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A STUDY OF THE ACTION OF CERTAIN BACTERIA, YEASTS AND MOLDS ON THE KEEPING QUALITY OF BUTTER IN COLD STORAGE

by

Michael Grimes

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A STUDY OF THE ACTION OF CERTAIN BACTERIA, YEASTS AND MOLDS ON THE KEEPING QUALITY OF BUTTER IN COLD STORAGE.

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M. Grimes.

The development of the practice of cold-storing butter, manufactured during the Spring and early Summer, for sale during the Winter months has resulted in much work being done to determine the factors which influence the keeping quality of butter in cold storage; for to the consumer, butter is as old as it tastes, whether it is a week old or six months old is immaterial to him, and if the butter has deteriorated in flavor it means a consequent depreciation of its commercial value.

Sayer, Rahn and Farrand 1908 (23) reviewed the literature relative to the keeping qualities of butter and Rahn and his co-workers 1909 (18) stated "It may and possibly is the type of micro-organism present that is of importance, and the enzyme or disintegration products therefrom." Rogers and co-workers 1912 (20) stated "Butter frequently undergoes marked changes, even when stored at very low temperatures. These changes are more marked as the acidity of the cream from which the butter is made increases." Brown 1915 (3) concluded that the casein in butter during storage is slowly broken down into amino acids and ammonia. Dyer 1916 (4) stated "The production of off flavors so commonly met with in cold storage butter

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A summary of work carried out in partial fulfilment of the requirements for the Degree of Doctor of Philosophy in the Faculty of Agriculture Iowa State College.

is attributable to a chemical change expressed through a slow oxidation progressing in some one or more of the non-fatty substances occurring in the butter. The extent of the chemical change is directly proportional to the quantity of acid present in the cream from which the butter was prepared." Hammer 1917 (7) reviewed the literature upon the production of fishy flavor in milk and butter and stated that Bact. ichthyosmius failed to produce fishiness when inoculated into butter. Washburn and Dahlberg 1917 (26) concluded that little if any relationship existed between the bacteria, the acidity and the score of the butter. Russell and Hastings 1920 (22) stated "In general it may be said that any and all types of organisms in the cream affect the keeping quality of the butter, to a greater or less extent, in an unfavorable manner, while the lactic bacteria seem to be the least injurious of all organisms." Hunziker 1920 (12) and Redfield and Stocking 1921 (19) agree that the quality of the cream largely governs the keeping quality of the butter in storage. Bouska and Brown 1921 (2) stated "The deterioration of butter is mainly the result of physical or biochemical causes. An indirect part may be played by microorganisms." Hammer 1921 (10) discussing butter flavors stated "When butter is held in storage it may go rancid, fishy, metallic, unclean, or any one of a number of other flavors. The temperatures usually used for the storage of butter are so low that the growth of microorganisms is very unlikely, and it is now believed that the deterioration in flavor is due to slow chemical change." Frymoffer 1922 (5) stated "One thing commonly agreed upon is that acid in cream is essential to the development of fishy flavor." As a result of work carried out at the Iowa State College, Mortensen 1922 (16) stated "Butter made from ripened

cream deteriorates faster than either butter made from sweet cream or from sweet cream and starter, but--where low ripening was employed, the ripened butter at the end of a two months cold storage period was about of the same quality as the sweet cream butter and the sweet cream and starter butter." This lack of agreement as to the cause of deterioration of butter in cold storage led to the work herein described.

STATEMENT OF THE PROBLEM.

The object of the work carried out was to determine the action of certain bacteria, yeasts and mold on the keeping quality of butter in cold storage. Bacteriological studies were made of the numbers and flora of the raw sweet cream and of the pasteurized sweet cream, and of the butter made therefrom, to seek to determine if any of these were a factor in the keeping quality of the butter in cold storage, and also serve as a check upon the other experiments. To determine the influence of the acidity of the cream the pasteurized sweet cream was divided into several portions and starter added and churning experiments made with cream of different acidities, both with and without the addition of various microorganisms. As the enzymes in the cream were not destroyed at the pasteurization temperature of 145° F. for 25 minutes it was sought to determine if the enzymes produced or other disintegration products of the bacteria were a factor in the keeping quality of the butter in cold storage. The starters used were prepared from Streptococcus lactis and Streptococcus paracitrovorus 1921 (8) as it was hoped that the use of pure cultures, which give a good flavor and aroma without rapid acid development, would result in the production of butter with exceptional keeping qualities.

