THESIS.

A Bacteriological Study of Milk and Its Products.

For the Degree of Bachelor of Science.

BY

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In view of the fact that there has been much discussion as to the cause of the changes which milk undergoes in souring, coagulating, and also difference of opinions as to what causes the flavoring of butter, it was determined to perform a series of experiments with bacteria in order to study their action in causing fermentative changes in milk, and also to determine their effect on the flavoring of butter .

The general plan of the work was divided into three parts.

First- Collecting Sterile Milk-

Second- Isolation of pure cultures of bacteria from ripened cream and also from butter and then inoculating cream and ripening it with these different species, then churning the butter and noting flavor, etc.- (as shown by table.)

Third- Testing acidity of skimmed milk at different periods of time after inoculating with pure cultures of all the species used in part II. This was for the purpose of determining the acid-forming qualities of different species.

Part I.

The bacteria which are found in milk exist in the air and dirt of the dairy and upon the animals, but not in the milk glands of the healthy cow's udder. If a cow is diseased it is highly probable that pathogenic organisms would appear in the milk. To determine whether or not the milk within the udder is sterile was the object of the first experiment - A healthy Jersey cow which had been milked for

some time was selected for the experiment . In order to destroy as many organisms as possible upon the cow the udder and surrounding parts were thouroughly brushed and washed, after which the cow was taken to an open, clean lot at least 100 yards from buildings where animals were kept. The udder and teats were then washed with a 1/10% water solution of corrosive sublimate, careful attention being given to the openings of the milk passages. To draw the milk an ordinary milking tube was used . This tube had been sterilized by heating in boiling water for twenty minutes, and when removed from the water was immediately wrapped in sterilized paper and kept covered until ready for use. As the milk flowed from the tube it was caught in sterile glass flasks provided with cotton plugs to exclude organisms. These flasks were set in a quiet place having a nearly constant temperature from 10°-15° . After standing for four weeks the milk in three flasks coagulated and on examination, a single organism was found contained in them. The remaining flasks after standing three months were still liquid without change in appearance, but had developed an odor resembling that of burned milk and had a slightly bitter taste.

Other samples were taken from milk, obtained under the usual dairy conditions, without precautions to prevent the entrance of organisms from the air etc.. This milk was put into sterile flasks which were plugged with cotton and then placed alongside the others. At the end of two weeks the milk in all the flasks had coagulated and soured, thus showing the presence of organisms. On examination of one of these flasks it was found to contain a large number of a single bacterial

species.

Part II.

It is now believed by investigators that the different flavors of butter and of cream are directly due to bacteria . In order to test this matter, organisms were isolated from ripened cream and from butter and used to ripen cream, which was churned and the flavor of the butter noted.

The advantage to the dairy industry of an organismwhich will produce uniformly good flavor in butter is evident.

All the bacteria experimented with were obtained either from ripened cream, or from butter, or from buttermilk immediately after the butter had been removed .

To obtain the species in pure culture, beef broth was inoculated with a small quantity of the material. This stood 24hrs. in the incubator at 35°c . A fresh beef broth tube was then inoculated from this and well shaken. After which a drop of this diluted material was taken up with a small sterilized pipette and dropped in a tube containing agar-agar. After allowing the liquid to flow over the surface of the agar, the tube was put in the incubator. At the end of 24 hrs. there usually appeared several small colonies distributed over the surface of the agar. Each colony usually contained only a single form of organism. Inoculations were made from the colonies into agar-agar and examined after 24 hrs. This method of isolation was found to be more certain than the ordinary Esmarch gelatin tube method because the tubes could be put in the incubator in a favorable temperature for growth, while

the gelatin tubes would melt down under such conditions.

Description of Species.

No. 2.

Source. From butter-milk obtained from our trial churning of Jan.16.'93.

Morphology. A very short bacillus with rounded ends. Often single but occasionally double or in short chains.

Growth. In beef broth after 24 hrs. in the incubator at 30° liquid milky with a small quantity of white sediment in the bottom of the tube.

In agar-agar. After 24 hrs. in the incubator at 30° there was a slightly spreading, polished, whitish, almost transparent entire edged, slightly raised growth.

In gelatin. After 12 days there was a white evenly growing growth along the track of the needle with a polished, white, raised, slightly spreading growth at the surface of the gelatin.

