearly shows that the average thermal resistance of the spores of the cultures employed was usually lowered when growth temperatures either above or below the optimum were used. In other words, unfavorable growth temperatures decreased the thermal resistance. Individual comparative trials likewise showed the same general tendency. These results substantiate those secured when two growth temperatures were studied.

If the cultures investigated are representative of those found in evaporated milk, it appears that a low resistance to heat can be effectively secured by maintaining low growth temperatures. From the data secured, temperatures of 21° C. or below would be considered as low temperatures. With all the cultures studied a temperature of 21° C. or below resulted either in no growth or in a low thermal resistance of the spores formed.

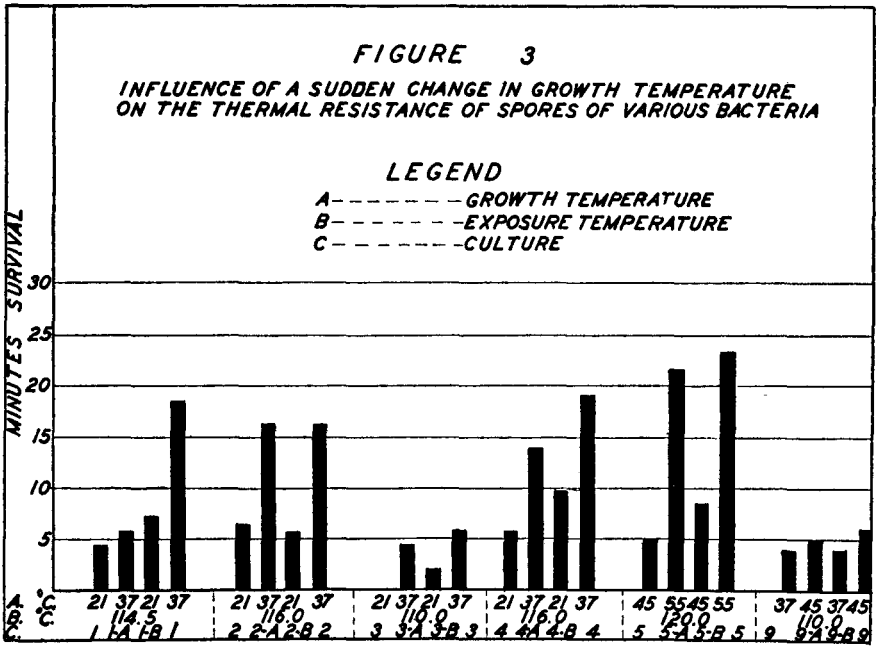
INFLUENCE OF A SUDDEN CHANGE IN GROWTH TEMPERATURE ON THE THERMAL RESISTANCE OF SPORES OF VARIOUS BACTERIA

In all the trials thus far reported each culture was carried through a series of at least three transfers or generations at a certain temperature



before it was used in the thermal resistance trials. Speculation as to the influence of a sudden change in the growth temperature naturally arose, and a series of trials was planned in an effort to evaluate this factor.

After cultures 1, 2, 3, 4, 5 and 9 had been growing for some time at two different temperatures, of which the higher temperature was the optimum temperature, the growth temperatures of the cultures were suddenly changed. The same growth temperatures were used for each culture as formerly, but the transfers of the cultures were held at a temperature different from the one used with the cultures from which the transfers came. For example, if a transfer was made from a culture growing at 37° C. (the optimum temperature for the culture), the transfer was grown at 21° C.; while if the transfer was made from a culture of the same organism growing at 21° C., it was grown at 37° C. Figure 3 shows that with all cultures studied a sudden change in growth temperature from below the optimum to the optimum caused an increase in the average thermal resistance of the spores. In contrast, a sudden change of growth temperature from the optimum to a lower temperature resulted in a decrease in the average thermal resistance of the spores of each culture. Individual comparative trials showed the same differences in resistance of the spores. Figure 3 shows also that, with the exception of culture 2, all cultures were more resistant to heat when grown for several generations at their optimum temperatures than when grown for only one generation. When evaluating this observation, it should be pointed out that all cultures with the letters



A or B after the culture number (e. g., 1-A, 2-A, 1-B or 2-B) represent only one generation at the specific growth temperature. Culture 1, when grown for several generations at 37° C., produced spores with an average thermal resistance of 18.5 minutes; but, when grown for only one generation at the same temperature (culture 1-A), the average thermal resistance of the spores was only 5.7 minutes. This same tendency was shown by cultures 3, 4, 5 and 9, but to a lesser degree. With culture 2, however, spores produced after only one generation at 37° C. exhibited the same resistance to heat as did the spores from cultures grown several generations at 37° C. In contrast there appeared to be some tendency for the cultures grown for only one generation at a temperature below the optimum to produce spores with a greater thermal resistance than those grown for several generations at the same temperatures. This tendency appeared with cultures 1, 3, 4 and 5. Culture 2, however, when grown for one generation at a temperature below the optimum, produced spores with a lower thermal resistance than the spores from cultures grown for several generations at the same temperature. Spores of culture 9 showed no difference in a comparison of this kind.

It is well to note that the average spore contents of the spore suspensions, in comparative trials, were approximately the same and that the only variable factor influencing growth or resistance to heat was the growth temperature.

EFFECT OF CONTINUED GROWTH OF VARIOUS BACTERIA AT A CHANGED GROWTH TEMPERATURE ON THE THERMAL RESISTANCE OF THE SPORES

Observations made when there was a sudden change in growth temperature suggested that spores of cultures grown for some time at the optimum temperature were more resistant to heat than spores of cultures grown for only one generation at that temperature. In order to measure this tendency cultures which had been suddenly changed to a lower or to a higher growth temperature were carried through a series of transfers at that temperature and then tested for heat resistance.

Table 2 shows the thermal resistance of the spores after the cultures had been carried through a series of from 5 to 11 transfers or generations at the changed growth temperature. In addition there is given, for each culture, the average thermal resistance of the spores when the culture was originally tested and also the average thermal resistance of the spores when the culture had grown only one generation after the growth temperature was changed. It should be noted that, when the cultures were originally tested, they had been carried through at least three generations.

Results presented in table 2 indicate that the average thermal resistance of the spores of the various cultures, when grown for a period at a changed growth temperature, tended to approximate the average thermal resistance of the spores of the culture when originally tested after growth at the same temperature. The spores of culture 1-A, which was culture 1

TABLE 2. *Effect of continued growth of various bacteria at a changed growth temperature on the thermal resistance of the spores*

No. of transfers	Growth temperature °C.	Spores per cc. milk heated	Periods of heating (minutes)	Survival (minutes)	Survival original culture	
					Several transfers (ave.)[1]	One transfer (ave.)[2]
Culture 1-A, 5 days old, heated at 114.5° C. pH of milk 6.3						
7	37	65,000	3, 5, 7, 10, 15, 20, 25	15.0	16.9	5.7
Culture 1-B, 5 days old, heated at 114.5° C. pH of milk 6.3						
7	21	71,000	2, 3, 4, 5, 7, 9, 12	3.0	4.6	7.2
Culture 2-A, 5 days old, heated at 116.0° C. pH of milk 6.3						
5	37	100,000	7, 9, 12, 15, 20, 25, 30	20.0	19.9	16.2
Culture 2-B, 5 days old, heated at 116.0° C. pH of milk 6.3						
5	21	120,000	3, 4, 5, 7, 9, 12, 15	7.0	8.5	5.7
Culture 3-A, 5 days old, heated at 110.0° C. pH of milk 6.4						
7	37	2,000,000	1, 2, 3, 4, 5, 7, 9	5.0	3.0	4.3
Culture 3-B, 5 days old, heated at 110.0° C. pH of milk 6.4						
7	21	1,500,000	1, 2, 3, 4, 5, 7, 9	3.0	0.6	2.0
Culture 4-A, 5 days old, heated at 116.0° C. pH of milk 6.3						
11	37	4,000	9, 12, 15, 20, 25, 30, 33	15.0	18.8	14.0
Culture 4-B, 5 days old, heated at 116.0° C. pH of milk 6.3						
11	21	20,000	3, 4, 5, 7, 9, 12, 15	3.0	6.3	9.8
Culture 5-A, 3 days old, heated at 120.0° C. pH of milk 6.4						
5	55	100	9, 12, 15, 20, 25, 30, 35	25.0	23.6	21.6
Culture 5-B, 3 days old, heated at 120.0° C. pH of milk 6.4						
5	45	200	3, 4, 5, 7, 9, 12, 15	5.0	6.1	8.3

A = Change from lower to higher growth temperature
B = Change from higher to lower growth temperature

[1] Data taken from fig. 1.
[2] Data taken from fig. 3.

grown at 21° C. and then changed to a temperature of 37° C., had a thermal resistance of 5.7 minutes after one generation; but after 7 generations the thermal resistance had increased to 15.0 minutes, or almost the same thermal resistance as that exhibited by the spores of the culture when originally tested after growth at 37° C. Culture 1-B, which was culture 1 grown at 37° C. and then changed to a temperature of 21° C., produced spores which had an average thermal resistance of 7.2 minutes after one generation; but after 7 generations the thermal resistance had decreased to 3.0 minutes, which was less than that of the spores of the culture when originally tested after growth at 21° C. Culture 1-A and 4-A, after 7 and 11 generations, respectively, at the changed growth temperature, produced spores which had a thermal resistance almost equal to that of the spores of the cultures when originally tested after growth at 37° C. Spores of cultures 3-A and 5-A, after 7 and 3 generations, respectively, exceeded slightly the thermal resistance of the spores of the cultures originally tested after growth at 37° C.; and spores of culture 2-A, after 5 generations, equalled the thermal resistance of the spores of the culture when originally tested after growth at 37° C. In contrast, spores of cultures 1-B, 2-B, 4-B and 5-B, after 7, 5, 11 and 5 generations, respectively, were lower in thermal resistance than the spores of the cultures when originally tested after growth at 21° C. Spores of culture 3-B, however, after 7 generations, were not quite as low in thermal resistance as the spores of the culture when originally tested after growth at 21° C.; in fact, their thermal resistance was slightly increased.

The results given in table 2 indicate that continued growth at a changed growth temperature generally resulted in a thermal resistance approximating that of the culture when originally tested after growth at the same temperature. Apparently the cultures, after a period of time, had become acclimatized and the growth temperature exerted its influence on the heat-resisting ability of the spores produced.

DISCUSSION OF RESULTS

The importance of low temperatures for holding milk which is to be manufactured into evaporated milk is indicated by the data showing the relatively low thermal resistance of aerobic, spore-forming organisms grown at temperatures below the optimum. It appears, therefore, that the maintenance of low temperatures would markedly lower the thermal resistance of bacteria likely to be present at the time of sterilization and thereby reduce, if not prevent, the spoilage of evaporated milk. An exception to the beneficial influence of low temperatures would be in the case of using temperatures low enough to inhibit the growth of spoilage organisms but still having present in the milk spores which had developed at high or optimum temperatures. In this instance the maintenance of low temperatures would perhaps not markedly lower the thermal resistance of the spores. The observation emphasizes the necessity of main-

taining low temperatures, wherever possible, from the time the milk is drawn until the time of sterilization. The data presented give support to the observations of Weil (13) that growth temperatures influence the ability of microorganisms to resist heat.

The results show that the temperature to be avoided in attempting to secure a lower thermal resistance of spores of organisms isolated from evaporated milk is the optimum growth temperature. Although growth temperatures above the optimum tend to reduce the heat resistance of the spores, such holding temperatures are objectionable since some of the organisms found in evaporated milk have a high optimum growth temperature and high temperatures (37° to 55° C.) adversely affect the quality of the milk by stimulating the development of bacteria which may lower the heat stability of the evaporated milk. Realization of the great thermal resistance exhibited by some of the aerobic, spore-forming bacteria gives a better appreciation of the possibility of milk spoilage even when employing a sterilizing procedure that is usually efficacious. In fact, culture 5, at its optimum growth temperature, produced spores that could survive the ordinary autoclaving process used in laboratory work at 120° C. for 20 minutes.

The observations indicating that each growth temperature may give a different plane of heat resistance to the spores of specific organisms are of interest. From the commercial viewpoint they emphasize the necessity of continually maintaining temperatures which are unfavorable for growth. The data presented show that growth of organisms at favorable temperatures for only one generation resulted in a material increase in the heat resistance of the spores. The observations, therefore, form a basis for an explanation of the sudden outbreaks of spoilage in evaporated milk. Briefly: (Assuming the presence of causal organisms on the farm or in the plant) the prevalence of a favorable growth temperature for several days (sufficiently long to produce spores) might increase the heat resistance of the spores enough so that if they should gain entrance into the milk they would survive the usual heat treatment given the evaporated milk.

The relative constancy of the results obtained in the various heat resistance trials with each culture, as long as the growth temperature was the same, is in harmony with the work of Morrison and Rettger (11) but is not in agreement with the observations of Esty and Williams (4) and Magoon (10). The latter investigators consider the resistance of spores to heat as not a fixed property but a variable characteristic, influenced by a host of conditions rather than by one factor such as temperature of growth.

SUMMARY AND CONCLUSIONS

The work reported involved a study of the influence of growth temperature on the thermal resistance of spores of certain aerobic, spore-forming bacteria isolated from normal and spoiled evaporated milk.

Within the limits of the study, as imposed by the number and species of organisms used, the following points were established:

1. The thermal resistance of spores was influenced by the temperature at which the cultures were grown.

2. Growth temperatures below the optimum decreased the thermal resistance of the spores.

3. Growth temperatures above the optimum tended to decrease the thermal resistance of the spores, and the decreases were generally as great as that caused by growth at temperatures below the optimum.

4. Maximum thermal resistance of the spores was obtained by growth at the optimum temperature.

5. Some cultures isolated from evaporated milk, grown at the optimum temperature, produced spores which, when present in large numbers, survived the sterilization exposure normally used in manufacturing evaporated milk. This was true of cultures 1, 2, 4, 5, 10, 11 and 12.

6. Sudden decreases in growth temperature from the optimum always decreased the average thermal resistance of the spores, while sudden increases in growth temperature to the optimum always increased the thermal resistance, thus establishing the important influence of growth temperature on thermal resistance.

7. Continued growth of cultures at changed growth temperatures generally resulted in spores with a thermal resistance approximating that of spores of the cultures when originally grown and tested at the same temperatures.

8. The results of the study suggest that, in order to decrease the thermal resistance of bacteria likely to be found in evaporated milk and thus minimize or prevent spoilage losses, low temperatures should be maintained in the raw milk from the time of production until it reaches the forewarmer or preheater. Likewise, during any storage period previous to the sterilization process, low temperatures should be maintained.

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The Influence of Growth Temperature and Age on the Thermal Resistance of Milk Cultures of *Streptococcus lactis*[1]

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IT IS generally considered that *Streptococcus lactis* is readily destroyed by such heat treatments as are employed in the pasteurization of milk. While some acid-producing streptococci survive this treatment, they differ from *S. lactis* in range of growth temperature and certain other characteristics.

The majority of investigations on the thermal resistance of bacteria in milk have been concerned with pathogens, thermophiles and spore formers. While some work has been carried on with types not falling into these classes, only a limited amount of study has been devoted to the conditions that may affect the thermal resistance of *S. lactis*.

Among the factors influencing the thermal resistance of microorganisms are growth temperature and age of the culture. The variations observed in the resistance of organisms, as a result of differences in environment, suggest the possibility of similar effects with *S. lactis*. Whether or not certain conditions of development will enable this organism to withstand pasteurization temperatures is of practical importance. Any consistent difference in its reaction to heat treatments, due to previous environment, would also be significant from the standpoint of classification of the streptococci.

Mattick and Nichols (2) found that, in rail-borne milk, pasteurization efficiencies were greater in warm weather than in cold weather. They attributed this in part to the age of the cells being heated and to the conditions of growth. They suggested that, in warm weather, with rapid growth of some types of organisms, the proportion of young cells would

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be greater than in cold weather when growth would be slow; hence the effect of heat would be greater. Anderson and Meanwell (1) believed that old cells derived from dirty utensils or those grown below their optimum temperature might be largely thermostable and, therefore, survive pasteurization. It was pointed out by Sherman and Hodge (3) that "Slow growth enable an organism to better adapt itself to its environment and hence to exhibit greater viability when exposed to deleterious environmental conditions."

METHODS

Six strains of *S. lactis* were isolated from raw milk and cream delivered to the Dairy Industry Department at Iowa State College. After isolation, cultures of each organism were grown in litmus milk at 37°, 21°, 15° and 10° C.

In studying the influence of the growth temperature and the age of *S. lactis* cultures on the thermal resistance, heat trials were conducted periodically with each of the six organisms which had been carried at 37°, 21°, 15° and 10° C. The trials were commenced after the initial development had begun and continued through the cycle of growth until the numbers of organisms had markedly decreased. With the cultures grown at 37° and 21° C. tests were made daily for about seven days. With the cultures carried at 15° and 10° the intervals between the trials were about two and four days, respectively, since the development of the cultures was slower; the tests continued for about 15 days with cultures grown at 15° C. and for about 30 days with cultures grown at 10° C.

TECHNIC OF HEATING

Thermal resistance trials were conducted at 61.7° C. This temperature was chosen because it is the lowest used in the pasteurization of market milk. Cultures to be heated were diluted 1-1,000 in sterile litmus milk to avoid the effect, on the thermal resistance, of the acid present in the cultures. Throughout the trials it would have been desirable to have equal numbers of organisms in the portions heated to permit more accurate comparisons of heat resistance. However, as the cultures themselves differed in numbers of organisms, it was impossible to obtain equal numbers in the dilutions. Deviations from the 1-1,000 dilution might have resulted in variations in pH values.

The diluted cultures were prepared for heating by placing 2 cc. portions in each of a number of 4 cc. agglutination tubes. These tubes were sealed in a blast lamp and cooled in air. They were then suspended from a metal rod by means of strings and wire hooks so that they hung about 6 inches from the rod. With the exception of the tubes containing cultures grown at 37° C., which were the last to be sealed in comparative tests, all tubes were placed in ice water after sealing to retard bacterial growth.

The length of time between sealing and the beginning of the heating was kept as short as possible and rarely exceeded 25 minutes.

The heating was accomplished by placing the tubes in a galvanized iron tank (20″ x 10″ x 12″) which was filled with water to within 2 inches of the top and heated by two adjustable burners. When the water reached the temperature of 61.7° C., the burners were so adjusted that the variation from this point was not greater than 0.2° C. A thermometer graduated in tenths of a degree Centigrade was suspended in the tank. By the use of a paddle for agitating the water several times a minute, it was possible to keep the temperature of the whole body of water reasonably constant.

In conducting the heat trials the sets of tubes suspended from the metal rods were removed from the ice water and plunged into the bath. The rods rested on the sides of the tank. In the early part of the work, tubes were withdrawn from the bath at intervals of 1, 2, 3, 4, 5, 6, 7, 9 and 11 minutes, respectively; while in later trials the periods of heating were varied as seemed desirable. The periods were timed with a stop watch. One tube of each set was unheated and used as a check. When the tubes were withdrawn from the bath, they were immediately dropped into ice water. After cooling, the cultures which had been previously grown at 37° or 21° C. were again incubated at those temperatures; while the cultures that had been grown at 15° or 10° C. were incubated at 21° or 37° C. in order to speed up development.

OBSERVATIONS

Observations on survival were made frequently for about 9 days with cultures grown at 37° and 21° C. and for about 12 days with cultures grown at 15° and 10° C. Cultures were considered sterile when they failed to change the appearance of litmus milk in the heated agglutination tubes during those periods.

PLATE COUNTS

In most cases plate counts were made on the diluted cultures at the time of heating. Plates were poured immediately after the 1-1,000 dilutions had been prepared. Tomato juice agar of a pH of approximately 7.0 was used throughout. The plates were incubated at room temperature for four days and counted by means of a 6X binocular.

