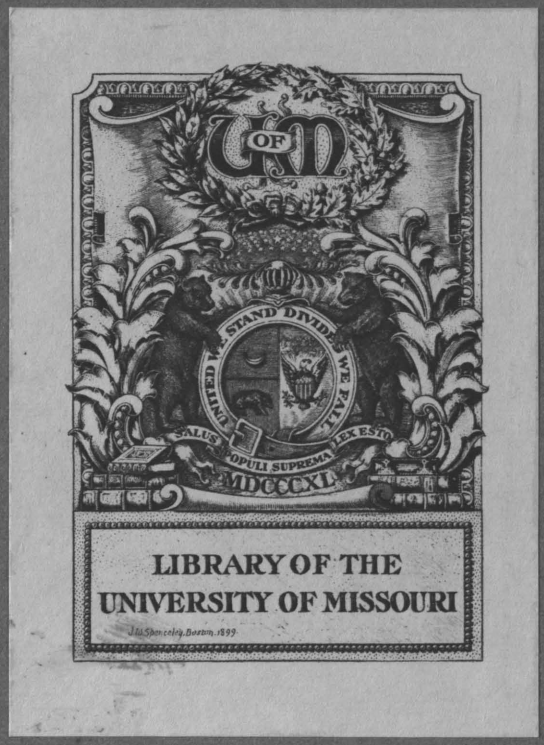

Bacteria Concerned in the Making of Salt-rising Bread

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SUBMITTED IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF
MASTER OF ARTS
in the

GRADUATE SCHOOL

of the
UNIVERSITY OF MISSOURI.

1912.

SALT-RISING BREAD.

Salt-rising bread is a kind of leavened bread, which is made light not by the introduction of yeast, as with yeast cakes or by mechanical aeration or by carbon dioxide liberated from chemical compounds, such as baking powders, but by some ferment which is present in the ingredients used in starting the bread. The most marked characteristics of it are its texture, odor and flavor. The texture is very close and fine, yet the bread is light. The odor and flavor are especially characteristic, being rather penetrating. This odor which suggests putrefaction is unpleasant to some people. The flavor is slightly sweet but peculiar and characteristic.

The making of salt-rising bread has always been regarded as an uncertain process at best. The most proficient salt-rising bread makers, while they protest that they never fail in making it, will usually upon questioning, confess to an occasional failure, and advise inexperienced persons not to attempt it. One will say it cannot be made in the winter time, another will not attempt it without a particular kind of cornmeal or flour, a third insists upon perfectly fresh milk, obtaining it directly from the cow even when the breadmaking is to be started at noon . At the time a starter is prepared one cannot tell definitely whether it will ferment or not. A strong fermentation should result in twelve hours, but this is not a certain indication that the bread will rise when made into loaves.

It is because the outcome is so uncertain in the making of salt-rising bread and because no scientific work on the subject has been done, that this work was undertaken. It was thought that if some definite knowledge of the organisms concerned in the making of salt-rising bread were obtained, results could be made more certain, and thus make this process of bread-making more accessible to a larger number of people.

The house keepers of the sections of the country where salt-rising bread is used have each their particular and individual methods of procedure. No two would be willing to exchange their methods; because the points where in they differ are held to be vital points. It is probably because of frequent failures that each housewife adheres so closely to her own methods which she has found to be successful.

As a result of the examination of numerous recipes for salt-rising bread, it was found that the bread is started with a variety of ingredients. Graham flour or cornmeal is always used; salt and sugar are often present. These are sometimes combined with water, sometimes with milk. From this it would seem as if there were no one ingredient responsible for the lightness. The ferment concerned therewith is connected with the Graham flour or with the cornmeal.

A review of the literature up to this time shows only a few comments on salt-rising bread, and no scientific investigation with the exception of Kohman's work.

In regard to the leavening agent there are various theories. Atwater[1], and the writers from the Ontario Agricultural

1) U.S.Dep't of Agr. Farmers' Bull. 389p21. Helen W.Atwater.