In milk. It is a marked acid former but did not cause coagulation .

No. 3.

Source. From butter that was made in our trial churning of Jan.16.'93.

Morphology. A medium sized micrococcus which grows in chains, singly, and in zooglea .

Growth. In beef broth after 24 hrs. at 30° liquid clear with a slight white sediment in bottom of tube .

In agar-agar. Very thin whitish, almost transparent, slightly raised growth .

In gelatin. Very slight whitish growth along track of needle, more distinct near bottom of tube .

In milk. It was a well marked acid former but did not produce coagulation .

No. 4.

Source. From butter made at H. B. Gurlers ,DeKalb, Illinois.

Morphology. A large micrococcus .

Growth. In beef broth forms a slightly cloudy liquid with no pellicle.

On agar-agar . Growth is smooth , spreading, polished, transparent at center with white edges which are somewhat raised; Edges smooth .

In gelatin . Liquefies.

In milk. Did not coagulate it and developed but little acid.

No.5.

Source. Ripened cream from H. B. Gurler's dairy.

Morphology. A straight rod shaped bacillus, not always of same length Usually about four times that of the diameter .

Growth. Beef broth: Liquid clear, smooth white detached pellicle .

On agar-agar . Growth is slightly raised, surface wrinkled, dull, light brown color, finely divided edges.

In gelatin. Liquefies gelatin, forming a rough white pellicle on surface .

In milk. Coagulates but does not acidify sterile skim milk.

No. 6.

Source. Isolated from milk from Chesters .

Morphology. Large oval bacillus, length about 1 1/2 times diameter.

Growth. Beef broth, turbid liquid, no pellicle.

On agar-agar. Growth is spreading, raised, polished, with portions white and others transparent . Edges smooth.

In gelatin. Whitish growth along track of needle, with a raised polished white growth on surface of gelatin .

No. 7.

Source. Butter from H. B. Gurler's dairy .

Morphology. A short rod like bacillus length about twice the diameter, often in chains .

Growth. Beef broth: Liquid translucent, no pellicle.

On agar-agar. Spreading, smooth, polished growth, white at center, transparent near outer edges which are roughly lobed.

In gelatin. A thick dense growth following track of needle, did not liquefy.

In milk. Does not coagulate it in 3 days. only slightly acid forming.

No. 8.

Source. Butter from H. B. Gurlers, DeKalb, Illinois.

Morphology. A small micrococcus which is found commonly in large zooglea but also in chains, pairs, and singles .

Growth. In beef broth after 24 hrs. in incubator at 35°c. liquid was opalescent with a white sediment in bottom of the tube.

On agar-agar. After 24 hrs. in incubator at 35°c. there was a white, slightly, raised, polished, spreading growth with edges entire.

In gelatin. After 24 hrs. at temperature of room there was a white evenly spreading growth along the track of the needle .

In milk. A marked acid former, also coagulated the milk in 40 hrs. at temperature of 28°c. .

No. 9.

Source. Butter from H. B. Gurler's dairy .

Morphology. A small oval bacillus, rarely in chains .

Growth. Beef broth: Forms a white, flocculent, detached, pellicle liquid clear .

On agar-agar. Growth much raised with smooth polished surface. Edges porcelain white .

In gelatin. White spreading growth on surface, slight growth along track of needle, does not liquefy .

In milk . Increases acidity greatly, but does not coagulate it.

No. 10.

Source. From butter made in Exp. no. 3. (not inoc.) .

Morphology. A small oval bacillus mostly in singles but often in pairs and occasionally in short chains.

Growth. Beef broth: After 24 hrs. at 35°c. liquid turbid, almost milky with white sediment in bottom of tube .

On agar-agar. After 24 hrs. in incubator at 35° there was a large raised, polished, even edged, spreading, yellowish white growth.

In gelatin. After 4 days there was a prominent white growth along track of needle with a white, raised, polished, growth on surface of gelatin.

In milk. Was a marked acid former, also produced coagulation.