RESULTS

ORGANISM 1

The thermal resistances obtained with organism 1 are graphed in the figure. Probably the most significant feature shown is that the heat resistance of the organism was inversely proportional to the growth temperature. The longest survival time at each growth temperature was 4 minutes at 37° C., 5 minutes at 21° C., 8 minutes at 15° C. and 13 minutes

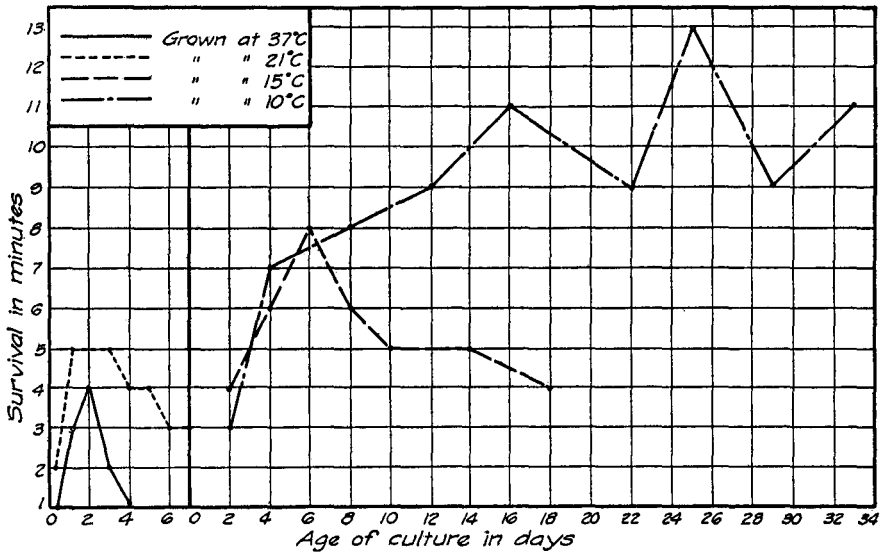

Fig. 1. Survival in minutes of organism No. 1 heated at 61.7° C.

at 10° C. At each temperature the heat resistance of the organism increased with the age of the culture to a maximum and then decreased. With the culture grown at 37° C., the survival time, while only 1 minute at ¼ day of age, reached its maximum of 4 minutes at 2 days and declined again to 1 minute at 4 days. The survival time of the culture grown at 21° C. increased from 2 minutes at ¼ day of age to its maximum of 5 minutes at 1¼ days; it remained at this point until 3 days and then declined to 3 minutes at 6 days. The survival time of the culture grown at 15° C. increased from 4 minutes at 2 days of age to 8 minutes at 6 days and then declined again to 4 minutes at 18 days. With the culture grown at 10° C. the survival time increased rapidly from 3 minutes at 2 days of age to 7 minutes at 4 days and then less rapidly to the maximum of 13 minutes at 25 days. While there was a decline in resistance to 9 minutes at 29 days, there was again an increase to 11 minutes at 33 days. The experiment was not carried on long enough to indicate the rate of decrease in resistance with this growth temperature. In general, the data show that the rate of increase in resistance, the maximum resistance attained and the rate of decrease were associated with the growth temperature of the organism.

Table 1 gives the counts on the diluted cultures at the time of heating and shows that, with the cultures grown at the three highest temperatures, there seemed to be a general relationship between the numbers of organisms and the resistance to heat; when the count was high the resistance was high. However, this did not apply to the culture grown at 10° C.

TABLE 1. *Numbers of bacteria in diluted cultures (1-1,000) at time of heating*

Age of culture in days	Organism No. 1			
	Bacteria per cc. in dilution of cultures grown at			
	37° C.	21° C.	15° C.	10° C.
¼	<1,000	4,000		
1¼	670,000	2,060,000		
2	530,000	2,860,000	1,080,000	<10,000
3	<10,000	1,970,000		
4	<10,000	1,550,000	1,880,000	150,000
5		1,450,000		
6		350,000	1,710,000	
7		32,000		
8			1,410,000	480,000
10			500,000	
12			50,000	620,000
14			35,000	
16				140,000
18			30,000	
22				130,000
25				59,000
29				30,000
33				16,000

At the time of its maximum resistance of 13 minutes the count was the lowest obtained at any time between the fourth and twenty-fifth day. Moreover, this count was much lower than the counts on the other three cultures at their periods of maximum resistance. The table shows that all counts made on the cultures grown at 37° and 10° C. were relatively low, while the counts on the cultures at 21° and 15° C. were relatively high, with those at 21° C. being the higher.

ORGANISM 2

While the heat resistance of this organism was not inversely proportional to the growth temperature, the culture grown at 10° C. was again the most resistant. The longest survival time of the organism at each growth temperature was 9 minutes at 37° C., 7 minutes at 21° C., 7 minutes at 15° C. and 13 minutes at 10° C. As was the case with organism 1, the heat resistance at each growth temperature increased with age to a maximum and then decreased. The same general relationship existed between the number of organisms and the heat resistance as was the case with organism 1.

ORGANISM 3

On the whole, this organism was generally less heat resistant than organisms 1 and 2. With the exception of the culture grown at 21° C., there existed the same relationship between heat resistance and growth

temperature as was the case with organism 1. The longest survival times obtained were: 2 minutes when grown at 37° C., 7 minutes when grown at 21° C., 6 minutes when grown at 15° C. and 9 minutes when grown at 10° C. There was not quite the same relationship between the number of bacteria and the heat resistance with organism 3 as in the case of organisms 1 and 2. With the three highest growth temperatures, the periods of high resistance did not coincide as closely with the high counts, as was the case with the other two organisms. The culture grown at 10° C. attained its maximum resistance at the time of the highest count; in this respect, it again differed from the other two organisms.

ORGANISM 4

This organism did not show the same general relationship between heat resistance and growth temperatures as organisms 1, 2 and 3. The maximum resistances obtained were: 7 minutes when grown at 37° C., 7 minutes when grown at 21° C., 6 minutes when grown at 15° C. and 7 minutes when grown at 10° C. With the cultures grown at 21° and 15° C., the resistances were high when the counts were high. In the case of the culture grown at 37° C. the numbers of bacteria had passed the maximum at the time the greatest resistance was obtained. While the culture grown at 10° C. reached a resistance of 7 minutes at the time the count was high and then became less resistant, it later again survived 7 minutes heating when the count had declined considerably. In this respect it acted similarly to organisms 1, 2 and 3.

ORGANISM 5

As was the case with organism 1, the maximum thermal resistances obtained were inversely proportional to the growth temperatures, being 4 minutes at 37° C., 6 minutes at 21° C., 7 minutes at 15° C. and 9 minutes at 10° C. On the whole, the same relationship between numbers of bacteria and heat resistance existed, as with organism 1. With the cultures grown at the three highest temperatures the resistances were high when the counts were high, but with the culture grown at 10° C. the two highest resistances were obtained near the end of the trials when the counts were very low.

ORGANISM 6

Irregularities in heat resistance were obtained with the cultures grown at 37° and 21° C. and were especially marked with the latter culture. However, the cultures grown at 15° and 10° C. gave results more in accordance with the previous trials, the latter culture finally showing the greatest resistance, as was the case with the other organisms. The highest survival times obtained were 4 minutes when grown at 37° C., 7 minutes when grown at 21° C., 7 minutes when grown at 15° C. and 9 minutes when grown at 10° C. With the exception of the culture grown

at 21° C. there was generally the same relationship between the numbers of bacteria and the heat resistance as with the other organisms. With the culture grown at 21° C., the irregularities in heat resistance did not always correspond to the irregularities in counts. The culture grown at 10° C. again showed the greatest resistance when the numbers of bacteria had greatly decreased.

DISCUSSION

The thermal resistance of *S. lactis* in milk is not high enough to enable the organism to survive pasteurization treatments; and, while such factors as growth temperature and age of the culture modify this resistance to a certain extent, it appears that they do not sufficiently increase the resistance to be of commercial importance.

The relatively high resistance obtained with cultures grown at 10° C. might have been attributable to slower development of the cultures and a correspondingly greater age of the cells. In addition, sufficient acid was not produced in the cultures to effect coagulation; and, while the dilutions of the cultures used in the heat trials were such that the amount of acid present in the heated portions was negligible, there may have been a less detrimental influence prior to heating. The greater resistance secured with cultures grown at 10° C. would seem to confirm the statement of Sherman and Hodge (3) that slowly growing cultures exhibit greater viability under unfavorable conditions than more rapidly growing cultures. It is also in accord with the suggestion of Anderson and Meanwell (1) that organisms, usually destroyed by pasteurization, might be largely thermostable and survive this treatment when grown below their optimum temperature.

SUMMARY AND CONCLUSIONS

1. At 10° C., *S. lactis* cultures grew more slowly than at higher temperatures but attained higher thermal resistances. The highest resistance obtained with an organism grown at 10° C. was 13 minutes.

2. The thermal resistances of *S. lactis* cultures increased with age to a maximum and then decreased. The rates of increase and decrease were influenced by the growth temperature, being slower at lower growth temperatures.

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The Limitations of Significance of Some of the Methods of Analyzing Ice Cream[1]

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THIS PAPER consists primarily of a plea for the use of common sense in the interpretation of the results of laboratory analyses of dairy products. There is an ever increasing emphasis being placed on the necessity for quality control of ice cream. The rapidly increasing number of public health regulations affecting the manufacture of ice cream, such as pasteurization requirements, bacterial standards, weight standards, stipulations of the quality of ingredients, composition of the mix, health certificates for employees, etc., should serve as warning to the ice cream manufacturer that he will soon be operating under as strict regulation as the market milk producer. In fact, the time may not be so far ahead when ice cream will be graded as market milk is now graded. This situation suggests that the ice cream manufacturer should become familiar with the significance and limitations of the various laboratory methods employed in the analysis of his product.

Any method of analysis has certain inherent limitations of greater or lesser magnitude. These limitations may consist of wide variations in the results of replicate analyses or the presentation of an indirect or perhaps only a partial index to the thing to be measured. It does not necessarily follow that a method is rendered valueless because of such limitations, but it does follow that intelligent interpretation of the significance of results obtained presupposes due recognition of the limitations imposed by the procedure employed.

The purpose in presenting a frank discussion of the limitations of some of these methods is not in the least to destroy confidence in them but,

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quite on the contrary, to increase confidence in them. Much of the dissatisfaction encountered with laboratory analyses can be traced to disappointments and embarrassments resulting from misinterpretation of their significance. It is always a safe practice, though it may not be as satisfying, to avoid speculations by confining conclusions to the data at hand.

The basis of the program for sanitary control should be inspection, and the effectiveness of that inspection should be enlarged by laboratory analysis. If one strips the sanitary control program of its technicalities, he finds that the central objective is to provide and insure a clean, wholesome, safe product. This objective can be attained most effectively by a suitable balance between inspection and laboratory analysis. Overemphasis of one or the other of these two factors is a common mistake; but, of the two, it is more common to find the sanitary control program resolving itself into a routine laboratory examination of samples collected periodically. Too frequently the plant is adjudged "clean" or "dirty," and the product regarded as "safe" or "dangerous" on a basis of laboratory findings not confirmed by inspection. Such a system totally ignores the fact that there are factors other than cleanliness which affect laboratory findings and which may have little if any very direct relation to the safety of the product. As valuable and indispensable as laboratory analyses are to a well-rounded sanitary control program, they cannot be used as a sole means of inspection.

STANDARD PLATE COUNT

The standard plate count reveals the number of organisms in a sample (of ice cream) capable of growing under rigidly prescribed conditions which are favorable for only a certain, and perhaps uncertain, percentage of the total bacterial flora of the sample. It is assumed that about the same percentage of the total number of bacteria will grow if all samples are plated under these strictly standardized conditions. Logic and experience justify the general applicability of this assumption, but likewise both logic and experience expose exceptions to the general hypothesis. This is not a serious limitation but it should not be entirely neglected.

Another limitation of the plate count as applied to ice cream is of interest perhaps only to the laboratory technician. For some reason, not entirely clear, ice cream frequently contains rather large numbers of saccharophilic, heat-resistant organisms which will not grow on plain agar, but will grow if a very small amount of sugar is added to the medium. Even the small amount of sugar carried over from the sample in the low-dilution plates is frequently enough to support growth, whereas the high-dilution plates in the same series will not show the expected number of colonies. It is not uncommon to find low-dilution plates containing so many colonies that it is impossible to count them, whereas the plates next higher in dilution are apparently sterile. Other samples of ice cream are commonly encountered which contain organisms manifesting this

same saccharophilic tendency in a slightly different way. If some plain agar plates, which at first appear to be practically sterile, are examined with a strong lens, literally myriads of very small colonies may be discerned. Unlike the colonies just described, they are present in the expected numbers in the series of dilutions but are too small to count without special lens equipment. If ice cream samples containing either of these types of organisms are replated on the same agar to which one percent of dextrose has been added, the colonies of the first type appear in the expected numbers in all dilutions; and the colonies of the second type develop sufficiently large to be counted without the aid of magnification.

Parallel bacterial counts, using standard agar and 1 percent dextrose agar, have been made on 271 samples of ice cream collected over a two-year period from practically every manufacturer in the state of Kansas. In approximately 6 percent of these samples the use of dextrose agar materially increased the count by enabling colonies to grow which either failed completely to grow on plain agar or were so extremely small that it was practically impossible to count them. With such samples the use of standard agar was entirely unsatisfactory and the use of dextrose agar completely solved the difficulty. With an additional 50 percent of the samples, the use of dextrose agar proved to be distinctly advantageous in counting, although the differences between the dextrose agar and plain agar counts were within the limits of normal variation. With the remaining 44 percent of the samples dextrose agar proved to be of no advantage or disadvantage. Before standard procedures for the plating of ice cream have become too firmly established to be changed, it would be well to investigate the advantages presented by dextrose agar.

Perhaps the most common objection to the plate count on milk or ice cream is the persistent tendency, even on the part of some inspectors, to misinterpret the significance of the results. This is attributable perhaps to a lack of familiarity with the limitations of the method and to the popular tendency to regard bacteria, dirt and disease as inseparable associates.

We must not conclude that all samples of ice cream with high bacterial counts have necessarily been produced in dirty plants and that they are absolutely dangerous to the consumer. We may sanely conclude that there exists in that plant a faulty practice which must be identified and corrected. A high plate count on a sample of finished ice cream may result from such things as the use of poor ingredients, ineffective pasteurization, inadequate refrigeration of the mix during aging or improperly sterilized utensils. These conditions are, of course, undesirable and should be corrected; but further inspection only will reveal which one or which combination of these factors is out of control.

Similarly, we must not conclude that all samples of ice cream with low bacterial counts have necessarily been produced in clean plants. The

demand for cleanliness of the plant can best be enforced by frequent and rigid inspection. The real value and place of bacterial counts in the program of sanitary control are to insure the inspector or the plant manager that previous inspections have been thorough, that instructions are being carried out and that the raw ice cream mix of unquestionable quality is being properly pasteurized, aged and frozen in clean equipment. These are indeed important factors in the safety and cleanliness of the final product, but they constitute only a part of complete sanitary control. Until we realize that bacteriological analyses alone do not constitute a complete sanitary control system, and learn to confine the interpretation of bacterial counts to their logical limitations, we cannot approach intelligently the problem of producing a clean, wholesome, safe product.

BACTERIAL STANDARDS

Bacterial standards for ice cream are extremely useful, providing they are properly interpreted. When municipal or state authorities set a bacterial standard of, let us assume, 100,000 per cubic centimeter by the plate count, that is a brief method of saying that in their opinion any ice cream containing more than the specified number has been improperly processed and the plant should receive the immediate attention of a qualified inspector. It does not say that the ice cream is unclean or unsafe or that the plant is dirty. Inspection may reveal the specific cause, and directions may be given for immediate correction.

The standard serves merely as an arbitrary basis of judgment, and excessive counts call the attention of the plant operator and the inspector to the immediate need for co-operative, corrective effort. The use of the bacterial standard beyond this interpretation is difficult to defend. Continued failure on the part of the manufacturer to correct the defects in his processing reflects his incapacity to handle human food properly, and his prompt removal is in the interest of public welfare. In the rare cases when prosecution of a manufacturer is justified, it should be based on his inability to handle human food or persistent unwillingness to co-operate with public health officials, never upon the flimsy pretext of an excessive bacterial count. The latter is merely evidence of the former. It is to the interest of the ice cream industry as a whole, as well as of the general public health, that persistently dirty ice cream plants be closed regardless of the bacterial count.

It is believed that much of the misinterpretation, misuse and dissatisfaction with bacterial standards are due to the phraseology with which they are incorporated in state and municipal regulations. The following is quoted from a milk ordinance:

"Milk containing in excess of 100,000 bacteria per cubic centimeter shall be regarded as unsafe and dangerous to the consumer, and shall not be offered for sale."

This clause implies a more imminent danger to the consumer of such products than experience would justify. In order to give the bacterial standard a legal status without erroneous implication, would it not be better to state that, "Bacterial counts in excess of 100,000 per cubic centimeter shall be officially regarded as excessive"? After all, that is essentially what the bacterial standard is—an official interpretation of an excessive count and not a line of demarcation between safety and danger.

DIRECT MICROSCOPIC METHOD

The direct microscopic examination of ice cream consists, briefly, of staining a known volume of the product which has been spread over a known area and counting the organisms in a few representative fields of a standardized microscope.

The principal limitation of this method as applied to ice cream is that the organisms killed by pasteurization are not distinguishable from the survivors, and all are counted. If one is interested in determining what the bacterial content of the mix was before pasteurization, this limitation becomes an asset to the method.

An important limitation of the microscopic method of analysis is the inevitably small sample ultimately employed for judging the sanitary quality. In the ordinary examination one actually observes only about 0.0001 cc. of mix or melted ice cream, but upon this small sample he passes judgment and perhaps condemns a large volume. The smallness of the sample may be forcibly illustrated by comparing the 0.0001 cc. sample used in judging the quality of a 300-gallon volume of mix to a proportionately small sample of wheat. By suitable calculation it can be shown that the analysis for protein of only one grain of wheat from 14.19 carloads would be comparable to judging a 300-gallon vat of mix on a basis of a 0.0001 cc. sample. If the illustration be based on the examination of 0.0001 cc. from a 10-gallon can of mix, it is comparable to using the grain of wheat as representative of 473 bushels, or approximately 10 wagonloads. It is needless to emphasize that due recognition should be given to the smallness of the sample when interpreting the results of the microscopic method of analyzing ice cream.

COLON TEST

The determination of the numbers of colon types of organisms plays such a very important part in judging the extent of fecal pollution of water, that dairy bacteriologists have attempted to apply the test to dairy products. Unfortunately, however, practically all, if not all, milk contains organisms of this type and these organisms grow rapidly at ordinary temperatures. Although the numbers found in a perfectly fresh sample of milk may reveal the extent of fecal pollution, the growth of these organisms while the milk or cream is reaching the market obscures the index to fecal pollution and reflects primarily the temperature of handling.

Since many strains of the colon types of organisms are destroyed by pasteurization, the test has been used as an index to ineffectively pasteurized milk and ice cream mix. More careful investigation reveals, however, that the original hypothesis is subject to criticism and that many colon types do survive pasteurization at 145° F. for 30 minutes. When higher temperatures are employed for a 30-minute period, the colon index may reveal more effectively contamination subsequent to pasteurization.

The value of the colon test on dairy products is essentially limited to the analysis of perfectly fresh milk or milk which has been so effectively refrigerated that its original bacterial flora has not changed materially, or, in the case of ice cream mix pasteurized at 155° F. or higher for 30 minutes or more, to the detection of contamination after the pasteurization process.

THE METHYLENE BLUE REDUCTION TEST

Although the methylene blue reduction is not frequently applied directly to ice cream, it is discussed here because of its wide use in many ice cream plants for judging the quality of milk used in compounding the mix. Its popularity may be due to its simplicity, its practicability and, supposedly, its accuracy. The test consists of adding a standard concentration of methylene blue to milk and incubating it at 37° C. until the blue color disappears.

This test is based upon the assumption that bacteria induce changes in milk which cause the oxidation-reduction potential to fall and, as a result, the dye to lose its color. The larger the original bacterial population, the shorter is the time required to bring about this visible change in the indicator. This assumption, however, overlooks three important considerations: (1) The necessary reducing intensity is much more effectively produced by some organisms than by others; (2) it may be induced altogether independently from bacterial action by the organic constituents of the milk; or (3) it may be induced by leucocytes in freshly drawn milk.