METHODS USED

The raw sweet cream delivered at the Iowa State College was pasteurized at 145° F. for 25 minutes and treated as follows:

1. Pasteurized sweet cream, acidity .14 to .21.
2. Pasteurized sweet cream with 10% starter added, not ripened.
3. Pasteurized sweet cream, starter added, ripened to serum x .0063.
4. Pasteurized sweet cream, starter added, ripened to an acidity 0.50 to 0.61.

Lots 2, 3, and 4 were divided into two or more portions of 110 to 140 pounds each, and to one or more duplicates before churning a pure culture of the microorganisms to be tested was added. The material added consisted of 50 to 100 c.c. milk culture, 72 hours old, plus the scraped off 72 hour old growth on two whey agar slants. The incubation was at room temperature. Every effort was made to standardize workmanship in manufacturing the butter. The moisture content was kept as near 16% as practicable and the salt content ranged between 1.5 and 2.1%. Bacteriological studies were made of the cream before churning and of the butter when manufactured and when finally scored. The butter was packed in ten pound tubs and stored for from six to seven months at -6° F. The bacteria used were, in addition to those in the starter, (S. lactis and S. paracitrovorus)

Bacterium ichthyosmius (7)

Peptonizing coccus. Digested milk in from two to four days. The mold used was Oidium lactis, as this is the mold usually found in dairy products.

Owing to the unsatisfactory condition of the classification of dairy yeasts, it is not possible to give definite names to the organisms used, but the following was used as a basis for the series of the experiments.

1. Forming regular white colonies on whey agar.
   - A. Lactose fermenters. (9)
   - B. Common white.

   Three types of common white yeasts were used in these experiments.
   - A. Large oval yeast, makes milk alkaline in from 18 to 21 days.
   - B. Large oval yeast, sweet curdles milk in from 11 to 17 days.
   - C. Large oval yeast, spore-forming, inert in milk.

2. Forming spreading colonies on whey agar.
   - A. Mycoderma Inert in milk.
   - B. Rapid liquefying yeasts. Liquefy milk in from 2 to 3 days.

3. Pink yeasts.

4. Type intermediate between yeast and mold. Inert in milk.

Whey agar was used for plating cream to which starter had been added and the butter manufactured therefrom. Whey agar

plus 1 c.c. 1% tartaric acid was used for estimation of yeasts and molds in cream and butter. Beef infusion agar was used for plating raw sweet cream, pasteurized sweet cream and the butter manufactured therefrom, as it was found to give higher counts than the whey agar, as there are types mainly micrococci that will not grow on the latter medium. The bacteria were differentiated into various groups by the litmus milk tube method. The acidity of the butter was estimated by dissolving 10 grams in 35 c.c. ether and 10 c.c. alcohol and, using phenolphthalein as indicator, titrating with tenth-normal sodium hydrate solution. All counts reported are total plate counts.

RESULTS OBTAINED

Data relative to the acidity, the bacterial count and the types of bacteria found in the raw and pasteurized sweet cream are given in Table 1. There were 28 lots of cream studied during the period from June to November 1922. The cream was pasteurized at 145° F. for 25 minutes.

In the raw cream the high bacterial counts and the predominance of S. lactis will be noticed. When the acidity of the cream reached 0.20 and over, S. lactis formed 80% or over of the total flora. There was no definite relationship between the flora of the raw cream and its acidity when the acidity was 0.18 or less, nor between the total bacterial count and the acidity. This is to be expected since the acidity depends on the acid-producing organisms present, and whether their environment is suitable or not, whereas the total count is the sum of

several different types. Rennet-acid digesting micrococci and proteolytic types were present to the extent of approximately 1% each in the raw cream; approximately 2% of the flora consisted of Bact. viscosum, Ps. fluor. liquefaciens, Eythrobacillus prodigiosus and members of the coli-aerogenes group. From 50 to 200 Oidium lactis was found in each lot of raw cream and from 60 to 3,000 yeasts. These yeasts were of the same general types as those used in the inoculation experiments.