College[1] think that the fermentation which takes place is due to ferments originally present in the ingredients or to ferments acquired from the air. Conn[2] and Bailey[3] state definitely that the fermentation is caused by yeasts from the air. Heinemann and Hefferan[4] believe the lightness of the bread to be due to carbon dioxide.

The preceding points are discussed by Kohman in a recently published paper so will need only brief mention here. Kohman proves that the organisms causing the fermentation do not come from the air as some of the above quoted writers believe, by the fact that a sterile medium when exposed to the air where the bread has been previously made does not ferment except in a few cases and then not so vigorously as the salt-rising starter.

If the organisms are lodged on the utensils and in this way get into the bread, we would have fermentation taking place much more frequently than is the case when food materials are allowed to stand in these utensils. It is not reasonable then to suppose that the organisms causing the fermentation are lodged on the utensils.

That the fermentation is not due to yeasts as has been suggested by Bailey and Conn, is proven by Kohman who found no yeasts when the fermenting material was examined microscopically; and furthermore plates made from the fermenting material have shown only bacteria.

1) Ontario Agr.Coll. Bull. 180,p17. R.Harcourt and M.A.Purdy.
" " " " 118,p16. F.C.Harrison.
2) Conn, H.W. Bacteria Yeasts and Molds. p 75
3) Bailey, E.H.S. Sanitary and Applied Chemistry. p 168
4) Science, Vol. 29, p1011. P.G.Heinemann and Mary Hefferan.

Heinemann and Hefferan believe that milk and sodium bicarbonate are essential ingredients in making salt-rising bread. These authors claim that the formation of lactic acid from the milk which combines with the sodium bicarbonate thus liberating carbon dioxide is the essential element in making the bread light. However, salt-rising bread can be made without either milk or sodium bicarbonate; in fact the recipes with one exception omit the sodium bicarbonate, proving that the above theory is untenable.

Kohman in the conclusion to his paper says,"The leaven in salt-rising bread is not yeast as is indicated by the literature on the subject, but certain bacteria. These bacteria aerate the bread by decomposing certain of its constituents, principally the sugars, into gaseous products and not, as has been suggested, by producing acids which liberate carbon dioxide from the soda. The microbic flora involved varies greatly, depending upon the temperature to which the meal is subjected in setting the "batter". The organisms that predominate in the batter when it is made by stirring the meal into boiling milk or water are only occasionally found upon plates made from batters that were not subjected to temperatures which destroy non-sporebearing organisms. The chief source of the bacteris is notthe air and utensils, as has been suggested in the literature, but the cornmeal used in making the batter. One organism was isolated which in pure culture produces the gas necessary to properly aerate bread. This bacterium seems to be a member of the coli groups and was never found in batters that were heated to 75°C. It in all probability

belongs to the same group as the organism described by Wolffin and Lehman, which they call Bacillus levans. This organism could be propagated in liquid media, such as milk, or could be grown in a batter and subsequently dried, to be used in the preparation of bread".

"When the liquid used in making the batter is taken sufficiently hot to bring the temperature of the batter to 75°C. or higher, certain spore-bearing organisms prevail which readily produce the gas necessary to aerate bread. These bacteria soon lose their gas producing power when kept in liquid media or when transferred to fresh media at intervals of 12 or 24 hours. From this fermenting batter no culture was isolated that retained its ability to produce gas when kept in the liquid state. A dry product consisting for the most part of starchy material was prepared, however, which could be used at will in making uniform bread".[1)]

Experiments and Observations.

This investigation was commenced in the spring of 1910 when two organisms were isolated from a fermenting batter used in making salt-rising bread. Both of these organisms in pure cultures could be used successfully to leaven bread. During the summer vacation and before their characteristics were studied very much in detail, the organisms were lost together with some other bacterial cultures, so that when the work was taken up again in the fall it had to be started from the beginning.