Under any circumstances bacterial reduction depends upon two important variables—the number of organisms and the kind of organisms. If the same kind of organism always dominated the bacterial flora of all samples of milk, then the only variable to be considered in the examination of several samples of milk would be the original number of bacteria in each of the samples. Unfortunately, the domination of some one type cannot be depended upon in fresh milk; but a dominant type is likely to occur when the milk is older.

By the time the bacterial populations of a number of samples of milk have reached 500,000 or 1,000,000 per cc., the odds are much in favor of the assumption that the dominating organism in each sample will probably be *S. lactis*. To the extent that this assumption is justified, the kind of organisms in relatively poor milks may be considered to be fairly uniform. In such samples practically the only variable then would be the

number of organisms; if this is so, the reduction time would reflect the original bacterial population. Under these conditions, the relatively small influence exerted by the reducing capacity of the milk constituents and by the leucocytes will be so greatly overshadowed by the reduction intensity induced by the large bacterial population that they will be unimportant. On the other hand, if the milk is of high sanitary quality and contains relatively few organisms, the dominance of a uniform type is less likely to have been established, and the reducing capacity of the milk constituents and the leucocytes plays a proportionately larger part in the reduction time. Under these conditions there are so many variables that the reduction time no longer can be relied upon to reflect the original bacterial population.

It is customary to recognize as Class I any milk in which the reduction time exceeds 5.5 hours; such milk, it is assumed, usually contains less than approximately 500,000 bacteria per cc. In the absence of an understanding of the fundamental principles of this test, there has been a tendency on the part of some inspectors to assume that if a 5.5-hour reduction time indicates a count of 500,000, reduction times of 10, 16 or 24 hours indicate correspondingly fewer organisms in direct order. Experiments which have been carried on at the Kansas Experiment Station indicate the fallacy of this assumption. A series of samples of sterile milk and sterile ice cream mix were inoculated with serially increasing numbers of a pure culture of *S. lactis*, adjusted to give reduction times varying from 5 to as high as 24 hours. Since the kind of organism introduced was uniform for each sample, the only variable was the number and, as might be expected, even the reduction periods of 24 hours reflected accurately the relative original bacterial populations. When this experiment was repeated, however, using similar serial inoculations of a mixed culture of common milk types of organisms, the reduction periods did not reflect the original populations. Such results tend effectively to defend the statement that the reduction period of milk depends upon the kind as well as the number of organisms; and, unless the kind of organisms in several samples of milk is uniform, their respective reduction periods cannot be relied upon as an index to their relative bacterial populations. This relative uniformity of flora cannot be relied upon until the count reaches the arbitrary minimum of approximately 500,000 or a reduction time of 5.5 hours.

This imposes a serious limitation on the methylene blue reduction test which should not be overlooked. Bacterial counts for milk of recognized quality in the United States are of the order of magnitude of 10,000 to perhaps 200,000 per cc. By the time a sample of milk reaches the more or less arbitrary lower limit of 500,000, where the methylene blue reduction test comes into play, our interest in this milk has diminished to the vanishing point. In other words, the methylene blue test begins to measure relative bacterial numbers in milk effectively only after it is too late.

SUMMARY

Again it should be emphasized that this discussion of the shortcomings of the various methods of milk analysis is not intended as destructive criticism, but to increase their utility by avoiding the pitfalls of misinterpretations. These are the best methods available; and, until better methods are provided, they must be used. The important part these bacteriological methods have played in the improvement of the milk supply of the United States during the past quarter of a century is in itself adequate recommendation for their continued use despite their limitations. The limitations of a method need not be a serious handicap so long as the limitations are recognized and not completely ignored. As stated in the beginning, most of the dissatisfaction experienced in the past with these methods is traceable to failure to recognize the limitations imposed by the procedure employed. For emphasis may it be repeated that intelligent interpretations of the significance of the results of any method presupposes an understanding of the limitations of the data.

Studies on *Bacillus coagulans*

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CANNED evaporated milk is usually considered, for all practical purposes, to be sterile. If bacteria, or their spores, survive the sterilization process, they seldom grow in the canned product. Cases are on record, however, in which heat-resistant bacteria survived the heat processing and caused spoilage of the canned milk. Dr. Hammer was one of the first dairy bacteriologists in this country to investigate the spoilage of evaporated milk by heat-resistant bacteria.

In 1915 an outbreak of coagulation in evaporated milk occurred in an Iowa condensery. The bacteriological studies on the cause of this outbreak were reported by Hammer (3); and the causative organism, *Bacillus coagulans,* was thoroughly described. Cordes (1) found *B. coagulans* responsible for an outbreak of "flat-sours" in the evaporated milk packed by a condensery in a neighboring state. Sarles and Hammer (4) found *B. coagulans* to be the cause of a serious outbreak of coagulation in the evaporated milk packed by a Wisconsin condensery.

In each of these outbreaks the cans of milk which coagulated during storage had received, according to the plant records, very thorough heat treatment. It was reasonably certain that some of the milk which spoiled during the 1915 outbreak had been heated to 113.3° C. (236° F.) for 36 minutes. The cans of spoiled milk from which *B. coagulans* was isolated by Cordes in 1928, had been subjected to 114.4° C. (238° F.) for 20 minutes in a batch sterilizer. In the last outbreak studied, which occurred during the late summer and early fall months of 1930 and was reported in 1932, cans of milk which had been run through hot water at 97.8° C. (208° F.) for 24 minutes and then through a continuous cooker for 15 minutes at 117.8° C. (244° F.), showed, after several days' storage, coagu-

lation caused by *B. coagulans*. These figures are given to illustrate that the heat resistance of this organism was high in each of the outbreaks studied.

The spoiled milk in each case usually showed a smooth, fairly firm curd with little whey. The cans of spoiled milk showed no signs of bulging, and no gas-escape was noted when they were opened. The quantity of gas present in cans of milk spoiled by *B. coagulans* was found to be similar to that in normal cans of milk. This gas was made up of about 15 percent carbon dioxide and 85 percent nitrogen. In cans of normal evaporated milk the gas was found to consist of 0.1 percent carbon dioxide and 99.9 percent nitrogen. The odor and taste of the spoiled milk was rather sour and cheesy but not at all disagreeable.

Isolation of the causative organism from cans of coagulated milk was usually not difficult because, in most cases, about 30,000 of *B. coagulans* per ml. were found by the plate count. This number, however, varied in different cans of spoiled milk from a few cells per ml. to over 9,000,000 per ml.

The original description of *B. coagulans*, made by Hammer in 1915 and corroborated and enlarged in 1932, is given in full below.

DESCRIPTION OF *BACILLUS COAGULANS*

MORPHOLOGY

Form and Size. Rods; 0.5 to 0.7 by 1.6 to 7.1 microns when grown on beef-infusion agar (24 hours at 37° C.); somewhat smaller when grown in milk (48 hours at 37° C.).

Arrangement. Singly and in short chains.

Motility. Motile; flagellation peritrichous.

Staining Reactions. Gram-positive in young cultures, often with distinct granulation; commonly gram-negative in old cultures although a few gram-positive cells sometimes persisted.

Spore Formation. In old beef-infusion agar slant cultures and in coagulated evaporated milk some cells contained spores. Preparations made from agar, or milk cultures grown under various incubation conditions, regularly showed spores in less than half of the cells. The spores were small, round, did not bulge the cells and were sub-terminal.

CULTURAL CHARACTERISTICS

Agar Slant. Beef-infusion and whey agars showed abundant, echinulate, white, nonviscid, shiny growth after two to three days at 37° C. Growth less abundant on standard agar.

Agar Stab. Beef-infusion agar and whey agars showed heavy, white, non-

viscid, surface growth with some growth along the line of inoculation after two to three days at 37° C.

Agar Colony. After two to three days at 37° C. surface colonies on beef-infusion agar were shiny, white, nonviscid, round, about 1 to 2 mm. in diameter, with entire edge. Sub-surface colonies were round to oval, white, nonviscid, and smaller than the surface colonies.

Gelatin Stab. On whey gelatin at 37° C. growth occurred; gelatin not liquefied.

Broth. Turbidity with sediment.

Potato. Dirty white, shiny, nonviscid, spreading growth.

Litmus Milk. Litmus milk was reduced. Reduction was followed by coagulation and appearance of red band at top of milk. Red band increased in depth, curd contracted, expressing small amount of whey. No apparent proteolysis. Coagulation in four to eight days at 37° C. and in two to four days at 50° C.

BIOCHEMICAL FEATURES

Indol. Not produced.

Nitrates. Not reduced.

Action on Carbohydrates and Alcohols. Dextrin, galactose, glucose, glycerol, lactose, levulose, maltose, raffinose, salicin, sucrose and soluble starch fermented with the production of acid but no gas; adonitol, arabinose, dulcitol, inositol, inulin, mannitol and sorbitol not fermented; starch hydrolyzed.

Oxygen Relationship. Organism facultative; grew well aerobically.

Growth Temperatures. Grew well between 37° and 55° C.; poorly, if at all, at 20° C.

The total and volatile acidities of evaporated milk coagulated by this organism were studied. It was found that the abnormal milk showed total acidities of from 0.85 to 1.06 percent, while normal evaporated milk varied from 0.42 to 0.49 percent acid calculated as lactic acid. The principal acid formed by *B. coagulans* was found to be *d*-lactic acid. The small quantity of volatile acid produced by this organism was shown to be largely acetic, with a trace of propionic acid.

Evaporated milk spoiled by *B. coagulans* showed no evidence of proteolysis, but it was found to contain about twice the soluble nitrogen and more than double the amino nitrogen found in the normal product.

Among the problems left unsolved by the published work on *B. coagulans* were the factors influencing spore-formation and the relation of spore-formation to the heat resistance of the organism. Working under the author's direction, Elliker (2) has reported studies on these problems.

The nine strains of the organism used in this work were those isolated in 1930 by Hammer and the author.

Three of the nine strains produced very few spores under any conditions, despite the use of most of the known methods for inducing sporulation. The other six strains seemed to sporulate readily. These cultures formed spores more readily when cultivated on agar media than when cultivated in milk or broth. The optimum temperature for spore-formation was found to be 47° C.

Attempts to correlate spore-formation with the heat resistance of the various strains illustrated that, in general, those which produced spores readily were more resistant than those which formed few spores. Few spores could be demonstrated with the staining methods used in cultures grown in milk, but these milk cultures exhibited the same heat resistance as those grown on agar. The readily sporulating strains probably formed spores in milk, but our methods for demonstrating them were inadequate.

In conclusion, it should be pointed out that the original source of *B. coagulans* is not known, and that the knowledge concerning its heat resistance is quite inadequate. However, outbreaks of spoilage of evaporated milk due to this organism are apparently few in number, and may be prevented by proper plant practice.

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Bacteria of the Escherichia-Aerobacter Group in Dairy Products[1]

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INTRODUCTION

THE Escherichia-Aerobacter group of bacteria, more commonly known as the colon group, is widely distributed in nature. While ordinarily saprophytic, its members may under certain circumstances invade plant and animal tissues. The importance of these organisms from the standpoint of disease, their value as an indicator of polluted water and shellfish supplies and their importance in dairy and food products both as the cause of defective conditions and as a measure of sanitary quality have served to make the group the object of extensive research.

Methods of detecting the colon group in water have frequently been applied to dairy products without recognition of the fact that methods suitable for water analysis are not necessarily suitable for the analysis of dairy products. Also, many workers have placed the same meaning on the presence of the colon group in milk as in water. The significance of the colon group in dairy products is entirely different from that in water and, furthermore, varies according to the kind of product.

HISTORICAL

The literature on the colon group is undoubtedly larger than that on any other group of organisms. While there are a number of good summaries covering this group as a whole such as that by Wilson (1), reviews of the literature are inadequate; and workers are handicapped by lack of knowledge of previous work. This may be, in part, responsible for the present confusion of ideas in regard to the group.

[1] Approved by the Director of the New York State Agricultural Experiment Station for publication as Journal Paper No. 129, March 13, 1936.

The best reviews in this country cover applications to water bacteriology, such as Levine (2) and Prescott and Winslow (3), and are valuable for general information. Some idea of the extensive amount of work that has been done may be gained from the fact that the latter authors cite over 500 references to the group.

While a complete review of the literature with respect to the group as found in dairy products is desirable, it is not possible in this article. Many important references must necessarily be omitted.

TAXONOMY

The Escherichia-Aerobacter group is commonly referred to either as the colon group or as the coli-aerogenes group except in England, where the term coliform is used. Since the term Escherichia-Aerobacter group is lengthy and awkward to use continually, the term colon group will be used as a synonym. The colon group is a member of the larger colon-typhoid group which Bergey (4) divides into nine genera based on fine distinctions in respect to the fermentation of carbohydrates. Between clearly defined members of these genera are borderline types which are difficult to classify satisfactorily according to Bergey's Manual.

Because of the close relation between all of the organisms in the colon-typhoid group, some workers—among whom may be mentioned Orla-Jensen (5) and Winslow et al. (6)—feel that all of the gram-negative, nonsporeforming, nonchromogenic rods which grow aerobically should be placed in a single genus. The generic term Bacterium has been widely used but is really invalid on the ground that the original definition of the group and of the type species was inadequate, Buchanan (7). Breed and Conn (8) have pointed out that "many difficulties in our present classifications would disappear if Bacterium is retained as a temporary genus without a designated type species other than the historic but non-recognizable *Bacterium triloculare* to include all species of nonsporeforming rods that cannot be placed at once in well recognized and reasonably well defined genera." This would give justification to those who wish to be conservative and retain such names as *Bacterium coli, Bacterium prodigiosum*, etc.

Several other genera made up of gram-negative nonsporeforming rods which are capable of growing aerobically are very closely related to the colon group and differ chiefly in respect to their chromogenesis (Serratia, Flavobacterium) or in respect to a mucoid encapsulated growth (Klebsiella). Some of the species placed by Bergey in the genus Achromobacter ferment lactose with gas production and correspond closely to descriptions of organisms in the colon group. A number of the plant pathogens listed in the genus Erwinia are probably strains of colon organisms.

In water analysis the value of generic differentiation (Escherichia from Aerobacter) is a much discussed point. At the present time it seems to be generally accepted that members of the genus Escherichia are sig-

nificant in indicating fecal pollution, while the presence of the Aerobacter section is of doubtful significance in this respect, as it indicates fecal pollution under certain conditions only.

In the case of dairy products, generic differentiation is of no practical value where the test is used as an index to sanitary conditions, since both sections are added to milk in about equal numbers at the time of production and since growth of either section may take place on utensils. In establishing the nature of organisms responsible for defects in milk such as ropiness, generic differentiation is important, since practically all defective conditions are due to the members of the genus Aerobacter.

Number of species. The number of species in the Escherichia-Aerobacter group is a much debated question and many different classifications have been proposed. The number of possible species based on a system of classification depends upon the number of key characters used and may be determined by the formula 2^n where "n" is the number of characters studied, Levine (2). Thus, the possible number of species in the genus Escherichia according to Bergey's scheme with 7 characters is 128 species in contrast with 16 species according to Levine's scheme (2) which would use only 4 key characters.

Bergey lists 22 species in the genus Escherichia and 7 in the genus Aerobacter, whereas Levine lists 5 species in the genus Escherichia and only 2 in the genus Aerobacter. Since there appears to be no practical value in drawing fine distinctions between closely related strains, the simple classification of Levine is more practicable and therefore more preferable to the complex scheme used by Bergey.

Species in dairy products. The species belonging to the genus Escherichia which are most common in dairy products according to Yale (9) are *Escherichia coli, E. pseudocoloides* and *E. communior,* based on Bergey's scheme of classification. If we accept Levine's classification, *E. pseudocoloides* might easily be regarded as an atypical strain of *E. communior* differing chiefly in its failure to ferment dulcitol. Other species have been reported by various workers but seem to be present in fewer numbers than the above.

In respect to the genus Aerobacter in dairy products, Yale (9) found *Aerobacter aerogenes, A. oxytocum* and *A. cloacae* to be the only species present.

The intermediates occupying a position between the genus Escherichia and the genus Aerobacter and comprising organisms with a positive methyl red test reaction but able to utilize citrate as the sole source of carbon are also found in dairy products, usually comprising between 10 and 35 percent of the total number of cultures in the entire colon group. While many workers, including the author, feel that these organisms are more closely related to the genus Escherichia than to the genus Aerobacter, Pont (10) found that the intermediate forms showed a marked simi-

larity to *A. aerogenes* in cream and brought about a marked and rapid deterioration in quality accompanied generally by ropiness.

METHODS OF EXAMINATION OF DAIRY PRODUCTS

The methods of detection and enumeration of colon organisms in dairy products which are used at the present time were first developed for water analysis and if suitable for dairy products are so by chance rather than by design.

Early in the history of water analysis, when water supplies were mostly untreated, it was found that the use of plain lactose broth sometimes gave spurious results primarily because of the presence of lactose-fermenting, sporeforming organisms. With the gradual development of chlorination, the use of lactose broth gave proportionately more false tests due to the presence in relatively greater numbers of lactose-fermenting sporeformers.

Water bacteriologists have devoted much attention to the development of selective liquid media which would restrain the growth of false test organisms. These media mostly contain various combinations of bile salts and dyes to inhibit the growth of gram-positive and sporeforming bacteria. As might be expected, certain strains of organisms in the colon group are sometimes inhibited. In a search for a perfect medium, one fluid medium after another has been developed. Whether or not much of this work is justifiable is becoming more and more questionable since there seems to be so little difference between many of these media that the differences in results obtained by various investigators appear to be due to experimental errors rather than to differences produced by the action of the media. Also, it is not always evident that it is worth-while to devote the time and energy necessary to establish the presence of a relatively small percentage of weaker colon organisms. As Prescott and Winslow (3) aptly state, "It is disheartening to the believer in human common sense to see investigator after investigator demonstrate the value of a differential procedure and then discard it because it inhibits 5 percent of the colon bacilli discovered by a lactose broth confirmed test." These remarks are just as applicable to media for examination of dairy products as for water analysis.

Standard Methods of Water Analysis (11) at the present time specify that plain lactose broth shall be used in water analysis for primary inoculation and in the event of gas production may be confirmed by the use of a number of different selective media. This procedure is unsatisfactory in milk analysis since the predominance of streptococci in a milk sample frequently results in the production of an amount of acid sufficient to inhibit the growth of organisms of the colon group before there is visible gas formation when plain lactose broth is used.

The selective liquid media which are used most frequently in this country for dairy products are gentian-violet-lactose-peptone-bile, Kessler and Swenarton (12); brilliant-green-lactose-peptone-bile, 2 percent recommended by Standard Methods of Milk Analysis (13); and the more recent formate-ricinoleate broth developed by Stark and England (14). McCrady and Archambault (15) found that brilliant-green-bile yielded a somewhat larger number of positive presumptives than did gentian-violet-lactose-bile. In another series using only brilliant-green-bile, 86 percent of the tubes which showed at least 10 percent gas in 48 hours completely confirmed in the case of pasteurized milk and 99 percent in the case of raw milk. Where less than 10 percent of gas was produced in 48 hours, 45 percent of the tubes confirmed in the case of pasteurized milk and 92 percent in the case of raw milk.

It is significant to note that the number of confirmations was increased by 11.5 percent by fishing a second colony from the eosin-methylene-blue plate. It is possible that failure to completely confirm all tests where gas was produced was caused by the failure to recover organisms of the colon group since they were overgrown by other types or died after gas was formed. It by no means follows that gas production is due to false test organisms where results cannot be compeltely confirmed. This is a point that many workers have overlooked.

On the other hand, Stark and Curtis (16) believe that when 0.1 cc. or 1.0 cc. quantities of milk are employed, false positive tests sometimes take place in the brilliant-green medium. Working with pure cultures of lactose-fermenting sporeformers, and also with organisms capable of producing spurious results due to "symbiosis," they found that the inhibiting effect of brilliant-green was reduced by the addition of milk, especially by 1 cc. quantities, to such an extent that growth of these false test organisms took place in many instances.

These objections to brilliant-green-bile, especially for the examination of pasteurized milk, are not strongly supported by work in the field and appear to be more theoretical than practical. This appears to be due to the fact that lactose-fermenting sporeformers are so infrequent in milk and that the conditions necessary for false tests due to symbiosis occur but rarely.

Stark and England (14) found that formate-ricinoleate broth inhibited the growth of false test organisms and that addition of protein material did not materially change the surface tension of the medium. Bacteria belonging to the Escherichia-Aerobacter and Salmonella groups were able to produce gas from formic acid in the absence of any other fermentable substance, and this markedly increased the total amount of gas produced.