It is evident that the cream after pasteurization contains relatively few organisms as compared with the original count, but these counts are higher than are usually considered to be present in pasteurized sweet cream. In 28 experiments the average pasteurization efficiency was 99.55%, which is slightly lower than the efficiency of 99.93% which was previously reported (6). The difference may be due to the media used; in the previous work the pasteurized sweet cream was plated on whey agar, and this did not permit various staplylococci to grow, thus giving lower counts and consequent higher pasteurization efficiency. The typical S. lactis type was not found in the pasteurized cream, and since it was present in the sweet cream butter, it is probable that the cells surviving pasteurization were missed in the dilutions used, or suffered injury during pasteurization and showed an abnormal lag phase, and were included in the slow S. lactis group. S. lactis var. maltigenae was not found in the pasteurized cream nor in the butter, so it seems evident that it does not survive a pasteurization temperature of

145° F. for 25 minutes. The main surviving types were the slow S. lactis and the acid-forming, non-coagulating types. In this latter group were identified S. paracitrovorus and lactic acid producing, non-citric acid fermenting Gram positive streptococci which included a thermophilic type which grew best at 45° C. grew well at 37° C. and poorly at 20° C. There were also found two types of Gram positive micrococci easily differentiated because one produced yellow pigment and the one did not on the media used. The percentage of the yellow pigment-forming type tended to increase with decreasing bacterial counts. They were not found in the raw cream, probably owing to the high dilution used (1: one-million).

The alkali-forming group, which according to Ayers and his co-workers (1) are primarily soil organisms, largely decreased in number during pasteurization, but increased in relative amount. No member of this group was found in 12 out of the 28 experiments. The group of bacteria inert in milk largely decreased in number during pasteurization, but held their relative percentage of the flora. They were found in 8 out of 28 experiments. The aerobic spore-forming bacteria were not identified in the pasteurized cream, but were found in some of the butter when taken out of cold storage. They were probably missed in the high dilutions used for plating the cream (1 to 10,000). No molds nor yeasts were found in the pasteurized cream. Lund (14) and Thom and Ayers (25) have shown that Oidium lactis will not survive heating at 145° F. for 10 minutes, and the number of yeasts originally present in the raw cream was too small to draw any conclusion as to their ability to survive pasteurization.

Table 1.

ACIDITY, BACTERIAL COUNT AND TYPES OF BACTERIA FOUND IN RAW AND PASTEURIZED SWEET CREAM

Raw Sweet Cream

Results of 28 experiments.

	Acidity	Bacteria per c.c. Millions	% Type A	B	C	D	E	F
Av.	.18	210	71	14	5	14	14	2
Max.	.21	388	96	33	22	50	31	17
Min.	.14	65	32	0	0	0	0	0

Pasteurized Sweet Cream

	Acidity	Bact.per c.c.: Thousands	Efficiency of Past.	% Type C	D	E	F	Y
Av.	.17	957	99.55	34	56	8	2	3
Max.	.20	4,100	99.93	91	94	47	23	11
Min.	.13	185	98.54	0	13	0	0	0

A=*S. lactis* B=*S. lactis var maltigenae* C=Slow *S. lactis* type D=Acid-forming, non-coagulating type E=Alkali-forming type F=Types inert in milk Y=Gram positive micrococci producing yellow pigment. Column Y is included under Column D since they are acid-forming non-coagulating. Column B is included under column A since *S. lactis var maltigenae* belongs to the *S. lactis* group. The organisms included under slow *S. lactis* are so-named for want of definite knowledge of this group. They slowly reduce litmus milk and finally coagulate it after from 4 to 8 days.

Table 2.

ACTION OF PROTEOLYTIC BACTERIA ON THE KEEPING QUALITY OF BUTTER IN COLD-STORAGE

A=Bact. ichthyosimius B=Peptonizing coccus *

Scoring	No. of Exps.	Acidity of Cream	Micro-organism inoculated	Score
Initial	2	.50	A	92.75
Final				91.75
Initial	1	.50	Check exp.	92.5
Final				92.5
Initial	3	.22	A	92.18
Final				91.83
Initial	1	.22	Check exp.	92.0
Final				92.0
Initial	3	.50	B	93.00
Final				92.17
Initial	1	.50	Check exp.	92.5
Final				92.5

* Peptonizing coccus was a Gram positive peptonizing micrococcus isolated from raw sweet cream.