In this first work, sterile milk was used but there was no attempt made to sterilize the flour. In beginning the work

1) Journal of Industrial and Engineering Chemistry. V.4, No.2. Page 106. H.A.Kohman.

again an attempt was made to sterilize the flour, using dry heat for varying lengths of time, moist heat chloroform, ether and alcohol. That these results were not successful is shown by the following table--table I. When the temperature was low enough not to destroy the elasticity of the gluten it was not sufficient to kill the micro organisms.

Table I. The Effect of Sterilizing Agents on the Bacterial Content of Flour.

Agent	Time	Temp.	Effect on Flour. Color	Gluten	Bacterial Content.
Hot Air	60min	140° C	brown	destroyed	not determined.
" "	60 "	120 C	slightly brown	"	" "
" "	120 "	96-108C	no color change	"	" "
" "	30 "	100 C	" " "	not "	" "
" "	50 "	"	" " "	" "	" "
" "	60 "	"	" " "	" "	" "
" "	70 "	"	" " "	" "	" "
" "	80 "	"	" " "	" "	" "
" "	90 "	"	" " "	" "	Probably 9/10
" "	170 "	"	" " "	" "	organisms killed.
Chloroform	48 "	20 C	" " "	" "	Many present
Ether	48 "	20 C	" " "	" "	" "
Alcohol	48 "	20 C	" " "	" "	" "
Moist heat	20 "	120 C	" " "	destroyed	not determined.

After these unsuccessful attempts to sterilize the flour it was decided to abandon all attempts at sterilization and do as had been done in the previous work, that is, use sterile milk to grow the culture in up to the time it was made into a sponge, then use non-sterile flour. This gives any organisms which

may be present in the flour only a few hours to start growing, which is not likely to be long enough for any fermentation to start.

As in some recipes for salt-rising bread, flour is used instead of corn-meal or raw milk instead of scalded milk, bread was started in several ways, as shown by the accompaning table, in order to determine whether it was better to use meal or flour, raw, scalded or sterile milk.

Ingredients used in Making Salt-rising Bread.

Milk	Cornmeal	Flour	Sterile Salt	Sterile Sugar	Result
Raw 50cc	10 gms.	____	1 gm.	2 gm.	Fermented.
" "	_____	10 gms	"	"	Not fermented.
Scalded 50cc.	10 gms.	____	"	"	Fermented.
" "	_____	10 gms	"	"	Not fermented.
Sterile	10 gms.	____	"	"	Fermented.
"	_____	10 gms	"	"	Not fermented.

The ingredients were placed in flasks and kept at 38° C. for 16 hours when the notes on fermentation were made. The flasks containing flour showed no fermentation while all those containing cornmeal were fermented, indicating that the cornmeal contains the organisms of fermentation. Scalded milk and cornmeal used as the starter made the quickest rising and most typical bread.

Lactose agar plates were made from the milk and corn-meal used in this experiment, from the fermenting batter and from the dough. From these plates twelve organisms which seemed to

have distinctive characteristics were transferred to lactose agar slopes, and were later examined more in detail. From this critical examination it was found that there were only six different organisms. Three of these organisms produce gas in lactose agar shake cultures while three do not produce gas.

The three gas producers were used alone and in all their combinations in bread making, using the following procedure. The organisms were grown in flasks containing 50cc. of sterile milk, with 1 gm. of salt, 2 gms. of cane sugar and 5 gms. of cornmeal added. These dry ingredients had been heated 5 hours at 140° C and were supposedly sterile . They were added to the milk cultures of the organisms after the organisms had been growing 6 hours. Then the cultures were incubated 15 hours longer at 38° C, when 100cc. of boiled water and non sterile flour enough to make a stiff dough were added, and the whole made into a loaf. When more than one organism was used, each one was grown separately in a small quantity of milk. The loaf was kept in a warm place, approximately 38° C. until light, then baked. The bread was raised in an hour and a half to twice its volume. Some samples doubled their bulk more readily than others as is shown in Table III.

Table III.