Minkin and Burgwald (17) found that yeasts are sometimes present in 1 cc. quantities of pasteurized milk and may grow in fluid media used to

determine the presence of the colon group. Yeasts are easily destroyed by pasteurization and, in the case of growth on equipment, are probably outnumbered by members of the colon group. Even if false tests should result from the growth of lactose-fermenting yeasts, they would be just as significant in revealing recontamination of pasteurized milk as are members of the colon group. The same statement holds true for certain strains of glucose-fermenting bacteria which may produce false tests in the formate-ricinoleate medium due to fermentation of the formate or which may cause spurious results in brilliant-green due to symbiotic action between organisms.

To sum up the situation with respect to liquid media, it would seem that both brilliant-green-bile broth and formate-ricinoleate broth are valuable for the examination of milk if used with an understanding of their real value and of their limitations.

Solid media. In order to secure reasonably accurate quantitative results where the number of colon organisms present may vary over a considerable range it is necessary to use several tubes of liquid media in each of three dilutions so selected that all tubes are positive in the lowest dilution and negative in the highest dilution. From the results obtained the most probable number of organisms can be derived from tables as given in Standard Methods (13).

McCrady and Archambault (15), having compared the relative precision of results of tubes and plates, observe that "one plate count of say 8 colonies, is a result quite as reliable as that obtained from 10 fermentation tubes." The chief difficulty involved in the use of a solid medium is to find one sufficiently selective and, at the same time, one which will permit plating of at least 1 cc. quantities of milk. Inasmuch as a plating medium containing lactose normally measures acid-forming rather than gas-forming organisms, it is not anticipated that a plating medium will be found which will be a perfect index to gas-forming organisms (colon group).

Unpublished data collected during the past two years by the author have shown that several plating media developed by two different agencies are sufficiently selective to permit plating of 1 cc. quantities of pasteurized milk when a special technic is used. Under these conditions the majority of the large red colonies which develop in 24 hours belong to the colon group.

A plating medium has advantages and disadvantages as compared with a liquid medium. Conditions under which advantages more than offset disadvantages are being studied at the present time by the author. Under practical conditions the degree to which accurate results are desired is a major factor in the selection of the method to be used. Also, from a research standpoint, it is quite evident that plating methods offer a much more accurate means of studying the relative numbers of different types of lactose-fermenting organisms than any enrichment scheme.

SANITARY SIGNIFICANCE IN RAW MILK

For many years it has been the popular opinion that the presence of *Escherichia coli* in milk indicated pollution with cow manure. Conn (18), who pioneered in dairy bacteriology in this country and made many important contributions, states as follows: "Thus, it follows that, although the presence of *B. coli* may render water unsafe, because it suggests sewage contamination, its presence in milk does not render the milk unsafe, but merely indicates that there may have been a certain amount of contamination with animal feces."

Ayers and Clemmer (19) in a classic piece of work showed convincingly that growth of the colon group in milk or on the surface of utensils makes a correct interpretation of results impossible as to where growth may have taken place. More recently Sherman and Wing (20) and others have confirmed this viewpoint and added further information.

Yale and Eglinton (21) concluded that even in fresh raw milk which has not been held longer than three to four hours following production the colon test has only a slight value when used as a routine test because: (1) Much time and energy are required to make the observations necessary to determine the true significance of high colon counts; (2) the test is misleading, since similar sanitary conditions sometimes result in low colon counts and at other times in high colon counts; (3) while colon infections of the udder are uncommon, samples taken during the early stages of the infection may show very high colon counts; and (4) high colon counts are usually not due to manurial contamination but to growth on the utensils and even in milk.

While not a reliable sanitary index, the conditions which account for the presence of these organisms in fresh raw milk are all undesirable.

SANITARY SIGNIFICANCE IN PASTEURIZED MILK

The significance of the colon group in pasteurized milk is entirely different from that in raw milk. The question whether or not these organisms survive pasteurization has been the subject of much study. While opinions on this point seem to be conflicting, they are more in agreement than many workers realize. Thus, Ayers and Johnson (22) in a classical study on the ability of colon bacilli to survive pasteurization found in test tube experiments that at 60° C. (140° F.) 95 cultures out of 174, or 54.6 percent, survived, while at 62.8° C. (145° F.) 12 cultures, or 6.9 percent, survived. The time of heating was 30 minutes.

Under present commercial conditions, colon organisms are seldom recovered from 1 cc. quantities of freshly pasteurized milk. This does not conflict with the results obtained by Ayers and Johnson since they showed that colon bacilli survived pasteurization on account of the resistance of a few cells (low majority thermal death point). They worked with much larger numbers than occur in commercial supplies, where occasionally

colon organisms are absent from 1 cc. quantities of raw milk previous to pasteurization. The recent work of Henneberg and Wendt (23) in Germany supports this viewpoint. They found that heat-resistant strains (63° C. for 24-30 minutes) were not common, and they failed to recover them in 1 cc. quantities of commercially pasteurized milk. However, an examination of 50 cc. to 100 cc. quantities of milk showed the presence of heat-resistant strains in 2 out of 30 samples.

The presence of colon organisms in bottled samples normally indicates recontamination following pasteurization, McCrady and Langevin (24), Slack and Maddeford (39) and Sherman (25). McCrady and Langevin have shown that frequently recontamination may be detected by the colon test in cases where the standard agar plate count is not sufficiently sensitive. This was also observed to be the case by Chilson, Yale and Eglinton (26).

Process samples yield more information than do street samples of bottled milk in which growth may have taken place. It is desirable that positive results from street samples be followed by the taking of process samples. The presence of colon organisms in street samples may be due to any one or to a combination of the following conditions: (1) Heat-resistance; (2) faulty pasteurization; (3) recontamination; (4) growth in the bottled milk.

The number of colon organisms may vary greatly in the bottled milk. In the case of contaminated equipment the first milk bottled may show the presence of considerable numbers of colon organisms, since the organisms may be rinsed from the equipment to such an extent that they are absent from 1 cc. quantities of milk bottled later in the run. Thus, conclusions as to the reason for positive tests should not be formed from tests of street samples until a series of samples has been examined.

DEFECTS IN MILK AND CREAM

In many cases the presence of colon organisms in dairy products results in the production of defects which cause a great economic loss to the industry.

Mastitis. Acute colon infections of the udder are not as rare as many investigators suppose, and there are numerous references to the subjects in the literature. Hardenbergh and Schlotthauer (27) have reviewed this problem and also report observations of their own. Recently Smith and Henderson (28) have reported a case which has interest in that analyses of the milk were made before, during and following the infection.

Soon after an acute colon infection takes place, the milk becomes so clearly abnormal in appearance that it should be easily detected and kept out of the supply. Shortly prior to this, the milk may contain millions of colon organisms per cc., Yale and Eglington (21). The inclusion of this milk in a city supply may have no direct public health significance due to

the dilution factor; but, if used undiluted or diluted only slightly, it may conceivably produce intestinal upsets or other disorders and is objectionable on other grounds also.

Flavor and keeping quality. Organisms of the colon group may produce a flavor in milk and cream termed "cowy," Pont (10), or "feedy," Sadler and Irvin (29). The English workers, Barkworth (30) and Hoy and Newland (31), feel that there is a correlation between the number of colon organisms and keeping quality. Hoy and Newland showed that milk of a low bacterial count may have a very poor keeping quality, which is connected with the presence of a high proportion of organisms of the colon group.

Ropiness. Considerable economic loss is caused in the industry each year through outbreaks of ropy milk and cream. In many cases this ropy condition is due to members of the Aerobacter section, principally *Aerobacter aerogenes* and *A. oxytocum,* as shown by Yale (9). More rarely, ropiness may be due to *Aerobacter cloacae* or to *Escherichia neapolitana,* as found by Sarles and Hammer (33). Stark and Foter (34) have shown the importance of feeds as a source of ropy milk organisms.

In the case of pasteurized milk and cream ropiness is usually caused by contamination rather than by survival of these organisms during the heat treatment. Kelly (35) suggests ways for preventing and controlling these outbreaks.

Cheese. Gassiness in cheese is a major problem which is as old as the cheese industry and was studied prior to 1900 by a number of investigators. Organisms of the colon group are the offenders in many instances. In addition to delaying the action of starters, gas and other by-products are produced which result in poor quality cheese.

Cheesemakers have had limited success in overcoming gassiness by control with "starters" or by the addition of chemicals such as potassium nitrate. In this instance an ounce of prevention is worth a pound of cure. Harrison (36) found that the addition of potassium nitrate (saltpeter) to colored cheese produced discoloration and recommended that the use of nitrates be discontinued.

Ice cream. Whether a routine test for organisms of the colon group in ice cream would be worthwhile in giving information concerning sanitary quality not afforded by the total count is uncertain. Cream and other materials used in the preparation of the mix may contain colon organisms in large numbers. Fabian and Coulter (37) found that in ice cream mix many strains survive a temperature of 62.8° C. for 30 minutes, while a few strains survived 65.5° C. Although ice cream has a greater protective action than skim milk, under commercial conditions this may have little significance since ice cream mixes are usually pasteurized at higher temperatures than milk. Recontamination with colon organisms may also result from addition of contaminated flavor extracts and fruits or

from contact with contaminated equipment such as pipe lines, freezers and containers.

Butter. For many years it has been thought that organisms of the colon group were sometimes responsible, in part at least, for defective butter. Very little has been published on the subject. At the Iowa Agricultural Experiment Station organisms belonging to the colon group have frequently been isolated from butter showing flavor defects. Hammer and Yale (38) identified 25 cultures from 17 samples of off-flavored butter and found that 60 percent were *Aerobacter aerogenes,* 12 percent *A. cloacae* and 16 percent *A. oxytocum,* while 12 percent belonged to the intermediate group. This indicated that organisms belonging to the genus Aerobacter were more important than organisms of the genus Escherichia in causing flavor defects. This opinion was confirmed when portions of cream were inoculated with pure cultures representing several species in each group and then churned. When the butter was held for 10 days at 7° C. and also at 18° C., species belonging to the genus Escherichia did not develop off flavors. On the other hand, members of the genus Aerobacter grew more rapidly than those of the genus Escherichia and regularly developed unclean odors and flavors in the salted and unsalted butter held at 18° C. No defects occurred in the case of the salted butter held at 7° C., but off flavors sometimes were apparent with the unsalted butter.

It should be pointed out that organisms belonging to the genus Aerobacter are practically always present in cream supplies used for buttermaking. When present in large numbers they are deleterious to cream quality. These organisms are practically always destroyed by pasteurization. Recontamination is undoubtedly responsible in many cases for the presence of colon organisms in butter.

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Observations on *Alcaligenes lipolyticus*[1]

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DURING the plating of a large number of samples of milk, cream, butter and various other materials for lipolytic organisms by the use of the nile blue sulfate technic (6) an organism was occasionally encountered which differed from the usual types found in that the colonies were very small in size. An outstanding character of the organism was its pronounced lipolytic ability as evidenced by the rapid hydrolysis of the fat globules under the colonies and by the extent of the zone of hydrolysis.

Evans (3) in 1916 made a study of the bacteria of milk freshly drawn from the normal udder and isolated a species which she designated *Bacillus abortus* var. *lipolyticus*. Evans found that the organism produced a very scanty growth in the form of small separate colonies on meat-infusion agar slants. Gelatin was not liquefied, nitrates were not reduced and acid was not produced from carbohydrates. Growth was slight in litmus skim milk, and the medium remained unchanged; in litmus whole milk there was good growth with slow acid development, which was first apparent in the cream layer. Thirty-seven degrees Centigrade was considered to be the optimum growth temperature.

Later Evans (4) pointed out that *Bacillus abortus* var. *lipolyticus* could be found in large numbers in milk and that it was killed by a temperature of 52° C. for 30 minutes or 63° C. for 30 seconds. The same author (5) in 1918 noted that this organism did not form endospores and accordingly belonged in the genus Bacterium. Since the variety

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designation was unwieldy and the organism was likely a distinct species, she suggested the name *Bacterium lipolyticus*. The organism was found to be nonpathogenic for guinea pigs.

Steck (7) in 1921 studied the bacteria present in the normal udder and reported the occurrence of the type described by Evans. Dorner (2) investigated the bacterial flora of aseptically drawn milk and found a large number of rods which he considered identical with the species isolated by Evans.

SOURCES OF CULTURES

Twenty-one cultures of the organism were isolated. Fifteen were obtained at various intervals from the raw milk of one producer supplying Iowa State College, and one came from the raw milk of another producer. Four cultures were isolated from raw mixed milk, and one was obtained from the water of a lake on the Iowa State College campus.

IDENTITY OF THE ORGANISM

A study of the morphology, cultural characters and biochemical features of the 21 cultures isolated indicated that they were identical with the organism described by Evans (3). This study has permitted an extension of the original description. The characters of the species are such that it probably belongs in the genus Alcaligenes, and the name *Alcaligenes lipolyticus* is suggested.

SPECIAL CHARACTER OF *ALCALIGENES LIPOLYTICUS*

HYDROLYSIS OF FAT

All of the 21 cultures hydrolyzed fat when tested by the nile blue sulfate technic (6) using cottonseed oil and beef-infusion agar, and they were relatively consistent in the type of lipolysis produced under spot colonies. Complete lipolysis beneath the growth occurred with all of the cultures, while all except one hydrolyzed the fat for a considerable distance beyond the edge of the colony.

HYDROLYSIS OF SIMPLE TRIGLYCERIDES

The 21 cultures were tested for their ability to hydrolyze various simple triglycerides dispersed in beef-infusion agar containing nile blue sulfate. All of them hydrolyzed triisovalerin, tricaproin, tricaprylin, tricaprin, trilaurin and triolein. Tripropionin and tributyrin were hydrolyzed by a majority of the cultures; those not bringing about hydrolysis were unable to grow on the media. Trivalerin, triheptylin, trimyristin, tripalmitin and tristearin were not hydrolyzed.

ACTION IN CREAM

Each of the 21 cultures was inoculated into a small amount of sterile cream. After seven days at 21° C. all of the cultures had developed pronounced rancidity.

ACTION IN BUTTER

Each of 11 cultures was inoculated into cream that had been pasteurized at 82° C. for 15 minutes. The cream was then churned and the unsalted butter stored at 21° C. After three days seven of the samples had developed rancidity, while after five days all of the samples were rancid.

PRODUCTION OF ACETYLMETHYLCARBINOL PLUS DIACETYL IN SKIM MILK

Each of four cultures was inoculated into skim milk and the milk incubated at 21° C. for three days. When 200-gm. samples of the cultures were steam distilled in the presence of ferric chloride and the distillates collected in a solution of hydroxylamine hydrochloride and sodium acetate, no nickel dimethylglyoximate was obtained when nickel chloride was added.

Each of two cultures was inoculated into three portions of skim milk to which had been added 0.4, 0.5 and 0.6 percent citric acid, respectively. After three days incubation at 21° C. no acetylmethylcarbinol plus diacetyl was found when the cultures were examined.

PRODUCTION OF VOLATILE ACID

The production of volatile acid was studied with three cultures by inoculating each of them into skim milk and incubating the milk six days at 21° C. When volatile acidity determinations were then made by steam distilling 250-gm. portions of the cultures after the addition of 15 ml. of approximately n/1 H_2SO_4, it was found that no increase had occurred.

EFFECT OF GLYCEROL AND THE SODIUM SALTS OF FATTY ACIDS ON THE GROWTH OF *ALC. LIPOLYTICUS*

The fact that the group of otherwise relatively inert organisms attacked fat so readily suggested that they must use one or more of the products of hydrolysis. This theory was tested by noting the effect of glycerol and of the sodium salts of various fatty acids upon the growth of the organisms. Beef-extract broth and beef-infusion agar were used as controls; glycerol and the sodium salts in various concentrations were added to other lots of broth and agar; and the reactions of the media were adjusted to pH 6.8. Each medium to be tested was inoculated with each of the 21 cultures. The results obtained after an incubation of approximately one week at 21° C. were as follows:

Glycerol. Concentrations of 0.5 percent of glycerol in the broth and agar did not influence the growth of the organisms.

Sodium Acetate. In broth containing 0.5 percent sodium acetate growth was much heavier than in the control tubes. Concentrations between 0.25 and 0.5 percent in agar slants also increased the growth. In the control slopes the growth was thin, dull, beaded and almost streptococcus-like; while in the slopes containing sodium acetate it was luxuriant, white and spreading.

Sodium Propionate. Concentrations of 0.5 percent of sodium propionate in broth greatly aided growth, while the same concentrations in agar completely inhibited development. When 0.25 percent sodium propionate was added to the agar there was good growth, but the growth was not quite as extensive as in the agar to which sodium acetate had been added.

Sodium Butyrate. The addition of 0.5 percent sodium butyrate to broth gave the best growth in the series, and with some cultures pellicle formation was noted. A 0.25 percent concentration in agar gave very good growth as compared with that in the control, while 0.5 percent inhibited growth.

Sodium Caproate. When 0.25 percent sodium caproate was added to broth, growth was greatly stimulated. The same concentration in beef-infusion agar inhibited development, while 0.1 percent gave remarkably good growth.

Sodium Caprylate. Sodium caprylate appeared to be extremely toxic, and the concentrations used were necessarily very low; 0.1 percent in broth and 0.05 percent in agar brought about much better development than that in the control tubes.

Sodium Caprate. Sodium caprate was relatively toxic, as compared with the other compounds used. Concentrations of 0.1 percent in broth and 0.05 percent in agar stimulated growth.

Sodium Oleate. Growth of all the cultures was distinctly aided by the addition of 0.5 percent sodium oleate to broth and 0.25 percent to the agar.

ABILITY TO USE VARIOUS FAT COMPONENTS AS THE SOLE SOURCE OF CARBON

The ability of *Alc. lipolyticus* to use glycerol or certain of the fatty acids as the sole source of carbon was investigated. The synthetic medium A of Ayres, Rupp and Johnson (1) was used as a control. It had the following composition:

Sodium ammonium phosphate	2.0 grams
Dextrose	10.0 grams
Potassium chloride	0.1 grams
Distilled water	1,000.0 ml.

The test media were made up by varying the source of carbon in the above medium. Instead of dextrose, the following compounds were used in the amounts designated: 0.25 percent glycerol, 0.25 percent sodium acetate, 0.25 percent sodium propionate, 0.25 percent sodium butyrate, 0.05 percent sodium caproate, 0.05 percent sodium caprylate, 0.05 percent sodium caprate and 0.1 percent sodium oleate.

All of the cultures were able to grow to a slight extent in the control medium containing dextrose as the sole source of carbon. The organisms were also able to utilize glycerol and the sodium salts of the fatty acids

designated as carbon sources, but the various salts differed in their ability to support growth. The development in the media containing sodium acetate, sodium butyrate and sodium oleate was very good as compared with that in the control tubes; while growth in the media containing glycerol, sodium propionate, sodium caproate, sodium caprylate and sodium caprate was relatively poor.

GENERAL DESCRIPTION OF *ALCALIGENES LIPOLYTICUS*

MORPHOLOGY (CULTURES GROWN AT 21°C.)

Form and Size. Rods; 0.6 to 1.0 by 1.0 to 1.4 microns after one day on beef-infusion agar; after approximately three weeks cells elongated measuring up to 0.8 microns in width and 2.4 microns in length.

Arrangement. Singly and in pairs.

Motility. Non-motile.

Staining Reactions. Generally gram positive in young cultures. Older cultures gram negative.

Spores. None observed in either young or old agar cultures.

CULTURAL CHARACTERISTICS (CULTURES GROWN AT 21°C.)

Agar Slope. Scanty, white, filiform, nonviscid, dull growth on beef-infusion agar after 1 to 2 days, the type of growth not changing on extended incubation.

Agar Stab. Small amount of surface growth on beef-infusion agar with growth extending downward along the line of inoculation.

Agar Colony. Growth evident on beef-infusion agar after approximately 2 days; after 4 days surface colonies white, nonviscid, round with entire edge and from 1 to 2 mm. in diameter. Subsurface colonies oval, white, nonviscid and smaller than surface colonies.

Gelatin Stab. No liquefaction. Scanty growth on surface with some growth following the line of inoculation.