To determine the effect of the different bacteria on the flavor of butter it became necessary to use cream which was as nearly sterile as possible . Now the different methods proposed by others for sterilizing cream do not seem practicable in that they could not be used in the dairy where large quantities of cream are handled . Also the method of collecting sterile milk given at the beginning of this paper is certainly not one which will come into general use. Milk or cream is very difficult to sterilize because many of the bacteria found in milk produce very resistant spores and therefore require heating at the temperature of boiling water for a few minutes on at least three successive days. When cream is heated to such high temperature it acquires a burned taste which is exceedingly unpleasant . Pasteurization consists in heating to about 82° C. and cooling quickly, but this, as has already been shown, is not sufficient for complete sterilization . In these experiments a method was followed which is believed to be, at once, practicable and sufficiently accurate to be used in any well regulated dairy . This consists in separating the cream immediately after milking, and

adding to the cream a "starter" containing a large quantity of a pure culture of bacteria. In this way the effect produced by a given species was obtained with a comparatively small amount of extra labor or trouble .

For separating the cream a De Lavel "Baby" Cream Separator was used, all parts of which were thoroughly sterized by heating in boiling water . The whole milk was taken immediately after milking and passed through the machine which separated the cream by means of centrifugal force; the cream issuing from one spout and the milk from another . The cream was caught in half gallon glass jars which had been previously sterilized and was kept and churned in these same jars. The cream was thoroughly mixed by pouring from one jar to the other so that each one was about half full . One jar of cream was inoculated with a pure culture of bacteria. This "starter" was prepared by sterilizing about 100cc. of skimmed milk by heating to the temperature of boiling water on three successive days. This sterile milk was inoculated with a pure culture of bacteria and allowed to stand in incubator for about 24 hours . Slides were made and examined before using the culture. In this manner a "starter" was formed which contained an immense number of organisms of the species to be experimented with . The remaining jar of cream was not inoculated but received the same treatment afterwards as the inoculated one . A sample of cream was preserved for testing the per cent of fat contained . Both jars were placed in an oven (or incubator) having a constant temperature of 25°-35°C. (varying for the different

experiments). After remaining at this temperature for about 24 hrs. the cream in both was churned.

The churn used consisted of two half gallon glass jars placed side by side in a frame which was provided with an axis and crank. When the churn was revolved the cream was agitated by dropping from end to end in the jars . The churning was done in a room the temperature of which averaged about 15°c.. The churn was revolved 60 times per minute. After the butter had collected into granules (or had "come") the temperature of the butter-milk was taken then the butter-milk was drained off by pouring the entire contents of the jar on to a sieve. The butter was then put into the jar and a quantity of water run in, and the whole shaken up. This washing process was continued two or three times until the wash water was almost clear . The butter was then kneaded with a paddle until most of the liquid was removed . Samples were then tested for odor and the butter put in tight glass bottles, corked and placed in a cool room.

The buttermilk was tested immediately after churning to determine the acidity developed in the inoculated and the non-inoculated jars.

The per cent of fat in the buttermilk was also determined after each churning by means of the Babcock test for butter fat.

The following table gives the results in a condensed form. It will be noted that some of the data are wanting in the first four experiments. This is due to the fact that the exact method of carrying out the experiments had not been decided upon at the time.

Great difficulty was experienced in describing odors.

CREAM FROM SEPARATOR.