Data relative to the action of Bact. ichthyosmius and a peptonizing coccus on the keeping quality of butter cold-stored for six months at -6° F. are given in Table 2. Although the butters to which proteolytic bacteria were added decreased somewhat in score, the decrease was not enough to indicate that the addition of the proteolytic bacteria was an important cause of deterioration in butter in cold storage. Bact. ichthyosmius was inoculated into cream ripened to .50% and into cream to which starter had been added but not ripened; the results indicate that the acidity of the cream did not favor the production in the butter of undesirable flavors as a result of the action of proteolytic bacteria.

Data relative to the action of various types of yeasts on the keeping quality of butter made from cream ripened to varying acidities and cold stored for six months are given in Table 3. Considering that flavor and aroma are qualities in butter that are difficult to give a definite value to when scoring at different intervals, it will be seen that the scores of the butter made from the uninoculated cream and the inoculated cream agree very well. Although in many experiments there was some decrease in score, a study of the figures show that the yeasts were not a factor in the keeping quality of the butter in cold storage and that their action was not influenced by the acidity of the cream since the yeasts were inoculated into cream of varying acidities and in several cases the check experiments decreased in score more than the butter made from inoculated cream. Some types of yeasts were more resistant to the adverse conditions than others. Pink yeast and Mycoderma did not survive. 80 to 98% of the lactose fermenting types (T.cremoris and T. sphaerica) and Common White types died off in cold storage. The most resistant type was the Digesting yeast of which an average of 43% survived. From 10 to 25% of the yeast count per c.c. in cream was found per c.c. in butter, except with the Pink yeast which apparently found conditions adverse from the start.

Table 3.

ACTION OF YEASTS ON THE KEEPING QUALITY OF BUTTER IN COLD-STORAGE

Scoring	No. of Exps.	Acidity of Cream	Microorganism inoculated	No. in Cream Per cc	No. in Butter Per cc	Score	Increase or Decrease in Score
Initial*	6	.27	Common White Yeast	10,600	930	92.83	
Final					33	92.25	-0.58
Initial	3	.28	Check Exps.			93.17	
Final						92.83	-0.34
Initial	6	.44	Common White Yeast	28,800	6,100	91.79	
Final					450	91.21	-0.58
Initial	5**	.44	Check Exps.			92.10	
Final						90.90	-1.20
Initial	2***	.56	Common White Yeast	34,400	6,000	91.00	
Final					110	91.50	+0.50
Initial	6	.50	Lactose-fermenting yeasts	33,900	4,620	92.00	
Final					840	91.75	-0.25
Initial	4	.51	Check Exps.			92.12	
Final						91.64	-0.48
Initial	5	.30	Digesting yeast	33,200	8,540	91.70	
Final					3,680	91.90	+0.20
Initial	2	.31	Check exps.			92.00	
Final						91.50	-0.50
Initial	4	.24	Pink yeast	32,750	1,200	93.12	
Final					none	91.87	-0.35
Initial	2	.24	Check exps.			92.00	
Final						92.00	±0.0
Initial	5	.31	Mycoderma	7,260	350	92.50	
Final					none	92.80	+0.30
Initial	2	.29	Check exps.			92.50	
Final						92.25	-0.25

* All figures given are the average figures for the experiments carried out.

*** These experiments were duplicates of experiments averaged in 5**. Three types of Common White yeast were used in the experiments, but as the results secured with them were essentially alike, they are included under one heading in above table.

Table 4.

ACTION OF *OIDIUM LACTIS* AND YEASTS AND *OIDIUM LACTIS* ALONE ON THE KEEPING QUALITY OF BUTTER IN COLD STORAGE

M = *Oidium lactis* Y = Yeasts

Scoring	No. of Exps.	Acidity of Cream	Micro-organism used	No. in Cream	No. in Butter	Score	Increase or Decrease in Score
Initial	7	.48	M plus L	33,360 Y 1.710 M	8.100 Y 234 M	92.70	
Final					820 Y	90.93	-1.77
Initial	7	.48	Check Exps.			92.57	
Final						91.00	-1.57
Initial	3	.44	M	2.730 M	1.470 M	92.33	
Final					None	92.18	-0.15
Initial	3	.44	Check Exps.			91.83	
Final						91.69	-0.14
Initial	2	.57	M	3.150 M	1.700 M	92.00	
Final					None	90.50	-1.50
Initial	2	.57	Check Exps.			92.00	
Final						90.75	-1.25

Date relative to the action of (a) *Oidium lactis* and yeasts and (b) *Oidium lactis* alone, inoculated into cream at varying acidities, in relation to the keeping quality of butter in cold storage are given in Table 4.