Bouillon. A slight cloudiness in the medium and a slight sedimentation after 3 to 4 days.

Potato. No visible growth.

Litmus Skim Milk. Beyond a slight precipitate in the tube on extended incubation, no visible action in litmus skim milk.

Litmus Whole Milk. Acid apparent in the cream layer after approximately 2 weeks, the acid later extending down through the milk.

BIOCHEMICAL FEATURES (CULTURES GROWN AT 21°C.)

Indol. Not produced.

Nitrates. Not reduced.

Hydrogen Sulphide. Not produced in agar.

Methyl Red Reaction. Negative.

Voges Proskauer Reaction. Negative.

Fermenting Power. Most of the cultures produced neither acid nor gas from the compounds used. Acid but no gas produced from arabinose, dextrose, lactose, levulose and maltose by a few cultures. Galactose, glycerol, inulin, mannitol, raffinose, salacin and sucrose not fermented and starch not hydrolyzed.

Hydrolysis of Fat. Fat hydrolyzed.

Hemolysis. Red cells not hemolyzed.

GROWTH CONDITIONS

Oxygen Relationships. Aerobic.

Growth Temperatures. Growth by all cultures at 10° C., 37° C., and at temperatures in between. At 40° C. slight growth by most of the cultures.

SUMMARY

A number of lipolytic cultures were isolated and were considered identical with *Bacillus abortus* var. *lipolyticus* described by Evans (3). The characters of the organism indicate that it belongs in the genus Alcaligenes; therefore, the name *Alcaligenes lipolyticus* is proposed. *Alc. lipolyticus* produced rancidity in butter and was characterized by its ability to rapidly hydrolyze fat and to use certain of the salts of fatty acids as the sole source of carbon. A description of *Alc. lipolyticus* is presented.

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Studies on Lactobacillus Cultures That Actively Coagulate Milk[1]

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IT HAS long been known that certain species of the genus Lactobacillus are important in the dairy industry. Cultures of *Lactobacillus casei* were early studied in connection with the ripening of Swiss, Cheddar and other types of cheese. *Lactobacillus acidophilus* is of particular importance in the production of fermented milk which has certain therapeutic uses. *Lactobacillus bulgaricus* is also used in the production of certain types of fermented milk and in the manufacture of Swiss cheese.

The species of lactobacilli mentioned above are primarily important in the dairy industry because of their ability to ferment lactose actively. These microorganisms are definitely homofermentative; that is, lactic acid is the chief product formed in milk and there are only relatively small amounts of additional compounds produced. Other lactobacilli are undoubtedly common in dairy products, but the types that are active lactose fermenters have a much better opportunity to bring about conspicuous changes in milk and its derivatives than types that ferment lactose slowly or not at all.

In view of the fact that little work has been done on the determination of the products other than lactic acid produced by lactobacilli, continued studies on the fermentation of milk by these organisms seem desirable.

The work herein reported involves a study of the amounts of total acid, volatile acid and acetylmethylcarbinol plus diacetyl produced in milk by representative strains of *L. casei* that actively coagulate milk.

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METHODS

CULTURES USED

A total of 36 cultures of lactobacilli were studied. Most of the cultures were isolated from dairy products such as cheese and milk, while a small number came from feces and ensilage. Tomato juice agar was generally employed for the isolation of the cultures; the colonies were picked from the plates and propagated in litmus milk. Cultures identified as lactobacilli which did not produce relatively large amounts of acid in a comparatively short period were discarded.

CHEMICAL ANALYSIS

The test organisms were inoculated into flasks of sterile skim milk and held 7 days at 37° C. The following determinations were then made on the cultures:

Total acidity. Nine gms. of cultures were titrated with N/10 NaOH using phenolphthalein. The results are expressed as the percentage of lactic acid in 9 gms. of culture.

Volatile acidity. The method used was that outlined by Michaelian, Farmer and Hammer (1). The results are expressed as the cc. of N/10 NaOH required to neutralize the first liter of distillate when a 250-gm. portion of culture was distilled with steam.

Acetylmethylcarbinol plus diacetyl. The method employed was that suggested by Michaelian, Farmer and Hammer (1). The results are expressed as the milligrams of nickel dimethylglyoximate equivalent to acetylmethylcarbinol plus diacetyl per 200 gms. of material.

RESULTS

THE PRODUCTION OF TOTAL ACID, VOLATILE ACID AND ACETYLMETHYLCARBINOL PLUS DIACETYL IN MILK

The data on the amounts of total acid, volatile acid and acetylmethylcarbinol plus diacetyl produced in milk by the 36 cultures of lactobacilli are given in table 1.

There was considerable variation in the amounts of total acid formed by the various cultures, the values ranging from 0.50 to 2.34 percent calculated as lactic acid. No differences were noted in the appearance of litmus milk cultures of the organisms at the end of the incubation period and all the organisms produced a smooth curd with no evidence of gas or proteolysis. Cultures that produced relatively large amounts of total acid sometimes showed a tendency to "whey off." There was very little difference in the rate of coagulation of litmus milk with the various organisms. Culture 25 when first isolated coagulated milk rather slowly, but after the second transfer in litmus milk the rate of coagulation was about the same as for the other cultures.

TABLE 1. *The production of total acid, volatile acid and acetylmethylcarbinol plus diacetyl in milk*

Cultures incubated at 37° C. for 7 days

Culture no.	Percent total acid	Volatile acid[1]	Mg. of Ni salt equiv. to amc + aa[2] per 200 gm.
1	2.02	39.0	trace
2	2.00	28.5	1.8
3	2.34	36.3	1.8
4	0.85	41.0	0.0
5	2.17	42.6	trace
6	2.10	35.7	1.2
7	2.31	31.8	0.0
8	1.50	34.5	trace
9	1.40	35.8	10.8
10	0.68	11.2	3.0
11	1.23	29.3	0.5
12	1.85	35.3	2.8
13	0.80	28.2	10.0
14	0.90	38.2	8.9
15	0.90	40.6	trace
16	1.11	28.6	1.3
17	0.96	27.3	0.0
18	1.21	16.9	trace
19	1.01	16.8	0.8
20	0.80	31.0	8.3
21	1.12	10.4	trace
22	1.34	30.9	1.5
23	0.90	22.4	0.0
24	0.80	21.5	2.1
25	0.50	10.0	trace
26	0.58	35.3	0.9
27	1.70	11.1	29.1
28	2.01	11.6	1.9
29	0.90	27.0	trace
30	1.19	27.5	0.0
31	2.05	36.0	11.8
32	1.80	29.4	17.4
33	1.50	27.8	0.0
34	1.90	29.1	0.0
35	2.10	28.9	5.5
36	0.80	21.4	1.6

[1] Volatile acid expressed as cc. N/10 NaOH required to neutralize the first liter of distillate obtained when a 250 gm. portion of culture was steam distilled after adding 15 cc. N/1 sulfuric acid.

[2] amc + aa = acetylmethylcarbinol + diacetyl.

All of the cultures produced an appreciable quantity of volatile acid. The value for the 36 cultures ranged from 10.0 to 42.6. A comparison of the production of total acid and of volatile acid is given in the following summary:

Cultures producing total acidities from	Produced volatile acidities from	Number of cultures
0.50 to 1.00	10.0 to 41.0	13
1.01 to 1.50	10.4 to 35.8	10
1.51 to 2.00	11.1 to 35.3	5
2.01 to 2.50	11.6 to 42.6	8

The summary shows that the production of volatile acid was not related to the total acid formed since the volatile acidities varied widely in each of the total acid groups and the minimum and maximum values for the different groups were much the same.

The yield of nickel salt equivalent to acetylmethylcarbinol plus diacetyl with the various cultures ranged from 0.0 to 29.1 mg., with 7 of the 36 cultures giving values of 0.0. Only five of the cultures yielded 10.0 or more mg. of nickel salt and only one yielded more than 20.0 mg. The following summary compares the production of total acid with the values for nickel salt equivalent to acetylmethylcarbinol plus diacetyl for the various cultures:

Cultures producing total acidities from	Yielded mg. of Ni salt from	Number of cultures
0.50 to 1.00	0.0 to 10.0	13
1.01 to 1.50	0.0 to 10.8	10
1.51 to 2.00	0.0 to 29.1	5
2.01 to 2.50	0.0 to 11.8	8

From the summary it is evident that the production of acetylmethylcarbinol plus diacetyl was not correlated with the formation of total acid. Each of the total acid groups contained organisms that did not yield acetylmethylcarbinol plus diacetyl and with three of the four groups, including those representing the highest and lowest acid producers, the maximum production by an organism in the group was essentially the same.

The results indicated that there was no direct relationship between the amounts of total acidity, volatile acidity, and acetylmethylcarbinol plus diacetyl formed by the cultures, regardless of the sources from which the organisms were isolated.

THE PRODUCTION OF VOLATILE ACID AND ACETYLMETHYLCARBINOL PLUS DIACETYL WHEN 0.15 PERCENT CITRIC ACID WAS ADDED TO THE MILK

The effect of the addition of citric acid on the production of volatile acid and acetylmethylcarbinol plus diacetyl in milk was studied with the 36 cultures by adding 0.15 percent citric acid to the milk at the time of

inoculation, incubating at 37° C. for 7 days and then determining the volatile acid and acetylmethylcarbinol plus diacetyl values. Table 2 gives the data obtained.

The addition of citric acid to the milk apparently had little effect on the production of volatile acid by the organisms. The values obtained

TABLE 2. *The production of volatile acid and acetylmethylcarbinol plus diacetyl when 0.15 percent citric acid was added to the milk*

Cultures incubated at 37° C. for 7 days

Culture no.	Milk alone[1]		Milk plus 0.15% citric acid	
	Volatile acid	Mg. of Ni salt equiv. to amc + aa per 200 gm.	Volatile acid	Mg. of Ni salt equiv. to amc + aa per 200 gm.
1	39.0	trace	35.0	11.1
2	28.5	1.8	19.3	trace
3	36.3	1.8	40.0	2.1
4	41.0	0.0	42.0	trace
5	42.6	trace	23.0	3.2
6	35.7	1.2	29.0	0.8
7	31.8	0.0	36.4	0.9
8	34.5	trace	37.1	1.9
9	35.8	10.8	12.2	2.0
10	11.2	0.0	15.1	1.0
11	29.3	0.5	23.5	0.9
12	35.3	2.8	30.4	trace
13	28.2	10.0	33.7	13.6
14	38.2	8.9	34.6	9.1
15	40.6	trace	44.5	2.1
16	28.6	1.3	27.3	8.0
17	27.3	0.0	30.2	1.5
18	16.9	trace	19.4	1.0
19	16.8	0.8	21.2	0.0
20	31.0	8.3	18.9	trace
21	10.4	trace	16.1	2.3
22	30.9	1.5	33.2	2.0
23	22.4	0.0	16.4	1.5
24	21.5	2.1	25.4	3.5
25	10.0	1.3	15.9	trace
26	35.3	0.9	31.4	1.1
27	11.1	29.1	21.2	1.8
28	11.6	1.9	13.5	2.1
29	27.0	trace	27.0	1.7
30	27.5	0.0	31.2	trace
31	36.0	11.8	39.0	9.3
32	29.4	17.4	37.2	21.2
33	27.8	0.0	19.8	trace
34	29.1	0.0	34.8	trace
35	28.9	5.5	29.5	3.5
36	21.4	1.6	28.4	2.2

[1] These data are taken from table 1.

with the citric acid added ranged from 12.2 to 44.5 while those in the controls ranged from 10.0 to 42.6. Twenty-two of the cultures gave higher volatile acidities with citric acid while 13 gave lower values and with 1 there was no difference; in general, the differences were not significant. The addition of 0.15 percent citric acid approximately doubles the citric acid content of the original milk; therefore, if citric acid is a source of volatile acid, the added citric acid should greatly increase the volatile acid formed.

The addition of citric acid had no significant effect on the production of acetylmethylcarbinol plus diacetyl by the organisms in milk. The values for the nickel salts obtained on the milk cultures with the citric acid added ranged from 0.0 to 29.1 mg. while those for the controls ranged from 0.0 to 21.1 mg. Twenty-six of the cultures gave higher values with citric acid added, while 10 gave lower values. There was considerable variation in the amounts of acetylmethylcarbinol plus diacetyl formed, both with and without citric acid added and, in general, the differences between the values for the milk with citric acid and without the acid were not significant.

It was interesting to note that some of the cultures which produced relatively small amounts of acetylmethylcarbinol plus diacetyl showed appreciable increases of these compounds when citric acid was added to the milk, while those cultures that produced comparatively large amounts of acetylmethylcarbinol plus diacetyl did not show large increases of these compounds.

THE PRODUCTION OF ACETYLMETHYLCARBINOL PLUS DIACETYL WHEN VARIOUS CONCENTRATIONS OF ACETALDEHYDE WERE ADDED TO THE MILK

The effect of adding various concentrations of acetaldehyde on the production of acetylmethylcarbinol plus diacetyl in milk was studied with 10 cultures of lactobacilli as follows: Five 100-cc. portions of sterile skim milk in bottles were inoculated with 0.5 cc. of an actively growing milk culture of an organism and incubated at 37° C. for 12 hours. Different concentrations of acetaldehyde were then added to four of the bottles and the remaining one was used as a control; the concentrations of acetaldehyde used were 0.05, 0.1, 0.3, and 0.4 percent. After incubating at 37° C. for 7 days, acetylmethylcarbinol plus diacetyl determinations were made on the various lots. The data obtained are given in table 3.

From the results, it appeared that the addition of various concentrations of acetaldehyde to the milk did not appreciably increase the production of acetylmethylcarbinol plus diacetyl. The values for nickel salt, equivalent to acetylmethylcarbinol plus diacetyl, varied widely both in the controls and in the milk to which the various concentrations of the acetaldehyde had been added; the values for the controls ranged from a trace to 23.2 mg., while those for the milk with acetaldehyde added ranged from 0.0 to 26.0 mg.

TABLE 3. *The influence of various concentrations of acetaldehyde on the production of acetylmethylcarbinol plus diacetyl in skim milk*
Cultures incubated at 37° C. for 7 days

Culture no.	Mg. Ni salt equiv. to amc + aa per 200 gm.				
	Concentration of acetaldehyde				
	Control	0.05%	0.1%	0.3%	0.4%
9	8.4	9.1	8.0	trace	0.0
10	2.8	5.5	6.2	3.1	0.0
11	0.7	1.8	0.9	trace	0.0
13	9.1	10.4	10.9	6.3	0.0
20	5.1	2.0	0.0	0.0	0.0
21	trace	3.6	4.0	0.0	0.0
27	23.2	25.1	26.0	21.2	0.0
28	2.3	2.5	8.4	3.9	0.0
31	12.4	11.2	12.9	8.4	0.0
32	16.1	19.5	20.7	19.2	0.0

With 0.05 percent acetaldehyde added the values for the nickel salt ranged from 1.8 to 25.1 mg. Eight of the cultures showed increases over the controls with the acetaldehyde added while two showed decreases, but in no instance was the difference great.

With 0.1 percent acetaldehyde added to the milk the nickel salt values ranged from 0.0 to 26.0 mg. Eight of the organisms gave increases, as compared with the controls, in acetylmethylcarbinol plus diacetyl with the aldehyde added while two gave decreases, but in no instance was the difference great. One culture failed to produce any acetylmethylcarbinol plus diacetyl with acetaldehyde while a considerable quantity was produced in the control.

With 0.3 percent acetaldehyde added to the milk the acetylmethylcarbinol plus diacetyl values ranged from 0.0 to 21.2 mg. nickel salt. In three instances there were increased amounts formed with the acetaldehyde while in seven there were decreases. Four of the organisms produced no more than a trace of acetylmethylcarbinol plus diacetyl with the aldehyde added. In general, the results obtained suggest that the acetaldehyde was slightly toxic in the concentration used.

With 0.4 percent acetaldehyde the milk failed to coagulate and none of the cultures produced any acetylmethylcarbinol plus diacetyl. This indicates that 0.4 percent acetaldehyde in milk was definitely toxic to the organisms used.

From the results obtained it was evident that concentrations of 0.05 or 0.1 percent acetaldehyde produced slight increases in acetylmethylcarbinol plus diacetyl in milk in many instances, while in several instances small decreases were noted but in every case the difference was small. The addition of 0.3 or 0.4 percent acetaldehyde to the milk appeared to be toxic for the organisms used.

CONCLUSIONS

1. Appreciable quantities of total acid, volatile acid and acetylmethylcarbinol plus diacetyl are formed in skim milk by most of the lactobacilli; the amounts of these materials produced by the different cultures vary greatly and there is no close correlation between the amount of total acid and the amounts of volatile acid or acetylmethylcarbinol plus diacetyl produced by a culture.

2. The addition of citric acid to milk has no significant effect on the amounts of volatile acid and acetylmethylcarbinol plus diacetyl formed by the organisms.

3. The addition of acetaldehyde does not significantly increase the production of acetylmethylcarbinol plus diacetyl by the organisms in milk. Low concentrations of the acetaldehyde result in slight increases in the amounts of acetylmethylcarbinol plus diacetyl produced while the higher concentrations appear to be definitely toxic.

LITERATURE CITED

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Determination of Acetylmethylcarbinol and Diacetyl in Dairy Products[1]

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STUDIES carried on by various investigators in recent years have definitely proved that a good quality of butter culture usually contains comparatively large quantities of acetylmethylcarbinol and some diacetyl; consequently, the determination of these compounds in dairy products has become the usual practice in dairy laboratories. Often, however, certain irregularities have been encountered with the technique employed in the determination of these compounds.

Attempts to determine the nature of these irregularities and, if possible, to find a working remedy herein are reported.

METHODS

Acetylmethylcarbinol and diacetyl are commonly determined together (3) because ordinarily small amounts of diacetyl also are present with the carbinol. To 200 gm. or 200 ml. of the sample for analysis 40 ml. of ferric chloride solution (40 gm. made up to 100 ml. with distilled water) were added to oxidize the carbinol to diacetyl and then steam distilled. The distillate was carried by means of an adapter on the end of the condenser into a mixture of one part of a solution of hydroxylamine hydrochloride (20 gm. made up to 100 ml.) and two parts of sodium acetate solution (20 gm. made up to 100 ml.). The distillation was continued until the volume of the distillate collected was approximately three-fourths of the original sample used. The mixture, consisting of distillate, hydroxylamine hydrochloride and sodium acetate, was heated to

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80°-90° C., and nickel chloride solution (10 gm. made up to 100 ml.) was added. The quantities of reagents used depended on the amount of diacetyl expected, always an excess being used. van Niel (7) has shown that the concentration of the reagents can fluctuate between wide limits; for about 100 mg. diacetyl he suggested 2 ml. hydroxylamine hydrochloride (20 percent solution), 3 to 5 ml. sodium acetate (20 percent solution) and 1 to 2 ml. nickel chloride (10 percent solution). The distillate with the reagents added was allowed to stand at room temperature at least two days, and preferably longer, in order to obtain complete crystallization. This can be accomplished also by heating the distillate containing hydroxylamine hydrochloride and sodium acetate to 80°-90° C. and, after adding nickel chloride, allowing it to stand overnight at 70°-80° C. (1). The nickel dimethylglyoximate was filtered into a weighed crucible, and the filtrate occasionally treated with more reagents to assure complete crystallization. The nickel salt was washed with distilled water, dried to constant weight at 105°-110° C. and the results recorded as grams of nickel dimethylglyoximate equivalent to acetylmethylcarbinol plus diacetyl per 200 gm. or 200 ml. of material.

Diacetyl was determined by the method used for the acetylmethylcarbinol plus diacetyl, except that ferric chloride was not added and, in the procedure preferred, the distillation was carried out after the sample had been saturated with an inert gas, such as carbon dioxide or nitrogen, to prevent the formation of diacetyl from acetylmethylcarbinol during the distillation process.

The volatile acid was determined by steam distilling a 200 ml. sample and the distillate collected (by means of an adapter on the end of the condenser) into a known amount of n/20 sodium hydroxide in a flask immersed in ice water; 200 ml. of the distillate were collected and the excess alkali titrated with n/20 hydrochloric acid, using phenolphthalein as the indicator. The results are expressed as milliliters of n/20 sodium hydroxide required to neutralize the acid in the distillate.