Experiment No.	Date, 1893.	Cream in incubator. Time, hrs.	Temperature incubator.	What cream was inoculated with.	Per cent fat. Cream.	Per cent fat. Butter milk.	Acidity of butter milk.	Temperature. Cream when started to churn.	Temperature. Butter milk when stopped churning.	Time of churning. hrs.	Time of churning. min.	Amount of inoculator added.	Remarks on flavor or aroma of butter on succeeding days.
2	Feb. 24		26.6°C.	No. 2								70 c.c.	A pleasant flavor; 48 hours had a sickening decaying odor.
	March 6	24	29.4°-32.2°C.	No. 3.			30			1	5	50 c.c.	Flat, odorless; 24 hours and 48 hours later, odors same.
3	"	24		Nothing			36.6				50	Nothing	Pleasant acid odor; 24 hours and 48 hours later odors same.
	May 2	18	22°	No. 4	27.7		38.6	12° C.					
4	"	18	22°	Nothing	27.7		28.8						
	March 11	24	21.1°-25.8°C.	No. 5		12	9	14.2° C.	16.6° C.		30	50 c.c.	Sour cream odor; April 7th, sickening fatty odor.
5	"	24	"	Nothing		10	9.2	14.2° C.	16.6° C.		30	Nothing	Sour cream odor; April 7th, musty cheese odor.
	March 28	20	40° C.	No. 6	27.4	0.7	3.3	11.1° C.	12° C.	1		25 c.c.	Hickory nut odor; 48 hours later almost gone.
6	"	20	40° C.	Nothing	27.4	1.4	43.6	11.1° C.	12.2° C.		38	Nothing	Sour cream odor.
	March 30	21	37° C.	No. 7	30.6	Lost	3.3	14.2° C.	17.7° C.		28	30 c.c.	Characteristic sour odor
7	"	21	37° C.	Nothing	30.6	3.1	Lost	14.2° C.	19.4° C.		26	Nothing	Characteristic sour cream odor.
	April 3	20	28° C.	No. 8	29.6	9.7	47.5	11.6° C.	15.5° C.	1	50	40 c.c.	Sour bread odor.
8	"	20	28° C.	Nothing	29.6	9.5	49.5	11.6° C.	18.8° C.	2	35	Nothing	Sour cream odor.
	April 6	19	35° C.	No. 9	26.2	1.2	45	12.2° C.	17.2° C.		24	25 c.c.	Odorless.
9	"	19	35° C.	Nothing	28.2	1	47	12.2° C.	17.7° C.		28	Nothing	Sour bread odor.
	April 10	19	25° C.	No. 10	28.8	4.7	22.6	13.3° C.	15.5° C.	1	40	100 c.c.	Strong yeast odor.
10	"	19	25° C.	Nothing	28.8	5.1	18.6	13.3° C.	16° C.	1	5	Nothing	Characteristic sour cream odor.

Part 3.

A well mixed sample of skimmed milk containing less than 0.1% of fat was obtained by means of the hand separator and about 40cc of this put in each of a number of 50cc flasks and sterilized by leaving in boiling water for 15 minutes on three successive days . In order to have a basis for comparing the acidity developed by the different species in skimmed milk, the acidity of one of these flasks was tested and found to require 5.3cc of deci-normal alkali to neutralize 25cc of the milk . To each flask of sterile skimmed milk 12 drops, of a 24 hour old beef broth culture of the different species of bacteria was added . These flasks were then put into the incubator and the acidity of the skimmed milk in each flask tested after growing for different numbers of hours as shown in the following table .

Acidity developed in Skim Milk by different Bacteria.

No. of c.c. deci-normal alkali required to neutralize 25 c.c. of skim milk treated as described on previous page.

Bacteria used.	Acidity sterile skim milk. used.	Acidity after standing in incubator.					
		6 hrs.	8 hrs.	24 hrs.	48 hrs.	Temperature of incubator.	Remarks.
No. 2	3.7	7	8.2	10.3	10.6	26°	Not coagulated
No. 3	5.3	5	5.4	8	11.5	28°	Not coagulated
No. 4	4.9	5	5	5.7	5.4	28°	Not coagulated
No. 5	5.3	4.8	5.3	5.4	5	25°	Coagulated,48 h.
No. 7	5.3	5.5	6.1	8.2	11.6	25°	Not coagulated
No. 8	5.3	5.4	5.4	5.5	8.3	28°	Coagulated,40 h.
No. 9	5.3	6.7	7.5	13	19.2	25°	Not coagulated
No. 10	4.9	5.4	7	10.9	13.8	28°	Coagulated

Conclusions.

Bacteria evidently give flavor to butter. This is shown by the fact that the flavor of butter from the non-inoculated jars was the same in all the experiments, while the flavor of the butter from the inoculated jars varied greatly . Exp. no. 7. is the only one in which the flavor of the butter from the inoculated jar was the same as its duplicate non-inoculated jar.

Of the different species experimented with no's. 6&7 . give promise of proving useful in giving an exceptionally good flavor.

It will be noted by examining the table that the different species had very different effects in developing acidity in the sterile skimmed milk , and also different effects in producing coagulation or other changes . For example no. 9 was a marked acid former but did not coagulate the milk. With no. 5. the milk was coagulated but not soured, while no.8. caused both souring and coagulation.