No.of organism	Lightness of Loaf after 1 1/2 hrs.	Character of Bread.
2	Not well risen	———
6	Double initial vol.	Porous,good texture,odorless.
9	Light, but not double volume.	" ,fair " "
2 and 6	Double initial vol.	" ,good " "

Table III continued.

No. of organism	Lightness of Loaf after 1 1/2 hrs.	Character of bread.
2 and 9	Double initial vol.	Porous, good texture, odorles
6 " 9	" " "	" " " "
2, 6 and 9	" " "	" " " "

All of the bread was nearly odorless and had little flavor and was therefore unlike salt-rising bread which has a characteristic odor and flavor. These organisms are a possible leavening agent in salt-rising bread, but it seems do not give the characteristic odor or flavor.

It was thought that perhaps the non-gas producing organisms were responsible for the characteristic odor and flavor, so it was decided to try combinations of these with the gas producing organisms.

In order that the nutrients ordinarily supplied in making salt-rising bread might be given to the organisms in a convenient form, a special medium was prepared, containing 1000 cc. of water, 35 gms. of cornmeal, 15 gms. of cane sugar and 10 gms. of salt. These ingredients were boiled together until the cornmeal was cooked then put into small flasks and sterilized by the intermittant method. It was thought better to have the ingredients combined and then sterilized, than to have each one sterilized separately and risk contamination in putting them together.

Combinations of the gas producing and non-gas producing organisms were used and bread was made with them. Each combination was made in sets of two, (1)Organisms grown in sterile

milk, (2) Organisms grown in sterile special medium. All the bread produced was very much alike. It lacked the characteristic salt-rising odor and flavor but was light and of good texture. All had a noticeably flat taste . Several trials with the organisms gave the same results. There was no noticeable difference between the bread made with sterile milk and that made with sterile special media.

As it seemed impossible to make bread having the characteristic salt-rising flavor with the organisms already isolated, another method of isolation was attempted. It was substantially as follows: the bread was made in the usual way. A portion of the starter was used to inoculate sterile milk and sterile special media, and when this had fermented transfers were made of it as before. The remaining fermenting material was used in breadmaking and the transferring was continued each day from the dilution of the preceding day. By this method of dilution it was thought that the organisms useful in making the bread would grow and supplant those not useful for this purpose, if favorable conditions were given them. After several transfers were made and a good salt-rising bread had been obtained from the resulting fermenting mixture, plates were made in order to isolate the organisms. Then synthetically using these organisms bread was made as before when pure cultures were used.

This method is applied to starters made up in the laboratory and to those obtained from others. Starters were obtained from two housewives well known for their ability to make this; one sample from one of them and two different samples from the other. The results from this study are given in Table IV.

Table IV.

Results of Breadmaking from Transferred Cultures

Date	Sample	Transfer	Medium	Appearance in initial medium	Time & Temp. of incubation
5/14/11	I	____	Milk	Light	Approx. 38°C. 16 hrs.
5/15/11	I	1	Milk	Light	" " 24 "
5/15/11	I	1	Special Medium	Light	" " " "
5/16/11	I	2	Milk	Light	" " " "
5/16/11	I	2	Special Medium	Light	" " " "
5/17/11	I	3	Milk	Light	" " 16 "
5/17/11	I	3	Special Medium	Light	" " 16 "
5/18/11	I	4	Milk	Light	" " " "
5/18/11	I	4	Special Medium	Light	" " " "
6/17/11	II	1	Milk	Light	" " 28 "
6/17/11	II	1	Special Medium	Light	" " " "
6/19/11	II	2	Milk	Light	" 20°C 52 "
6/19/11	II	2	Special Medium	Not Light	" " " "
6/20/11	II	3	Milk	Light	" 38°C 36 "
6/20/11	II	3	Special Medium	Light	" " " "
6/23/11	III	1	"	Light	" " 18 "
6/26/11	III	2	Milk	Not Very Light	" " " "
6/23/11	IV	1	Special Medium	Light	" " " "
6/26/11	IV	2	Milk	Not Very Light	" " " "
7/7/11	V	____	pasteurized M.	Very Light	" " " "
7/8/11	V	1	Milk	Very Light	" " 3 "

Table IV.