Although there was a decrease in score of the butter, it is evident that the combination of yeasts and *Oidium lactis*, or *Oidium lactis* alone did not cause deterioration in cold storage, and that the acidity of the ripened cream did not favor deterioration by these microorganisms. These results confirm those of Rogers 1909 (21) who was unable to produce fishy butter by the inoculation of *Oidium lactis* into cream although O'Callaghan 1907 (17) had claimed that *Oidium lactis* produces fishiness in butter. The fact that no *Oidium lactis* survived in the cold storage butter shows that it was in an unfavorable environment.

Table 5.

BACTERIAL COUNT, FLORA AND SCORE OF SWEET CREAM BUTTER AT INITIAL AND FINAL SCORINGS

Scoring	Bact. per c.c. thousands	S. lactis	Slow S. lactis	Non-coagulating acid forming	Alkali forming	Inert	Peptonizing
Initial*	167	0	20	70	6	4	0
Final *	141	6	8	80	2	1	3

	Acidity of cream	Bact. per c.c. in Butter Thousands		Acidity of Butter		Score	
		Initial	Final	Initial	Final	Initial	Final
Av. *	.18	167	141	1.08	1.17	91.71	91.76
Max.	.21	630	600	1.40	1.50	93.0	93.0
Min.	.14	54	30	0.90	1.00	90.5	90.0

*Average of 19 experiments.

Data relating to the bacterial count, flora and score of sweet cream butter are given in Table 5. The data shows that organisms of the various groups can survive in butter that has been cold stored six months at -6° F. The acid-forming, non-coagulating type formed 70% of the flora at the initial scoring and 80% at the final scoring. It is evident that S. paracitrovorus, lactic-acid producing, non-citric acid fermenting streptococci and various types of micrococci can resist pasteurization and cold storage conditions, and thus show themselves to be very resistant to unfavorable conditions. S. lactis was not found in the butter when manufactured, but was found after cold storage. The butter manufactured from the sweet cream retained 5 to 30% of the bacteria per c.c. of the cream; usually between 20 and 30% was found; and 35 to 100% of the bacteria per c.c. originally in the butter was found after the cold storage period. It is evident from the score that the

butter showed excellent keeping quality, having a slightly higher average score when it came out of cold storage than it had when it went in and that the flora of the pasteurized sweet cream did not cause deterioration in cold storage.

Data relative to the bacterial count per c.c. of the cream, buttermilk and butter; the acidity of the cream and butter, and the score of the butter at the initial and final scorings are given in Table 6. The number of bacteria per c.c. as determined by the plate method, in the cream to which starter had been added, had no definite relation to the acidity of the cream. This is to be expected since the production of acidity is a function (a) of the ratio of S. lactis to S. paracitrovorus (b) the rate of acid production of the S. lactis inoculated and (c) whether these streptococci grow in long or short chains. The buttermilk, except in a few instances, showed a higher bacterial count per c.c. than the cream did, but no definite relationship existed. The following causes are suggested as to why a higher count should be obtained per c.c. in the buttermilk than in the cream: (a) removal of the butterfat containing comparatively few bacteria, (b) breaking up of bacterial clumps by agitation during churning. The flora of the cream to which starter had been added was found to consist of S. lactis and S. paracitrovorus, the S. lactis forming 90 to 100% of the flora. Other organisms must have been present, but were probably missed owing to the high dilution necessary for plating (1 to 1 million).