The pH determinations were made electrometrically, using quinhydrone.

RESULTS OBTAINED

When the filtrates from nickel dimethylglyoximate equivalent to diacetyl or acetylmethylcarbinol plus diacetyl are saved and allowed to stand for some time, often new crystals of the nickel salt appear. This is particularly true with the determination of diacetyl.

Acetylmethylcarbinol is fairly volatile (2, 5, 6); therefore, it is probable that a considerable fraction of the carbinol distills over with the diacetyl when both are present in the material being analyzed. During the preliminary trials of this investigation it was found that, when ferric chloride was added to the material and then steam distilled, some of the carbinol also passed over into the distillate with the diacetyl.

TABLE 1. *Nickel dimethylglyoximate equivalent to acetylmethylcarbinol recovered in successive filtrates*

Determination for	Grams nickel dimethylglyoximate obtained			
	Butter culture 232[1]	Cultures of citric acid fermenting streptococci		Aqueous acetyl-methylcar-binol solu-tion
		9[2]	29[3]	
Acetylmethylcarbinol + diacetyl	0.1143	0.1980	0.0911	0.1297
1st filtrate	0.0048	0.0221	0.0037	0.0105
2nd filtrate	0.0016	0.0050	0.0019	0.0009
3rd filtrate	none	0.0007	none	none
Diacetyl	0.0139		0.0025	0.0087
1st filtrate	0.0298		0.0310	0.0355
2nd filtrate	0.0067		0.0071	0.0081
3rd filtrate	none		trace	none
Residue from diacetyl determination + 40 ml. $FeCl_3$	0.0607		0.0473	0.0643
1st filtrate	0.0042		0.0032	none
2nd filtrate	0.0012		0.0008	
3rd filtrate	none		none	

[1] Butter culture made from skim milk to which 0.15 percent citric acid had been added.

[2] Skim milk culture of a citric acid fermenting Streptococcus to which 0.85 percent citric acid had been added.

[3] Skim milk culture of a citric acid fermenting Streptococcus to which 0.15 percent citric acid and 0.3 percent sulfuric acid had been added.

To determine the amount of acetylmethylcarbinol that passed over into the distillate with the diacetyl, the filtrate from nickel dimethylglyoximate was acidified with concentrated sulfuric acid, 40 ml. ferric chloride added and the mixture steam distilled in the usual way.

The data in table 1 show that nearly all of the carbinol in the filtrate was oxidized by ferric chloride to diacetyl and thus recovered as nickel dimethylglyoximate, while a small amount of the carbinol again passed over into the distillate. The data indicate also that the carbinol was present in the second and sometimes even in the third successive filtrate.

Attempts to recover all of the carbinol in the filtrate with one distillation by heating it with steam to 95°-99° C. and holding it at that point for about five minutes with a small flame or by occasionally letting steam in were fairly successful. Almost all of the carbinol in the filtrate was oxidized to diacetyl with this treatment. The recovery of the carbinol in filtrates reported in this work was done in the manner described above.

Table 2 shows that a considerable fraction of acetylmethylcarbinol also distills over with the diacetyl during the determination of diacetyl

TABLE 2. *Recovery of acetylmethylcarbinol from filtrates of diacetyl or acetylmethylcarbinol plus diacetyl determinations*

Material used	Trial number	Determination for	Grams nickel dimethylglyoximate obtained		
			Recovered from		Total recovered
			distillate	filtrate	
Butter culture made from skim milk	1	Ac_2[1]	0.0026	0.0178	0.0697
		Residue + $FeCl_3$	0.0470	0.0023	
		Amc[2] + ac_2	0.0669	0.0059	0.0728
	2	Ac_2	0.0019	0.0154	0.0650
		Residue + $FeCl_3$	0.0452	0.0025	
		Amc + ac_2	0.0680	0.0054	0.0734
	3	Ac_2	0.0019	0.0157	0.0588
		Residue + $FeCl_3$	0.0399	0.0013	
		Amc + ac_2	0.0622	0.0042	0.0671
Butter culture made from skim milk to which 0.15% citric acid had been added	1	Ac_2	none	0.0418	0.1116
		Residue + $FeCl_3$	0.0642	0.0056	
		Amc + ac_2	0.1154	0.0128	0.1282
	2	Ac_2	none	0.0433	0.1072
		Residue + $FeCl_3$	0.0574	0.0065	
		Amc + ac_2	0.1003	0.0106	0.1109
	3	Ac_2	0.0018	0.0274	0.1012
		Residue + $FeCl_3$	0.0679	0.0041	
		Amc + ac_2	0.0975	0.0079	0.1054
Skim milk cultures of citric acid fermenting streptococci to which citric acid had been added	1	Ac_2	0.0083	0.1230	0.2512
		Residue + $FeCl_3$	0.1130	0.0069	
		Amc + ac_2	0.2708	0.0260	0.2968
	2	Ac_2	0.0043	0.0184	0.0600
		Residue + $FeCl_3$	0.0329	0.0044	
		Amc + ac_2	0.0591	0.0073	0.0664
Aqueous solution of commercial acetylmethylcarbinol	1	Ac_2	0.0023	0.0637	0.1823
		Residue + $FeCl_3$	0.1090	0.0073	
		Amc + ac_2	0.1909	0.0204	0.2113
Aqueous solution of purified acetylmethylcarbinol[3]	1	Ac_2	none	0.0789	0.2434
		Residue + $FeCl_3$	0.1460	0.0185	
		Amc + ac_2	0.2319	0.0258	0.2577
Commercial diacetyl added to skim milk and acidified with sulfuric acid	1	Ac_2	0.0406	none	0.0406
		Residue + $FeCl_3$	none	none	
		Amc + ac_2	0.0375	none	0.0375
Aqueous solution of commercial diacetyl	1	Ac_2	0.1419	0.0024	0.1443
		Residue + $FeCl_3$	none	none	
		Amc + ac_2	0.0969	0.0012	0.0981

[1] Ac_2 = diacetyl.

[2] Amc = acetylmethylcarbinol.

[3] Commercial acetylmethylcarbinol was washed with cold anhydrous ether (6).

alone. Runs made on butter cultures or pure cultures of citric acid fermenting streptococci or solutions of commercial acetylmethylcarbinol consistently show that larger quantities of carbinol were recovered from the filtrates of nickel dimethylglyoximate equivalent to diacetyl than from that of acetylmethylcarbinol plus diacetyl. The carbinol present

in the distillate may cause some error in the determination of diacetyl unless air is excluded. When nickel dimethylglyoximate is filtered into a crucible by suction, air mixes well with the filtrate and when the latter is held for some time the acetylmethylcarbinol is oxidized slowly to diacetyl which reacts with the excess reagents and forms new crystals of nickel dimethylglyoximate.

The sum of nickel dimethylglyoximate obtained from (1) diacetyl determinations and its filtrate, (2) the residue from (1) to which ferric chloride had been added and its filtrate was always less than the sum of nickel dimethylglyoximate obtained from the regular determination of acetylmethylcarbinol plus diacetyl and its filtrate on aliquot samples of material containing both the carbinol and diacetyl. This was particularly true with the cultures and also with the solutions of commercial acetylmethylcarbinol used in table 2. This situation was reversed, however, when commercial diacetyl solutions were used. Table 2 also shows that when ferric chloride was added to a solution of commercial diacetyl and steam distilled, the nickel dimethylglyoximate obtained was considerably less than that obtained when no ferric chloride was used. Apparently the ferric chloride destroyed some of the diacetyl during the distillation. It is therefore probable that ferric chloride also destroyed some of the diacetyl formed during the determination of acetylmethylcarbinol plus diacetyl and likewise during the recovery of the carbinol from the filtrates. When attempts were made to recover commercial diacetyl as nickel dimethylglyoximate, traces of acetylmethylcarbinol also were consistently recovered (not all data are presented) in the filtrates. This suggests the possibility that a small amount of carbinol may exist as a result of chemical equilibrium between the carbinol and diacetyl, or that traces of the carbinol exist as an impurity with the commercial diacetyl.

Some attempts to recover commercial acetylmethylcarbinol as nickel

TABLE 3. *Commercial acetylmethylcarbinol recovered as nickel dimethylglyoximate in distillates and filtrates*

Grams amc[1] used	Grams amc found in distillate	Percentage recovered	Grams amc found in filtrate	Percentage in filtrate	Total percentage recovered
0.2020	0.1706	84.45	0.0097	4.80	89.25
0.2020	0.1627	80.54	0.0167	8.26	88.80
0.2020	0.1654	81.88	0.0162	8.02	89.90
0.1029	0.0844	82.02	0.0057	5.54	87.56
0.2018	0.1654	81.96	0.0163	8.08	90.04
0.0943	0.0804	85.25	0.0064	6.78	92.03

[1] Amc = acetylmethylcarbinol.

dimethylglyoximate (6) have been successful to the extent of about 84 percent only. This percentage recovery was materially increased as is indicated in table 3.

In order to obtain the acetylmethylcarbinol in a pure state, the commercial product was washed with cold anhydrous ether (6). This purified carbinol was weighed quantitatively and recovered as nickel dimethylglyoximate by steam distillation and then calculated in terms of grams of acetylmethylcarbinol recovered.

Some of the determinations in table 3 were carried out by holding the material in the distillation flask at about the boiling point for 5 to 10 minutes and then distilling by steam.

The percentage carbinol recovered in the distillates in table 3 ranged from 80.54 to 85.25, while that in the filtrates ranged from 4.80 to 8.26. The total carbinol recovered ranged from 87.56 to 92.03 percent.

In table 4 are given the results of some attempts to oxidize more completely the acetylmethylcarbinol to diacetyl in the distillation flask, thus preventing the carbinol from passing over into the distillate, which in turn would eliminate the additional step of recovering any carbinol from the filtrate of nickel dimethylglyoximate.

A stock solution of acetylmethylcarbinol in distilled water was prepared. To 200 ml. portions of the carbinol solution in the distillation flasks, 40 ml. ferric chloride were added, the flasks were stoppered, held at room temperature for various periods and the determinations were made. The data in table 4 indicate that the holding of this mixture at room temperature from ½ to 75 hours had no significant effect on the total recovery of the carbinol.

Assuming that the oxidation of acetylmethylcarbinol by ferric chloride is more intense at higher temperatures, some trials were undertaken under various conditions. The temperature of the material in the distillation flask was raised to 95°-99° C. with steam and held there either by letting in steam occasionally or by keeping a small flame under the flask.

The results obtained in table 4 indicate that holding the material at 95°-99° C. for 5 to 10 minutes seemed to give the maximum yield of the carbinol in the distillate. Refluxing acetylmethylcarbinol solutions for 5 minutes and a skim milk culture of a citric acid fermenting Streptococcus for 5 to 10 minutes also seemed to yield the highest recovery of the carbinol in the distillate. However, as the period of holding the material at 95°-99° C. or refluxing it was further increased the recovery of the carbinol from the filtrates decreased and the total amount of nickel dimethylglyoximate equivalent to acetylmethylcarbinol decreased also. This probably indicates that the longer some of the diacetyl, formed from the oxidation of the carbinol by ferric chloride in the distillation flask, is held at this range of temperature, the more will be destroyed.

Holding the material at 90°-95° C. for 20 minutes gave essentially the same result as holding it at 95°-99° C. for 5 minutes. Similar results

TABLE 4. *Attempts to affect the complete oxidation of acetylmethylcarbinol to diacetyl*

Material used	Material treated prior to distillation	Grams nickel dimethylglyoximate ⇌ acetylmethylcarbinol: Recovered from distillate	Recovered from filtrate	Total	Percentage recovered from filtrate
Aqueous solution of purified commercial acetylmethylcarbinol	200 ml. + 40 ml. $FeCl_3$	0.2319	0.0258	0.2577	10.01
	" " " " " " held at R. T.[1] for 30 min.	0.2375	0.0265	0.2640	10.04
	" " " " " " held at R. T. for 60 min.	0.2374	0.0254	0.2628	9.66
	" " " " " " held at 95-99° C. for 5 min.	0.2409	0.0214	0.2623	8.16
	" " " " " " held at 95-99° C. for 10 min.	0.2447	0.0155	0.2602	5.95
	" " " " " " distilled without steam	0.2472	0.0114	0.2586	4.41
Aqueous solution of commercial acetylmethylcarbinol	200 ml. + 40 ml. $FeCl_3$	0.1909	0.0204	0.2113	9.65
	" " " " " " held at 95-99° C. for 5 min.	0.2085	0.0104	0.2189	4.75
	" " " " " " held at 95-99° C. for 10 min.	0.1989	0.0109	0.2098	5.19
	" " " " " " held at 95-99° C. for 30 min.	0.1912	0.0048	0.1960	2.42
	" " " " " " refluxed 5 min.	0.2127	0.0079	0.2206	3.58
	" " " " " " refluxed 10 min.	0.1994	0.0034	0.2028	1.67
	" " " " " " refluxed 20 min.	0.1906	0.0031	0.1937	1.60
	" " " " " " refluxed 30 min.	0.1717	0.0002	0.1717	.12
Aqueous solution of purified commercial acetylmethylcarbinol	200 ml. + 40 ml. $FeCl_3$	0.1764	0.0213	0.1977	10.77
	" " " " " " held at R. T. for ½ hr.	0.1769	0.0192	0.1961	9.79
	" " " " " " held at R. T. for 1 hr.	0.1797	0.0169	0.1966	8.59
	" " " " " " held at R. T. for 2 hrs.	0.1759	0.0212	0.1971	10.75
	" " " " " " held at R. T. for 8 hrs.	0.1762	0.0207	0.1969	10.51
	" " " " " " held at R. T. for 24 hrs.	0.1769	0.0189	0.1958	9.65
	" " " " " " held at R. T. for 72 hrs.	0.1792	0.0097	0.1889	5.13
	" " " " " " distilled at a very slow rate	0.1864	0.0028	0.1892	1.48
	" " " " " " refluxed 2 min.	0.1778	0.0189	0.1967	9.61
	" " " " " " refluxed 5 min.	0.1995	0.0064	0.2059	3.11
	" " " " " " held at 95-99° C. for 5 min.	0.1853	0.0158	0.2011	7.86
	" " " " " " held at 90-95° C. for 5 min.	0.1801	0.0169	0.1970	8.58
	" " " " " " held at 90-95° C. for 10 min.	0.1820	0.0129	0.1949	6.62
	" " " " " " held at 90-95° C. for 20 min.	0.1886	0.0077	0.1963	3.92

TABLE 4. Continued

Material used	Material treated prior to distillation	Grams nickel dimethylglyoxime ≎ acetylmethylcarbinol			
		Recovered from		Total	Percentage recovered from filtrate
		distillate	filtrate		
Aqueous solution of purified commercial acetylmethylcarbinol	200 ml. + 40 ml. $FeCl_3$	0.2554	0.0321	0.2875	11.16
	" " " " " " held at 95-99° C. for 5 min.	0.2711	0.0266	0.2977	8.94
	150 ml. + 50 ml. water + 40 ml. $FeCl_3$	0.1936	0.0231	0.2167	10.66
	100 ml. + 100 ml. " " " " "	0.1289	0.0153	0.1442	10.61
	50 ml. + 150 ml. " " " " "	0.0638	0.0061	0.0699	8.73
	25 ml. + 175 ml. " " " " "	0.0311	0.0041	0.0352	11.65
	25 ml. + 175 ml. " " " " "	0.0340	0.0036	0.0376	9.57
Skim milk culture of a citric acid fermenting Streptococcus to which 0.85 percent citric acid had been added	200 gm. + 40 ml. $FeCl_3$	0.1849	0.0212	0.2061	10.29
	" " " " " " distilled at a slow rate	0.2012	0.0174	0.2186	7.96
	" " " " " " distilled at a slow rate	0.1961	0.0110	0.2071	5.31
	" " " " " " held at 95-99° C. for 5 min.	0.1915	0.0148	0.2063	7.17
	" " " " " " held at 95-99° C. for 10 min.	0.1933	0.0113	0.2046	5.52
	" " " " " " refluxed for 5 min.	0.1914	0.0223	0.2137	10.44
	" " " " " " refluxed for 10 min.	0.1907	0.0135	0.2042	6.61
	" " " " " " refluxed for 30 min.	0.1719	0.0041	0.1760	2.33
	200 gm. + 60 ml. $FeCl_3$	0.1968	0.0160	0.2128	7.52
	" " " " " " held at 95-99° C. for 5 min.	0.2005	0.0104	0.2109	4.93
	200 gm. + 80 ml. $FeCl_3$	0.1976	0.0173	0.2149	8.05

[1] Room temperature.

TABLE 5. *The effect of hydrogen ion concentration on the oxidation of acetylmethylcarbinol to diacetyl during the distillation*

Material used	Material treated prior to distillation	Grams nickel dimethylglyoxime ≎ acetylmethylcarbinol			
		Recovered from		Total	Percentage recovered from filtrate
		distillate	filtrate		
Aqueous solution of purified acetylmethyl-carbinol	200 ml. + 40 ml. $FeCl_3$	0.1764	0.0213	0.1977	10.77
	200 ml. + 40 ml. $FeCl_3$	0.1769	0.0192	0.1961	9.79
	200 ml. + 0.5 ml. conc. H_2SO_4 + 40 ml. $FeCl_3$	0.1865	0.0158	0.2023	7.81
	200 ml. + 0.5 ml. conc. H_2SO_4 + 40 ml. $FeCl_3$	0.1885	0.0110	0.1995	5.51
Aqueous solution of purified acetylmethyl-carbinol + 0.04% K_2HPO_4. pH 7.80	200 ml. + 40 ml. $FeCl_3$	0.2622	0.0195	0.2817	6.92
	200 ml. + 0.05 ml. conc. H_2SO_4, pH 2.21, + 40 ml. $FeCl_3$	0.2740	0.0191	0.2931	6.52
	200 ml. + 0.10 ml. conc. H_2SO_4, pH 1.90, + 40 ml. $FeCl_3$	0.2749	0.0213	0.2962	7.19
	200 ml. + 0.25 ml. conc. H_2SO_4, pH 1.56, + 40 ml. $FeCl_3$	0.2779	0.0163	0.2942	5.54
	200 ml. + 0.5 ml. conc. H_2SO_4, pH 1.32, + 40 ml. $FeCl_3$	0.2796	0.0159	0.2955	5.38
	200 ml. + 0.75 ml. conc. H_2SO_4, pH 1.14, + 40 ml. $FeCl_3$	0.2805	0.0228	0.3033	7.52
	200 ml. + 1.0 ml. 5n. NaOH, pH 9.60, + 40 ml. $FeCl_3$	0.2590	0.0266	0.2856	9.31
Skim milk culture of a citric acid fermenting Streptococcus to which 0.85% citric acid had been added. pH 4.45	200 gm. + 40 ml. $FeCl_3$	0.1980	0.0271	0.2251	12.04
	200 gm. + 0.5 ml. conc. H_2SO_4, pH 3.36, + 40 ml. $FeCl_3$	0.1746	0.0306	0.2052	14.91
	200 gm. + 0.5 ml. conc. H_2SO_4, pH 3.36, + 40 ml. $FeCl_3$	0.1740	0.0318	0.2058	15.45
	200 gm. + 0.5 ml. conc. H_2SO_4, pH 3.36 + 60 ml. $FeCl_3$	0.1987	0.0198	0.2185	9.06
	200 gm. + 1.0 ml. conc. H_2SO_4, pH 2.29, + 40 ml. $FeCl_3$	0.1576	0.0318	0.1894	16.79
	200 gm. + 2.0 ml. conc. H_2SO_4, pH 1.12, + 40 ml. $FeCl_3$	0.1585	0.0343	0.1928	17.79
	200 gm. + 5.0 ml. conc. H_2SO_4, + 40 ml. $FeCl_3$	0.1520	0.0344	0.1864	18.45
	200 gm. + 1.0 ml. 5n. NaOH, pH 4.91, + 40 ml. $FeCl_3$	0.2011	0.0180	0.2191	8.22
	200 gm. + 3.5 ml. 5n. NaOH, pH 6.52 + 40 ml. $FeCl_3$	0.1953	0.0197	0.2150	9.16
	200 gm. + 5.0 ml. 5n. NaOH, pH 9.20, + 40 ml. $FeCl_3$	0.2003	0.0136	0.2139	6.36

were obtained when the distillation was carried out at a relatively slow rate (about 80 to 100 minutes as compared with 30 to 50 minutes for the regular steam distillation) and also when the material was distilled without steam.