Results of Breadmaking from Transferred Cultures

Odor before making into Loaves	Time for Loaves to Rise.	Finished Bread. Odor-Color-Texture-Taste.
Good salt-rising odor.	Slow. Several hours.	Coarse and dark.
Some str. Putre.	Slow. Sev. hrs.	Very bad.
Some strongly putrefactive	Slow. Several hours.	" "
Some stronglyput:	Slow. Several hours.	Not baked.
Some strongly putrefactive	Slow. Sev. hrs.	" "
Some strongly putrefactive	Slow. Several hours.	" "
Some strongly putrefactive	Slow. Several hours.	" "
Some strongly putrefactive	Slow. Several hours.	" "
Some strongly putrefactive	Slow. Several hours.	" "
Slightly putrefactive	Very very slow	Bad odor-dark-coarse.
Slightly putrefactive	Very very slow	Bad odor-dark-coarse.
Strongly putrefactive.	Did not get light:	Not Baked.
Strongly putrefactive	Did not get light:	Not Baked .
Strongly putrefactive	Not made into loaves	
Strongly putrefactive	Not made into loaves	
Strongly putrefactive	5 hrs. 2 X Vol.	Good salt-rising odor. Slightly dark- fine grain-good flavor.
Strongly putrefactive.	6 1/2 hrs. 1 1/2 X Volume.	Very dark and flat.
Strongly putrefactive	5 hrs. 2 X Volume:	Good odor-slightly dark-fine. texture-good flavor.
Strongly putrefactive	6 1/2 hrs. 1 1/2 X Volume.	Very dark and flat.
Rather strongly putrefactive	3 hrs. 2 X Vol.	Slightly sour & dark--fine texture--good flavor.#
Putrefactive & Sour	4 hrs. 2 X Volume.	Good odor and color--fine texture--good flavor.

Not real salt rising bread.

The noticeable thing in this set of experiments is that bread could not be made with transferred cultures after the first dilution, although the starter was always light. It may be that the cultures had been incubated too long before being made into bread and the organisms had formed some product in which they could not grow so well.

In this connection experiments were tried in which transfers were made in milk as soon as the mixture was light, approximately every 1 1/2 or 2 hours. The fermenting mixture was not used in making bread, but lactose agar plates were made from it. The organisms thus isolated were of no use whatever in breadmaking. Perhaps the gas producing organisms cultivated by this method were not able to grow on the lactose agar medium in which it was plated.

The fermenting mixture before making into loaves always had a putrefactive odor even when the mixture was left only until light. It might be that putrefactive organisms are present in such numbers as to retard the growth of the gas producers and thus make the bread slow to become light in the loaf. Plates were made from only one of these transfers, namely sample V., transfer 1. Only three different organisms appeared on the plates. None of these showed any signs of gas production when incubated 24 hours in milk, and none produced any gas when made into loaves. Later when tested in lactose-sucrose agar shake cultures no gas was produced.

It was noticed that whenever bread was made with sterile milk after the ordinary process or when pure cultures were used it was always slightly darker than when made with scalded milk and seemed to have a rather flat taste. There was a tendency to sourness when scalded milk was used which may be due to the period of incubation

which was usually 18 hours.This may be too long and products giving the sourness may be developed. The scalded milk cultures were seldom raised as quickly as should be the case and not as quickly as the sterile milk cultures.