The butter made from cream to which starter had been added and (a) not ripened and (b) ripened to varying acidities, retained per c.c. from 0.5 to 2% of the bacteria per c.c. in the cream, generally about 1%. The flora of the butter was found to consist of S. lactis and S. paracitrovorus, the S. lactis forming 70 to 100% of the flora, usually over 90%. The dilution used

for plating (1 to 10,000) probably accounts for other organisms not being found. The average dying off of bacteria during the six months cold storage at -6° F. was over 98%. This, considering the larger initial bacterial count of the butter, is in marked contradistinction to the results obtained with the sweet cream butter. When taken out of cold storage, the sweet cream butter often had a higher bacterial count per c.c. than the butter made from cream to which starter had been added. This seems to show that the normal flora of pasteurized cream find an acid environment unfavorable; also the utilization of the lactose by S. lactis and S. paracitrovorus may be a factor. The reason that S. lactis and S. paracitrovorus die off so markedly may also be due to what Sherman and Albus (24) term "physiological youth," that is where bacteria are reproducing rapidly they are most sensitive to an unfavorable environment. The flora of the butter when taken out of cold storage was found to consist chiefly of S. lactis and the acid-forming non-coagulating types and usually contained from 100 to 2,000 yellow pigment forming micrococci per c.c. S. paracitrovorus was usually present. The % of S. lactis varied from 0 to 100%, but was usually present in some amount. The acid-forming non-coagulating type varied from 0 to 100%; usually the lower the bacterial count of the butter the greater the percentage of this type.

The butter made from sweet cream to which starter had

been added, but not ripened, had a higher initial score than the sweet cream butter. The score decreased on the average 0.44 points whereas the sweet cream butter increased its score on the average 0.05 points, yet the final score was higher by 0.36 points than the sweet cream butter. This shows that this method of butter manufacture is practicable and it has the advantage that the butter has a flavor which makes it more desirable.

The average score of the butter made from the uninoculated cream, ripened to serum x .0063, was 92.34 points. This type of butter lost 1.34 points in cold storage, thus having a lower average score by 0.76 points than the sweet cream butter, but the average score of 91.0 points after six months cold storage shows better keeping quality than is usually credited to this type of butter.

The average score of the butter made from the uninoculated cream, ripened to 0.50 to 0.61, was 92.21 points. This type of butter lost 1.64 points during six months cold storage, and the corresponding decrease for the butter made from inoculated ripened cream was 0.47 points. The final average score of 90.57 points for the uninoculated butter and 91.62 points for the inoculated butter shows better keeping quality than is usually credited to this type of butter, and also that the addition of the microorganisms did not cause deterioration. It is also evident that while lactic acid may be a factor in deterioration it is not a cause of it.

Table 6

BACTERIOLOGICAL COUNT OF THE CREAM, BUTTERMILK AND BUTTER, ACIDITY OF THE CREAM AND BUTTER AND SCORE OF BUTTER AT INITIAL AND FINAL SCORINGS.

Sweet Cream plus 10% Starter (8 Experiments)

Acidity of Cream	Bacterial Count: Cream (Millions)	Buttermilk (Millions)	Butter: Initial (Thousands)	Butter: Final (Thousands)	Acidity of Butter: Initial	Acidity of Butter: Final	Score: Initial	Score: Final
Av. .28	278	336	4,100	95	1.30	1.32	92.56	92.12
Max..35	530	610	8,500	285	1.50	1.50	94.0	93.0
Min..22	77	90	650	3	1.20	1.20	91.0	90.0
Sweet Cream plus 10% Starter plus added Microorganisms (23 experiments.)								
Av. .27	256	302	3,250	91	1.28	1.32	91.94	91.84
Max..35	510	600	7,800	460	1.45	1.50	93.5	94.0
Min..22 Sw	70	78	600	3	1.20	1.15	91.0	90.0
Sweet Cream Ripened to Serum x .0063 (22 Experiments)								
Av. .44	196	211	2,813	69	1.55	1.68	92.34	91.00
Max..46	510	540	12,400	336	2.00	2.20	93.5	92.5
Min..42	67	73	520	1	1.35	1.50	91.5	88.0
Sweet Cream Ripened to Serum x .0063 plus added Microorganisms (17 Experiments)								
Av. .44	105	127	1,170	28	1.64	1.75	92.03	91.41
Max..46	160	205	1,850	86	2.20	2.20	93.0	93.5
Min..42	63	73	440	2	1.40	1.40	91.0	88.0
Sweet Cream ripened to from 0.50 to 0.61 (7 Experiments)								
Av. .55	232	282	2,650	52	1.96	1.96	92.21	90.57
Max..61	550	730	5,300	270	2.20	2.40	92.5	91.5
Min..50	123	144	960	3	1.75	1.75	91.5	90.0
Sweet Cream Ripened to from 0.50 to 0.61 plus added Microorganisms (16 Experiments)								
Av. .53	319	391	3,305	78	1.81	1.96	92.09	91.62
Max..61	800	960	7,300	305	2.35	2.35	93.0	93.0
Min..50	74	87	630	2	1.45	1.50	90.5	90.0