The use of smaller samples of the acetylmethylcarbinol solution did not reduce the recovery of the carbinol in the filtrates.

The addition of 60 or 80 ml. of ferric chloride to 200 gm. of skim milk culture of a citric acid fermenting Streptococcus increased the recovery of the carbinol in the distillate as compared with that of 40 ml. This increase, however, was not essentially greater than when 40 ml. were used and distilled slowly or when it was held at 95°-99° C. for 5 minutes and then distilled in the usual way. When 60 ml. of ferric chloride were added and the mixture held at 95°-99° C. for 5 minutes, the recovery of the carbinol in the distillate and the total recovery was about the same as when 40 ml. were added and it was distilled slowly or refluxed for 5 to 10 minutes and then distilled in the usual way.

The use of various amounts of acids, to facilitate a more complete oxidation of the carbinol in the distillation flask, looked encouraging with commercial acetylmethylcarbinol solutions. The results obtained from the trials on the above solution and on a skim milk culture of a citric acid fermenting Streptococcus are given in table 5.

A solution of commercial acetylmethylcarbinol with distilled water was adjusted to pH 7.80 by adding 0.04 percent K_2HPO_4. Portions from this adjusted carbinol solution were taken out, various amounts of concentrated (c.p.) H_2SO_4, or 5n. NaOH added and pH determinations made. To 200 ml. of this solution (after the pH had been adjusted) 40 ml. of ferric chloride were added and the mixture steam distilled.

From a lot of skim milk culture of a citric acid fermenting Streptococcus (0.85 percent citric acid had been added) with a pH 4.45, portions were taken out and treated as above. To 200 gm. of the treated culture 40 ml. ferric chloride were added and the mixture steam distilled.

The additions of 0.05 ml. up to 0.75 ml. of concentrated sulfuric acid to the commercial acetylmethylcarbinol solution brought about a gradual increase in the recovery of the carbinol in the distillate as is evident in table 5.

The percentage recovery of the carbinol in the filtrates apparently was not influenced by the pH of the solution.

The addition of sulfuric acid to a skim milk culture of a citric acid fermenting Streptococcus tended to decrease the recovery of the carbinol in the distillates and increase it in the filtrates.

When 0.5 ml. of concentrated sulfuric acid and 60 ml. of ferric chloride were added to the culture, the recovery of the carbinol in the filtrate decreased but the total recovery was less than that obtained when 40 ml. of ferric chloride alone were used.

TABLE 6. *Attempts to increase the recovery of acetylmethylcarbinol by aeration*

Material used	Material treated prior to distillation	Grams nickel dimethylglyoximate ≎ acetylmethylcarbinol			
		Recovered from distillate	Recovered from filtrate	Total	Percentage recovered from filtrate
Aqueous solution of purified acetylmethyl-carbinol	200 ml. + 40 ml. $FeCl_3$	0.2677	0.0247	0.2924	8.45
	" " " " " " aerated	0.2683	0.0208	0.2891	7.19
	" " " " " " aerated	0.2890	0.0123	0.3013	4.08
	" " " " " " held at 95-99° C. for 5 min., aerated	0.2933	0.0092	0.3025	3.04
Skim milk culture of a citric acid fermenting Streptococcus to which 0.85% citric acid had been added	200 gm. + 40 ml. $FeCl_3$	0.1849	0.0212	0.2061	10.29
	" " " " " " aerated	0.1890	0.0220	0.2110	10.43
	" " " " " " aerated	0.1784	0.0307	0.2091	14.68
	" " " " " " aerated during slow rate distillation	0.2066	0.0081	0.2147	3.77

The recovery of the carbinol in the distillate decreased and in the filtrate increased with the addition of 1.0 ml. 5n. NaOH to the carbinol solution (pH 9.6). With the skim milk culture of a citric acid fermenting Streptococcus the addition of 5n. NaOH in amounts of 1.0 ml., 3.5 ml. or 5.0 ml. gave results about the same as the control.

It seems probable that the destruction of some of the diacetyl is one of the factors in preventing the complete recovery of the carbinol in cultures or in solutions of commercial acetylmethylcarbinol.

Some attempts to accelerate the removal of diacetyl formed in the distillation flask by aeration are reported in table 6. The receiving flask containing the mixture of hydroxylamine hydrochloride and sodium acetate was immersed in ice water. By means of a pump, attached to the receiving flask, a slow stream of air was bubbled into the distillation flask and passed through the system during the entire period of steam distillation.

The aeration definitely increased the recovery of the carbinol in the distillate in one of the trials in which a solution of commercial acetylmethylcarbinol was employed, but the duplicate run of the same solution was about the same as the control. The holding of the same carbinol solution at 95°-99° C. for 5 minutes and then aerating during the steam distillation significantly increased the recovery of the carbinol in the distillate. The aeration, however, did not seem to have any appreciable effect on the recovery of the carbinol in the distillate when a skim milk culture of a citric acid fermenting Streptococcus was used. The aeration during a slow distillation of this culture increased the recovery of the carbinol in the distillate. Similar increases with the same culture without aeration were reported in table 4.

It was previously mentioned that when 60 or 80 ml. of ferric chloride were used during the distillation there was some increase in the recovery of the carbinol in the distillate. This led to studies to determine how much acetylmethylcarbinol could be oxidized by 40 ml. or more of ferric chloride solution (40 gm. made up 100 ml. with distilled water). The results are given in table 7.

When to 200 ml. of a relatively strong solution of commercial acetylmethylcarbinol 40 ml. ferric chloride were added, 576 mg. of nickel dimethylglyoximate equivalent to acetylmethylcarbinol were obtained in the distillate. When 60, 100 or 120 ml. of ferric chloride were used, the recovery of the carbinol in the distillates increased accordingly, while the percentage recovery in the filtrates decreased. However, when 200 ml. of ferric chloride were used, the recovery in the distillate decreased, the recovery in the filtrate was the lowest and the total recovery was lower than when 120 ml. of ferric chloride were used. Again it seems probable that this decrease is caused by the destruction of some diacetyl formed from the oxidation of the carbinol by the ferric chloride in the distillation flask.

TABLE 7. *The effects of various amounts of ferric chloride on the recovery of acetylmethylcarbinol*

Ml. ferric chloride used	Grams nickel dimethylglyoximate ÷ acetylmethylcarbinol			
	Recovered from		Total	Percentage recovered from filtrate
	distillate	filtrate		
40	0.5760	0.0725	0.6485	11.18
60	0.6215	0.0561	0.6776	8.28
100	0.6444	0.0430	0.6874	6.25
120	0.6601	0.0336	0.6937	4.84
200	0.6539	0.0254	0.6793	3.74

The attempts, discussed so far, to oxidize acetylmethylcarbinol completely to diacetyl in the distillation flask and thus eliminate the recovery of carbinol from the filtrate were only partly successful. Holding the material in the distillation flask at 95°-99° C. for 5 to 10 minutes or refluxing it for about 5 minutes seemed to yield the best recovery of the carbinol in the distillate; however, a small amount of the carbinol regularly passed over into the distillate. When the period for this type of treatment was prolonged, the recovery in the distillate tended to decrease because of a possible destruction of some of the diacetyl.

Other attempts to prevent the carbinol from passing over into the distillate were: (1) Using various oxidizing reagents along with ferric chloride, (2) increasing or decreasing the concentration of the latter compound, (3) using superoxal in the distillate, and (4) using various devises such as a fractionating column with or without glass beads to hold back the unoxidized carbinol. None of these attempts were successful.

The data shown in table 8 suggest the possibility that a correction factor may be used in place of recovering acetylmethylcarbinol from the filtrate. The percentage carbinol recovered from the filtrates of the regular steam distillations (table 8) ranged from 7.36 to 12.04 and averaged 9.66. In the majority of trials the recovery ranged between 9 and 11 percent. These percentage recoveries of the carbinol from the filtrates may vary with the conditions under which the distillations are performed. The percentage recoveries of the carbinol from the filtrates, therefore, ought to be determined by making several runs and then the average recovery applied as a correction factor for other determinations.

As previously mentioned, with regular steam distillations about 80 to 84 percent of the commercial acetylmethylcarbinol could be recovered as nickel dimethylglyoximate in the distillates, and about 9 to 11 percent in the filtrates. However, there still remains some carbinol that cannot be accounted for but which probably is oxidized to diacetyl and then destroyed during the distillation. Data supporting this assumption are given in table 9.

TABLE 8. *Percentage acetylmethylcarbinol recovered from filtrates of regular steam distillations*

Material used	Trial number	Grams nickel dimethylglyoximate ⇔ acetylmethylcarbinol + diacetyl			
		Recovered from		Total	Percentage recovered from filtrate
		distillate	filtrate		
Aqueous solution of purified acetylmethylcarbinol	1	0.2319	0.0258	0.2577	10.01
	2	0.2375	0.0265	0.2640	10.04
	3	0.2374	0.0254	0.2628	9.66
Aqueous solution of commercial acetylmethylcarbinol	1	0.1909	0.0204	0.2113	9.65
Aqueous solution of purified acetylmethylcarbinol	1	0.1764	0.0213	0.1977	10.77
	2	0.1769	0.0192	0.1961	9.79
	3	0.1797	0.0169	0.1966	8.59
	4	0.1759	0.0212	0.1971	10.75
	5	0.1762	0.0207	0.1969	10.51
	6	0.1769	0.0189	0.1958	9.65
Butter culture made from skim milk	1	0.0669	0.0059	0.0728	8.10
	2	0.0680	0.0054	0.0734	7.36
Three butter cultures made from skim milk to which 0.15% citric acid had been added	1	0.1154	0.0128	0.1282	9.98
	2	0.1003	0.0106	0.1109	9.56
	3	0.0975	0.0079	0.1054	7.49
Skim milk cultures of citric acid fermenting streptococci to which about 0.85% citric acid had been added	1	0.2708	0.0260	0.2968	8.76
	2	0.1980	0.0271	0.2251	12.04
	3	0.1953	0.0197	0.2150	9.16
	4	0.0591	0.0073	0.0664	10.99
	5	0.1849	0.0212	0.2061	10.29
				Average	9.66

TABLE 9. *Destruction of diacetyl by ferric chloride during steam distillation*

Material used	Lot no.	Material treated prior to distillation	Grams Ni dimethylglyoximate ≙ diacetyl	Percentage destroyed
Aqueous solutions of commercial diacetyl	1	200 ml. + no $FeCl_3$	0.1419	0.00
		200 ml. + 40 ml. $FeCl_3$	0.0969	31.71
		200 ml. + 40 ml. $FeCl_3$, held at 95-99° C. for 15 min.	0.0883	37.77
	2	200 ml. + no $FeCl_3$	0.1589	0.00
		" " " " "	0.1586	0.00
		" " " " " held at 95-99° C. for 30 min.	0.1446	8.99
		200 ml. + 40 ml. $FeCl_3$	0.1055	33.61
		" " " " " "	0.1065	32.98
		" " " " " " held at 95-99° C. for 30 min.	0.0974	38.70
		200 ml. + 80 ml. $FeCl_3$	0.1033	34.99
		200 ml. + 40 ml. $FeCl_3$, refluxed for 20 min.	0.0902	43.24
	3	200 ml. + no $FeCl_3$	0.0322	0.00
		200 ml. + 40 ml. $FeCl_3$	0.0181	43.78
		" " " " " "	0.0178	44.72
	4	200 ml. + no $FeCl_3$	0.1421	0.00
		200 ml. + 40 ml. $FeCl_3$	0.0936	34.13
	5	200 ml. + no $FeCl_3$	0.1930	0.00
		200 ml. + 40 ml. FeC_3	0.1215	37.04
		" " " " " "	0.1202	37.72
Commercial diacetyl added to skim milk	1	200 gm. + no $FeCl_3$	0.0291	0.00
		" " " " "	0.0298	0.00
		200 gm. + 40 ml. $FeCl_3$	0.0168	43.62
		" " " " " "	0.0165	44.63
Commercial diacetyl added to skim milk and acidified with sulfuric acid	1	200 gm. + no $FeCl_3$	0.3351	0.00
		200 gm. + 40 ml. $FeCl_3$	0.2960	11.66
	2	200 gm. + no $FeCl_3$	0.0431	0.00
		200 gm. + 40 ml. $FeCl_3$	0.0375	12.99

The addition of 40 ml. of ferric chloride to aqueous solutions of commercial diacetyl caused the destruction, of the latter compound, which ranged from 31.71 to 44.72 percent with regular steam distillations. Holding this mixture at 95°-99° C. for 15 or 30 minutes or refluxing 20 minutes increased the percentage destruction to some extent. However, when a diacetyl solution without ferric chloride was held at 95°-99° C. for 30 minutes, there was also a reduction of 8.99 percent in the recovery of diacetyl.

When diacetyl was added to skim milk and steam distilled, the destruction by ferric chloride was about the same as in aqueous solutions. This destruction was only 11.66 and 12.99 percent in two separate trials with acidified mixtures of skim milk and diacetyl.

While a large percentage of commercial diacetyl is destroyed by ferric chloride during steam distillation, it is probable that the extent of this destruction is much less during the oxidation of acetylmethylcarbinol to diacetyl by the ferric chloride.

Attempts to determine the products formed from the destruction of diacetyl by ferric chloride are given in table 10.

Data in table 10 indicate that while small amounts of volatile acids were obtained from the steam distillation of diacetyl solution alone, these amounts were significantly increased when 40 ml. ferric chloride were added to the same solution. Refluxing the latter mixture for 30 minutes, before steam distillation, gave volatile acid values which were higher than those obtained by regular steam distillation. The addition of 1 ml. of concentrated sulfuric acid to diacetyl solutions with or without 40 ml. of ferric chloride gave volatile acid values which were higher than the controls. These values were greater when 40 ml. of ferric chloride were used along with 1 ml. of sulfuric acid. The addition of 1 ml. of 5n. NaOH along with 40 ml. of ferric chloride to the diacetyl solution also increased the volatile acidity. When 4 ml. of concentrated sulfuric acid were added to the residues from various volatile acid determinations of the diacetyl solution, volatile acid values were obtained which were higher than those obtained when the same amount of sulfuric acid was added to boiled distilled water and steam distilled. The values obtained from various other volatile acid determinations on boiled distilled water were insignificant as compared with those obtained from diacetyl solutions under identical conditions.

Trials to determine the presence of carbon dioxide, due to complete oxidation of diacetyl, were negative. It seems probable that volatile acids were the chief products formed from the destruction of diacetyl by ferric chloride; therefore, attempts were made to determine the nature of the volatile acids formed.

To a strong aqueous solution of diacetyl some ferric chloride was added and steam distilled. Other trials, such as refluxing the above mixture for 30 minutes or adding concentrated sulfuric acid or 5n. sodium

TABLE 10. *Volatile acid formed from the destruction of diacetyl by ferric chloride*

Material used	Trial no.	Material treated prior to distillation	Volatile acid[1] Detn. 1	Volatile acid[1] Detn. 2
Aqueous solution of commercial diacetyl	1	No $FeCl_3$	5.1	3.0
	2	No $FeCl_3$	4.6	
		Residue + 4 ml. conc. H_2SO_4	6.6	
	3	No $FeCl_3$, refluxed 30 min.	3.6	6.6
		Residue + 4 ml. conc. H_2SO_4	8.9	6.6
	4	40 ml. $FeCl_3$	13.5	14.6
		Residue + 4 ml. conc. H_2SO_4		11.6
	5	40 ml. $FeCl_3$, refluxed 30 min.	17.1	23.9
		Residue + 4 ml. conc. H_2SO_4		11.7
	6	No $FeCl_3$ + 1 ml. conc. H_2SO_4	10.4	7.4
	7	40 ml. $FeCl_3$ + 1 ml. conc. H_2SO_4	17.3	13.1
	8	40 ml. $FeCl_3$ + 1 ml. 5n. NaOH	19.3	20.0
Boiled, distilled water	1	No $FeCl_3$	1.2	
		Residue + 4 ml. conc. H_2SO_4	1.2	
	2	No $FeCl_3$, refluxed 30 min.	1.5	
		Residue + 4 ml. conc. H_2SO_4	1.6	
	3	40 ml. $FeCl_3$	2.1	
		Residue + 4 ml. conc. H_2SO_4	2.0	
	4	40 ml. $FeCl_3$, refluxed 30 min.	3.3	
		Residue + 4 ml. conc. H_2SO_4	1.8	
	5	No $FeCl_3$ + 4 ml. conc. H_2SO_4	1.8	
	6	No $FeCl_3$ + 1 ml. 5n. NaOH	0.8	

[1] Milliliters n/20 NaOH required to neutralize the acid in 200 ml. distillate.

hydroxide along with ferric chloride and then steam distilling, were undertaken also.

In four different trials it was possible to obtain enough volatile acids to make four different identification tests by the partition method (4). In all four trials the only volatile acid identified was acetic acid, and the tests for formic acid were negative.

SUMMARY

1. During the determination of diacetyl, in a material containing both diacetyl and acetylmethylcarbinol, the carbinol also distilled over with the diacetyl. This caused erroneous results, because of the oxidation of the carbinol to diacetyl, unless air was excluded from the distillate.

2. During the determination of acetylmethylcarbinol plus diacetyl some of the unoxidized carbinol also passed over into the distillate with the diacetyl. This carbinol which was recovered from the filtrates of the regular determinations ranged from 7.36 to 12.04 and averaged 9.66 percent in 20 trials.

3. The percentage recovery of commercial acetylmethylcarbinol as nickel dimethylglyoximate ranged from 80.54 to 85.35 in the distillates and 4.80 to 8.26 in the filtrates. The total carbinol recovered ranged from 87.56 to 92.03 percent.

4. Attempts to bring about the complete oxidation of acetylmethylcarbinol to diacetyl, and thus to eliminate the necessity of recovering the carbinol from the filtrates, were only partly successful.

5. The data obtained support the assumption that some of the diacetyl, formed from the oxidation of acetylmethylcarbinol by ferric chloride, was destroyed during distillation.

6. The percentage destruction of the commercial diacetyl by ferric chloride ranged from 31.71 to 44.72 with regular steam distillations in 16 trials.

7. Volatile acid seemed to be the chief product formed from the destruction of commercial diacetyl by ferric chloride. Acetic acid was present, but tests for formic acid were negative.

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A Study of the Distribution of Strains of *Streptococcus lactis* Which Are Sensitive to a Filterable Inhibitory Principle from Slow Starters[1]

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BUTTER cultures which undergo a sudden loss of vitality and require excessive periods of time to ripen to the desired degree of acidity are encountered rather frequently in dairy plants, especially where considerable quantities of culture are ripened in large containers. The longer ripening period which is necessary under these conditions is associated commonly with a decrease in desirable flavor and aroma and with characteristic off-flavors. Butter cultures of this type are usually designated as "slow" cultures and may be the cause of considerable difficulty in the manufacture of butter, cheese and cultured milks. Some butter cultures are more subject than others to this type of retarded acid development. This study was undertaken in an effort to determine the distribution of types of *Streptococcus lactis* sensitive to the inhibitory action of bacteria-free filtrates from slow butter cultures.

HISTORICAL

In 1933 the Iowa Agricultural Experiment Station (1) reported that a bacteria-free filtrate from a slow butter culture would delay the coagulation of a normal one, resulting in a characteristic slow culture. The restraining action was found to be destroyed by boiling the filtrate.