Since the isolation of organisms from the transfers of salt-rising bread mixture were unsuccessful and could not be used in the breadmaking, it was decided to return to the former method of plating directly from the fermenting salt-rising mixture. The first step was to make typical salt-rising bread. Three samplesof meal were used and two samples of milk. Three combinations were used in making the starter. The milk was (1)boiled and added to the meal while hot, (2)ccoled before adding to the meal and (3) used raw. A salt-rising starter was again procured from a well known bread maker and used along with those made in the laboratory. All these starters made bread which was very similar. None of it had the strong putrefactive odor which is so often developed in breadmaking and which many people consider essential to salt-rising bread. The bread was fine grained, of good odor and flavor and became light quickly. It was not noticeably different from the pure culture breads which were being made at the same time . In fact only the persons with the keener sense of taste could tell the difference. But as it was my point to make a product which the persons with the keenest sense of taste could not tell from ordinarily made salt-rising bread, other plates for the isolation of new organisms were made.

Eleven different organisms were isolated, none of which produced gas in lactose agar shake cultures. As they were not gas producers they were used in combination with the old gas producing

organisms in breadmaking. The product with these organisms was not different from the other pure culture breads. At another time eight different organisms were isolated, six of which produce gas. In breadmaking these act very much like previously isolated organisms. One of the non-gas producing organisms gave a noticeably bad odor when grown in milk, coagulating it in 8 hours. This organism when used with a gas producer gave a better flavored bread than had been produced heretofore.

Some sixty four baking tests were made with pure cultures of the isolated organisms to determine which produced the best bread. They were used in various combination, in different media and incubated for different lengths of time. The results of these baking tests are given in Table V.

Table V.

Date	No. of Organisms.	Time incubated in test tube of milk.	Time incubated in flask of milk.
6/20/11	2	16 hrs. 38°C.	8 hrs. 38°C.
"	6	" "	" "
"	9	" "	" "
"	2 6	" "	" "
"	2 9	" "	" "
"	2 6 9	" "	" "
"	6 9	" "	" "
"	2 6 9 1 3 10	" "	" "
6/21/11	2 6 9 1 3	24 hrs. 38°C	16 hrs. 38°C.
"	2 6 9 1 10	" "	" "
"	2 6 9 1	" "	" "
"	2 6 9 3	" "	" "
"	2 6 9 10	" "	" "
"	2 6 9 3 10	" "	" "
6/22/11	2 1 3 10	" "	" "
"	6 1 3 10	" "	" "
"	2 1	" "	" "
"	2 3	" "	" "
"	2 1 3	" "	" "
"	2	" "	" "
"	2 10	" "	" "
7/6/11	2 6 inscalded milk	48 "	" "
"	2 6 in special media	" "	" "
"	11 12 scalded milk	" "	" "
"	11 12 special media	" "	" "

Table V.

Odor from flask	Time for loaves to raise.	Finished Bread. Odor	Color	Texture	Taste.
Mild	Not light in 4 hrs. Left over night. Each had risen and fallen.	Little unpleasant	Yellowish	Fine	Agreeable
Slightly putrefactive		"	Light Yellow	"	"
" "		Little putrefactive	Yellowish	"	Unpleasant
" "		"	"	Medium	Agreeable.
Odor of 2		"	"	"	"
" " "		"	"	"	"
Putrefactive		"	"	"	"
"		"	"	"	Unpleasant.
Slightly unpleasant	2 1/2 X Vol. in 4 hours.	Pleasant	White	Fine	Agreeable.
"	" " " "	"	Dark	"	"
Strong odor	" " " "	"	"	Coarse	Unpleasant
Mild-very little unpleasant	" " " "	"	"	"	"
" " "	" " " "	"	"	"	"
" " "	" " " "	"	"	"	"
" " "	" " " "	Good	Good	"	"
Mild good odor	" " " "	"	"	Fine	"
Slightly unpleasant.	" " " "	"	"	"	"
" "	" " " "	"	"	"	"
" "	" " " "	"	"	"	"
" "	" " " "	"	"	"	"
" "	" " " "	"	"	"	"
Pleasant	1/3 Vol. 6 hrs.	Soured	____	____	____
"	2 X Vol. 4 hrs.	Pleasant	Dark	Fine	Pleasant.
"	Raised very little	Soured	____	____	____
"	" " "	"	____	____	____

Table V. (Continued).