That lactic acid may aid as a factor in deterioration is shown by the fact that several churnings decreased from a 92 to an 88 point score, while the check churning made from the sweet cream without the addition of starter retained its score or only slightly decreased in score. It is this fact that has led to the association of acidity of cream with deterioration of butter in cold storage, and the increasing demand for sweet cream butter for storage purposes. A proof that S. lactis and S. paracitrovorus are not a direct cause of deterioration is shown by the keeping quality of the butter made from cream to which starter had been added but not ripened.

The acidity of the butter increased as the acidity of the cream increased, but the individual range is so wide, that it seems that the acidity of the butter is not in proportion to the acidity of the cream, but is influenced by other factors, such as the treatment during manufacture.

CONCLUSIONS

1. There was an average pasteurization efficiency of 99.55% when sweet cream was pasteurized at 145° F. for 25 minutes.
2. The bacterial count of the ripened cream had little or no relation to the acidity of the cream.
3. The bacterial count per c.c. of the butter made from ripened cream had no definite relation to the bacterial count per c.c. of the ripened cream.
4. The butter retained per c.c. from 0.5 to 2% of the bacteria per c.c. of the ripened cream, generally about 1%.

5. The butter made from pasteurized sweet cream retained per c. c. from 5 to 30% of the bacteria per c.c. of the pasteurized sweet cream, generally between 20 to 30%.

6. From 95 to 99% of the bacteria of the ripened cream died off during six months cold storage, generally from 98 to 99%.

7. The average decrease in the bacteria in the butter made from pasteurized sweet cream during six months cold storage was approximately 20%, the maximum decrease was 65%; the bacterial count per c.c. of some butters did not show any decrease.

8. Resistant strains of S. lactis, slow S. lactis type, S. paracitrovorus, lactic acid forming, non-coagulating, non-citric acid fermenting streptococci, various types of micrococci, inert types, alkali-forming types and proteolytic types lived over in the butter, cold-stored for six months.

9. Taking the acidity of the butter as a criterion, there was little or no detectable hydrolysis of the butterfat during six months cold storage.

10. The acidity of the butter increased as the acidity of the cream increased, but there was no definite relation between the acidity of the butter and the acidity of the cream.

11. Proteolytic bacteria, various types of yeasts found in dairy products, and Oidium lactis were added to ripened and unripened cream containing starter before churning and were found not to be a significant factor in the deterioration of butter in cold storage for six months at -6° F.

12. There was no evidence that the enzymes produced during the growth of the microorganisms or the disintegration products produced on the death of the microorganisms affected the keeping quality of the butter in cold storage.

13. Sweet cream butter was found to keep well in cold storage and for the most part to retain its original score. One hundred per cent of the lots of butters scored 90 and over both when put into and taken out of cold storage.

14. Butter made from sweet cream plus 10% starter, unripened, was found to have good keeping quality and to have a slightly higher average score when taken out of cold storage than the churnings made from pasteurized sweet cream. One hundred per cent of the butters scored 90 and over, both when put into and taken out of cold storage.

15. Butter made from sweet cream ripened with a mixture of S. lactis and S. paracitrovorus showed good keeping quality in cold storage, but marked deterioration of occasional churnings occurred. One hundred per cent of the butters scored 90 and over when put into cold storage, and 83% of these butters scored 90 and over when taken out of cold storage.

16. Where deterioration of butter occurs in cold storage it is not due to the normal flora of pasteurized cream, nor to S. lactis or S. paracitrovorus, nor to the lactic acid formed during the ripening of cream by a starter.

17. The occurrence of erratic decreases in score of butter made from ripened cream, which rarely occurs when the cream is not ripened, suggests that the deterioration of butter made from ripened cream is due to some as yet undetermined cause, which may be aided in its action by the lactic acid produced during the ripening of the cream.

18. The quality of the cream when delivered at the creamery is probably the main factor that determines the keeping quality of butter in cold storage.

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