Harriman (3) made an exhaustive study of the suddenly-appearing type of slow butter culture and found that the growth of some freshly inoculated butter cultures could be markedly restrained by the addition, at the time of inoculation, of certain other cultures, especially those which

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had previously shown slow acid development or had been grown in large lots. By preliminary filtration of the coagulated cultures through filter paper, followed by filtration through grade N Berkfeld ultrafilters, bacteria-free filtrates were obtained from some of the cultures which showed the inhibitory effect when added to other cultures. Using seven butter cultures and one culture of *S. lactis* as test organisms, 11 of the 19 filtrates from mother cultures and 22 of the 23 filtrates from large lots of butter culture were found to have a marked inhibitory effect on the acid production of one or more of the test cultures after a 16-hour period of incubation at 21° C. These filtrates were shown to cause a marked inhibition of growth, the number of organisms in the cultures at the 16-hour interval being markedly less than the number in the check cultures to which no filtrate had been added. The ratio of citric acid-fermenting streptococci to *S. lactis* frequently was unusually high in the retarded cultures. The surviving *S. lactis* strains were apparently normal in their ability to grow and coagulate milk, the result being that at 24 hours the acidities and counts of the retarded starters approached those of the control cultures. Using pure *S. lactis* cultures and inhibitory filtrates, counts under 500,000 per ml. were obtained frequently at the 16-hour interval, when the check cultures without added filtrate gave counts ranging from 219,000,000 to 1,750,000,000 per ml. Since the filtrates varied in their action toward the butter cultures and the *S. lactis* culture used as test organisms, Harriman (3) suggested that possibly different filtrates had varying degrees of restraining ability and that the ability to resist the action of filtrates varied among cultures, the inference being that resistance was a quantitative rather than a qualitative characteristic. Studies of the filtrates themselves showed that some of them had a marked restraining effect in dilutions as great as 1 part of filtrate in 20,000 parts of milk. The inhibitory principle was found to be completely inactivated by heating to 60° C. for five minutes in all but one case, in which 30 minutes were required. Holding at 50° C. for 30 minutes resulted in a decrease in the "virulence" of the filtrate but not in complete inactivation. Attempts to increase the titers of the filtrates by adding them to butter cultures and recovering them following coagulation were unsuccessful. The active principle apparently was closely related to a bacteriophage, but this investigator did not designate it as a bacteriophage.

Whitehead and Cox (6) succeeded in isolating from a slow starter a bacteriophage active against *Streptococcus cremoris* and considered this bacteriophage to be the causative agent of slowness in starters of the type which they were then investigating. After several propagations at the expense of a sensitive culture of *S. cremoris* the bacteriophage was obtained in such strength that one part in several hundred million parts of milk "sufficed to show the characteristic lysis with a susceptible streptococcus." Characteristic plaques were obtained on solid media. These investigators suggested that the isolation of nonsensitive strains of lactic acid strepto-

cocci for use in butter cultures might be the means of solving the problem of slow butter cultures.

The literature contains a few other references to bacteriophages active against *S. lactis* and related organisms. In 1926 Hadley and Dabney (2) reported the isolation of a bacteriophage active against *S. lactis*. They obtained their bacteriophage from sewage. Another bacteriophage from the same source was active against *Streptococcus fecalis* and was inhibitory toward *S. lactis* and toward *Bacterium coli* also. McKinley (5) mentioned *S. lactis* and *S. fecalis* among the organisms susceptible to bacteriophage activity. Klingmüller (4) was unable to isolate bacteriophages inhibitory toward *S. lactis* or *S. mastiditis* from old cultures of the organisms or from dung filtrates.

METHODS

The *S. lactis* cultures used in this study were isolated from butter cultures from various sources, from raw milk allowed to sour spontaneously and from raw-milk whey. The samples were plated on tomato juice agar and representative colonies picked and inoculated into sterile litmus milk. Unless a culture appeared as a typical *S. lactis* organism in litmus milk and coagulated the milk within 48 hours at room temperature, it was discarded.

Inhibition of the separate strains of *S. lactis* was determined by the addition of 0.1 ml. of filtrate and one drop of a 24-hour litmus milk culture to approximately 8.0 ml. of litmus milk in a test tube. Included in each series were controls from which the filtrate had been omitted. The cultures were incubated at room temperature (about 22° C.) during these experiments. When the reduction or coagulation of the litmus milk cultures to which filtrate had been added required an appreciably longer period than was necessary for the reduction and coagulation of the control cultures to which no filtrate had been added, the filtrate used was considered to be inhibitory to the test organism. Occasionally delayed reduction of the litmus was the only means of detecting inhibition, for the secondary growth was sufficiently rapid to cause coagulation to occur as rapidly in the inhibited culture as in the control. This was especially true of cultures which required more than 30 hours to coagulate under normal conditions.

When a preliminary run on a group of organisms from a single source showed no differences among the members of the group, only one or two representative organisms were used for further studies.

The filtrates used in testing any one group of organisms varied from time to time because an endeavor was made to use the greatest possible variety of filtrates for each group and the filtrate collection was constantly increasing in numbers because of new additions. The origins of the various filtrates used are indicated in table 1. These filtrates were, with three exceptions, originally isolated from butter cultures which suddenly

TABLE 1. *The sources of the bacteria-free filtrates*

Filtrate	Date obtained	Source	Remarks
1	10-19-34	B. C. 122 + 232	Slow in 10-gal. can in butter laboratory
2	10-19-34	B. C. 232	Slow in 10-gal. can in butter laboratory
10	10-25-34	B. C. 232	Slow in 140 ml. aerated sterilized milk
13	10-27-34	B. C. 15/3+F191	
14	10-27-34	B. C. 232+F1	
21	11-13-34	B. C. 103	Slow in laboratory
29	11-23-34	F10	Propagated one generation on *S. lactis* 42
30	11-23-34	F14	Propagated one generation on *S. lactis* 42
32	11-23-34	F191	Propagated one generation on *S. lactis* 42
35	12-16-34	F10	Propagated two generations on *S. lactis* 42
41	1-16-35	B. C. 15/3	Slow in 10-gal. can in butter laboratory
51	1-25-35	B. C. 15/1	Normal but gave slow transfer in laboratory
52	1-25-35	B. C. 15/1	Slow in 140 ml. past. milk in laboratory
53	1-25-35	B. C. 26	Slightly slow in 140 ml. past. milk in laboratory
54	1-25-35	B. C. 232	Slow in 140 ml. past. milk in laboratory
55	1-25-35	B. C. 15/3	Slow in 140 ml. past. milk in laboratory
61	2-13-35	B. C. 15/3	Slow in 1-gal. can of past. milk in laboratory
80	3- 9-35	F191	Propagated once on *S. lactis* 163 and once on *S. lactis* 42
82	3- 9-35	F21	Propagated once on *S. lactis* 97 and once on *S. lactis* 42
124	4-11-35	F191	Propagated once on *S. lactis* 99
134	5- 2-35	F54	Propagated once on *S. lactis* 111
135	5- 2-35	Commercial starter	Propagated once on *S. lactis* 97
157	5-31-35	F191	Propagated four times on *S. lactis* 99
158	5-31-35	F191	Propagated three times on *S. lactis* 147
159	9-26-35	B. C. 232	Slow in 400-gal. vat
191	.....	B. C. 15	Slow (filtrate from Harriman)
206	11- 5-35	F61	Propagated five times on *S. lactis* 97
207	11- 5-35	B. C. 232	Plaque isolation propagated five times on *S. lactis* 97
208	11- 5-35	F191	Propagated five times on *S. lactis* 147
209	11- 5-35	Slow starter	Bacteriophage R from Whitehead
210	11- 5-35	Slow starter	Bacteriophage RW from Whitehead
256	12-12-35	F191	Propagated once on *S. lactis* 153A
257	12-12-35	F54	Propagated once on *S. lactis* 111 and once on *S. lactis* 153-0.
315	2- 6-36	B. C. 122F	Slow in 10-gal. can in butter laboratory

B. C. = butter culture.
F = filtrate.

had become slow, either in the laboratory or in the college dairy plant where they were used for buttermaking purposes. The coagulated cultures first underwent a preliminary filtration through filter paper to remove the coagulated casein and were then filtered through grade N Berkfeld filters to remove all bacteria. The utensils used in handling the filtrates both before and after filtration were sterilized before use at 15 pounds pressure for 25 minutes in an autoclave. The filtrates were stored at approximately 6° C.

The filtrates which are designated as having been propagated on strains of *S. lactis* were obtained by the addition of a loopful of the designated sensitive *S. lactis* culture and 1.0 ml. of the designated filtrate to 100 ml. of sterile litmus milk, the interval between inoculation and addition of filtrate being as much as 12 hours in the cases of some of the more "virulent" filtrates. The cultures were held at room temperature for approximately 24 hours following the addition of the filtrate before they were filtered. Addition of lactic acid to cause coagulation before the preliminary filtration was necessary in most cases, the growth of the organisms having been sufficiently inhibited by the presence of the added filtrate to prevent the formation of appreciable amounts of lactic acid.

EXPERIMENTAL RESULTS

The results of the tests of the susceptibility of various strains of *S. lactis* to inhibition by a series of bacteria-free filtrates from slow butter cultures are summarized in table 2.

Strains of *S. lactis* isolated from milk or whey which had been allowed to sour without the addition of butter culture were never found to be susceptible to any of the filtrates with which they were tested. The filtrates tabulated are only representative of a considerably larger number which were used in the study in an effort to find one or more filtrates to which members of this group of organisms might be sensitive. Apparently naturally-occurring organisms are not susceptible to inhibition by filtrates obtained from slow butter cultures, although it is possible that filtrates which would inhibit organisms of this group could be obtained if a sufficiently large number of filtrates from a greater range of sources were examined.

Examination of the cultures of *S. lactis* obtained from butter cultures revealed that some butter cultures yielded only strains which were subject to inhibition by some of the filtrates used in this study, some yielded only nonsusceptible strains, while both sensitive and nonsensitive strains were obtained from others. The last group included the majority of the butter cultures examined in any detail during the studies herein reported.

The results obtained from a study of the organisms obtained from butter culture 146 will be discussed in some detail, as they are more or less characteristic of those obtained with the groups isolated from other butter cultures in the series. At the time of the first isolations four different sensitivity types were recognizable among the six cultures studied. One type represented by two cultures was not sensitive to any filtrate then available; a second type represented by a single culture was sensitive to filtrate 191 only; a third type represented by two cultures was sensitive to two filtrates, 13 and 191; a fourth type represented by a single culture was sensitive to filtrates 13, 52, 53 and 191 and not sensitive to filtrates, 21, 205, 206, 207, 208, 209 and 210, which formed the remainder of the test group. Seven days after the first isolations were made, 18 other cultures were obtained from the same butter culture (No. 146) which

TABLE 2. *Summary of tests to determine the susceptibility of S. lactis strains to inhibition by bacteria-free filtrates*

Source	Date of isolation	No. of cultures isolated	Filtrates which inhibit	Filtrates which do not inhibit
Raw milk whey	11- 5-34	8		10, 14, 30, 32, 35, 53, 61, 124, 134, 135, 157, 159, 191
Soured raw milk	11- 5-34	10		10, 14, 30, 32, 35, 53, 61, 124, 134, 135, 157, 159, 191
Soured raw milk P1	10- 6-35	5		13, 21, 52, 53, 61, 134, 135, 157, 158, 159, 191
Soured raw milk P5	10- 6-35	5		13, 21, 52, 53, 61, 134, 135, 157, 158, 159, 191
Soured raw milk P6	10- 6-35	6		13, 21, 52, 53, 61, 134, 135, 157, 158, 159, 191
Soured raw milk P7	10- 6-35	6		13, 21, 52, 53, 61, 134, 135, 157, 158, 159, 191
Soured raw milk P8	10- 6-35	6		13, 21, 52, 53, 61, 134, 135 157, 158, 159, 191
B. C. 146	11- 6-35	6 2		13, 21, 52, 53, 191, 205, 206, 207, 208, 209, 210
		1	191	13, 21, 52, 53, 205, 206, 207, 208, 209, 210
		2	13, 191	21, 52, 53, 205, 206, 207, 208, 209, 210
		1	13, 52, 53, 191	21, 205, 206, 207, 208, 209, 210
	11-13-35	18		35, 134, 157, 191, 192, 205, 206, 207, 208, 209, 210
	3- 4-36	13 7		35, 55, 134, 157, 191, 206, 208, 315
		4	55, 134, 315	35, 157, 191, 206, 208
		1	55, 315	35, 134, 157, 191, 206, 208
		1	55, 134, 206	35, 157, 198, 208, 315
B. C. 122F	11-13-35	15	134, 191	35, 157, 192, 205, 206, 207, 208, 209, 210
	3- 4-36	9 1	315	35, 134, 157, 191, 206, 208, 315
		3	55, 191, 208	35, 134, 157, 206, 315
		3	55, 191, 315	35, 134, 157, 206, 208
		2	55, 134, 191, 315	35, 157, 206, 208
B. C. 232	11- 8-34	9	10, 14, 30, 32, 35, 41, 53, 61, 134, 157, 191	2, 21, 54, 132, 135, 159
	11-15-34	5 4	10, 14, 30, 35, 54, 61, 134, 135, 157, 191	32, 159
		1	10, 14, 30, 54, 61, 134, 135, 159	32, 35, 157, 191
	1-14-35	3 2	29, 35, 53, 61, 134, 135, 157, 191	30, 159, 92
		1	41, 53, 61, 92, 134	29, 30, 35, 54, 135, 157, 159, 191

P = patron.

B. C. = butter culture.

TABLE 2. (Continued)

Source	Date of isolation	No. of cultures isolated		Filtrates which inhibit	Filtrates which do not inhibit
B. C. 232	12- 4-35	22	21		13, 14, 35, 41, 51, 134, 157, 191, 206, 209, 210
		1	1	41, 134, 191, 257	13, 14, 35, 51, 157, 206, 209. 210
B. C. 15/3	11-15-34	5	2	14, 32, 191	2, 10, 30
			1	10, 14, 30, 35, 41, 53, 61, 157, 191	2, 32, 159
			2	10, 32, 35, 41, 53, 61	2, 14, 30, 157, 159
	3-18-35	1		13, 53, 61, 134, 157, 191	21, 52, 135, 158, 159
	12- 3-35	25	21		13, 14, 35, 41, 51, 134, 157, 191, 206, 209, 210
			2	191, 256	13, 14, 35, 41, 51, 134, 157, 206, 209, 210, 257
			2	41, 134, 257	13, 14, 35, 51, 157, 191, 206, 209, 210, 256
	3- 4-36	5	3		35, 55, 134, 157, 191, 206, 208, 315
			2	315	35, 55, 134, 157, 191, 206, 208
B. C. A	4- 8-35	4			13, 21, 52, 53, 61, 134, 135, 157, 158, 159, 191
B. C. LOL	3- 4-36	14			35, 55, 134, 157, 191, 206, 208, 315
B. C. M_1	11-19-35	25			13, 14, 35, 41, 51, 134, 157, 191, 206, 209, 210
B. C. 103	11-13-35	25			35, 134, 157, 191, 192, 205, 206, 207, 208, 209, 210
B. C. 19/1	11-15-34	5			10, 14, 35, 41, 53, 61, 132, 134, 135, 157, 158, 159, 191
B. C. H	11-15-34	7	4		2, 10, 14, 30, 32, 52, 53, 61, 134, 135, 157, 158, 159, 191
			1	134	2, 10, 14, 30, 32, 52, 53, 61, 135, 157, 158, 159, 191
			2	10, 14, 30, 32, 53, 61, 134, 157, 191	2, 132, 135, 158, 159
B. C. S	1-14-35	4	1	13, 61, 135, 158, 191	21, 35, 51, 52, 53, 80, 82, 134, 157, 159
			1	80, 82, 191	21, 35, 52, 53, 61, 134, 157, 158, 159
			2	13, 41, 61, 80, 135, 158, 191	21, 35, 52, 53, 134, 157, 159
B. C. 233	12- 7-35	12	11		13, 14, 35, 41, 51, 134, 157, 191, 206, 209, 210
			1	13, 41, 191, 256, 257	14, 35, 51, 157, 206, 209, 210
B. C. 15/1	12-10-35	23	4		13, 14, 35, 41, 51, 134, 157, 191, 206, 209, 210
			13	13, 14, 41, 134, 157, 191, 256	35, 51, 206, 209, 210, 257

TABLE 2. (Continued)

Source	Date of isolation	No. of cultures isolated	Filtrates which inhibit	Filtrates which do not inhibit
		2	13, 41, 134, 191, 256, 257 (13 neg. for one cult.)	14, 35, 51, 157, 206, 209, 210
		4	13, 14, 134, 157, 191 (14 neg. for one cult.)	35, 41, 51, 206, 209, 210
	2- 4-36	14		35, 55, 134, 157, 191, 206, 208, 315
B. C. FL	11-25-35	26 22		13, 14, 35, 41, 134, 157, 191, 206, 209, 210
		2	41, 134, 206	13, 14, 35, 51, 157, 191, 209, 210
		2	14, 41, 134, 206, 257	13, 35, 51, 157, 191, 209, 210, 256
B. C. 22	12- 6-35	23 7		13, 14, 35, 41, 51, 134, 157, 191, 206, 209, 210
		2	13, 41, 191, 206, 256	14, 35, 51, 134, 157, 209, 210, 257
		14	41, 134, 257	13, 14, 35, 51, 157, 191, 206, 256

had been transferred daily in specially pasteurized whole milk. This second group of cultures was uninhibited by any of 11 representative filtrates, apparently being identical with the nonsensitive group obtained from the previous isolations. About four months later 13 additional cultures were obtained from the same butter culture. Seven of these cultures were uninhibited by any filtrate used; a second group of four organisms was inhibited by filtrates 55, 134 and 315 and uninhibited by filtrates 35, 157, 191, 206 and 208; a third type represented by a single culture was inhibited by filtrates 55 and 315; and a fourth type also represented by only one culture was sensitive to filtrates 55, 134 and 206. The *S. lactis* organisms in this butter culture (No. 146) were shown to change from dominantly sensitive types with only a small percentage of nonsensitive cultures to dominantly nonsensitive types with the percentage of sensitive types too small to be detected by a random picking of 18 cultures and then back to sensitive and nonsensitive types in almost equal numbers. Possibly further changes were undergone between the periods of observation. The organisms obtained at any one time showed a group similarity in that all sensitive cultures were inhibited by some one filtrate, 191 in the first group and 55 in the third group, even though their sensitivity to other filtrates varied. The organisms obtained in the first group showed no similarity in sensitivity characteristics to those in the third group, indicating that the period of nonsensitivity during which the second group of organisms was obtained was apparently a definite entity following which a new group

of sensitive organisms inhibited by other types of filtrates was developed.

The *S. lactis* strains obtained from butter cultures 15/3 and 232 were also studied quite intensively and showed a marked tendency toward variation of the sensitivity types, not only among the organisms isolated at one time but also among the organisms obtained over rather extended periods of time. Butter culture 122F is of special interest because it at one time apparently contained only *S. lactis* cultures of a single sensitivity type. This condition proved to be only transitory, however, since four different types were obtained from the next group of organisms which was isolated almost four months later. Butter cultures A, M_1, 103, LOL and 19/1 were unusual because they seemed to contain only nonsensitive organisms. Had these cultures been studied more intensively and at intervals instead of only once, sensitive strains quite possibly could have been obtained from them.

Among the butter cultures used in this study, there was no absolute relationship between the record of a culture with regard to slowness and the type of sensitive or nonsensitive organisms obtained from it. Cultures M_1, A and 103 had been typically slow at least once under laboratory conditions during the 18 months of observation and still contained no demonstrable sensitive organisms, although examination at other times or the use of other filtrates of different inhibition characteristics might have shown these cultures to contain sensitive organisms. Cultures 233, H, S and 22 had shown no tendency toward retarded acid production during the 18 months during which they were observed, but sensitive strains of *S. lactis* were obtained from them. Possibly they could become noticeably slow under some circumstances, since they apparently possessed at least part of the latent potentialities, needing only the proper supplementary conditions, which are at present unknown, to bring about considerable loss in vitality.

CONCLUSIONS

1. All of the 46 cultures of *S. lactis* isolated from spontaneously-soured raw milk and raw-milk whey were resistant to the inhibitory action of bacteria-free filtrates from slow butter cultures.

2. Of the 317 cultures of *S. lactis* obtained from 14 different butter cultures at various times, 170 were inhibited by one or more bacteria-free filtrates from slow butter cultures.

3. The cultures of *S. lactis* isolated at one time from a butter culture may be quite varied in their sensitivities to inhibitory filtrates.

4. The cultures of *S. lactis* isolated from a butter culture at different times may be entirely different in their susceptibility to inhibition by bacteria-free filtrates from slow starters

5. The range in sensitivity of the strains of *S. lactis* studied varied in an almost continuous manner from those uninhibited by any of the fil-

trates used to those sensitive to the inhibitory action of a large percentage of the filtrates against which they were tested.

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