Date	No. Organisms	Time incubated in test tube of milk.	Time incubated in flask of milk.	Odor from flask	Time for loaves to raise.	Finished Bread. Odor	Color	Texture	Taste
7/7/11	2 6 9 scalded milk	24 hrs. 38° C.	16 hrs. 38° C.	Not noticed	2 X Vol. in 3 hrs.	Pleasant	White	Fine	Good
7/7/11	2 6 9 special media	" " " "	" " " "	" "	" " " " "	"	Slightly dark	"	Little flat
7/8/11	2 6 9 scalded milk	" " " "	" " " "	Strong-putrefactive	" " " 3 1/2	"	White	"	Good
7/8/11	2 6 9 scalded milk	8 " " "	" " " "	" "	" " " 3 hrs.	"	"	"	"
7/8/11	2 6 9 special media	" " " "	" " " "	" "	" " " 2 3/4	"	"	"	"
7/8/11	11 12 13 14 scalded milk	24 " " "	" " " "	———	Not raised 7 hrs.	Not baked.			
7/8/11	11 12 13 14 special media	" " " "	" " " "	———	" " " "	" "			
7/18/11	2 scalded milk	8 " " "	" " " "	Pleasant	2 X Vol. in 4 1/2.	Good	White	Fine	Good
7/18/11	6 " "	8 " " "	" " " "	"	" " " 2 1/2	Good	"	"	Very Good
7/18/11	9 " "	8 " " "	" " " "	"	" " " "	"	"	"	Good
7/18/11	2 6 9 " "	" " " "	" " " "	"	" " " "	"	"	"	"
7/20/11	6 15	" " " "	" " " "	Not noticed	" " " 3	"	"	"	"
7/20/11	6 16	" " " "	" " " "	" "	" " " "	"	"	"	"
7/20/11	6 17	" " " "	" " " "	" "	" " " "	"	"	"	"
7/20/11	6 18	" " " "	" " " "	" "	" " " 2 1/2	"	"	"	"
7/20/11	6 19	" " " "	" " " "	" "	" " " "	"	"	"	"
7/20/11	6 15 16 17 18 19	" " " "	" " " "	" "	" " " 3 hrs.	"	"	"	"
7/21/11	6 20	" " " "	" " " "	Odor of 6	" " " 2 hrs.	"	"	"	Pleasant.
7/21/11				" " "	" " " " "	"	"	"	"
7/21/11	6 21	" " " "	" " " "	" " "	" " " " "	"	"	"	"
7/21/11	6 22	" " " "	" " " "	" " "	" " " " "	"	"	Not Fine	"
7/21/11	6 23	" " " "	" " " "	" " "	" " " 3 "	Sour	"	" "	Sour
7/21/11	6 24	" " " "	" " " "	Not real salt-rising odor.	" " " 2 "	Good	"	Fine	Pleasant.
7/25/11	9 15 16	" " " "	" " " "						

Table V. (Continued).

Table V. (Continued).

Date	No. Organisms	Time incubated in test tube of milk.	Time incubated in flask of milk.	Odor from flask.	Time for loaves to raise.	Finished Bread
7/25/11	9 17 18	8 hrs. 38° C	16 hrs. 38° C.	Not salt-rising	2 X Vol. in 2 hrs	Good bread but not salt rising.
7/25/11	9 19 20	" "	" " " "	" " "	" " " " "	" " " " "
7/25/11	9 21 22	" "	" " " "	" " "	" " " " "	" " " " "
7/25/11	9 23 24	" "	" " " "	" " "	" " " " "	" " " " "
7/26/11	2 9	" "	" " " "	Good	" " " " 3 "	Two people who tasted couldn't tell it from salt-rising bread made same day
7/27/11	2 9 Cornmeal added when made into loaf.	" "	" " " "	"	" " " "2 1/2"	Several could not tell it from salt-rising bread made the same day.
7/28/11	25 26	" "	" " " "	"	" " " " "	Good texture and flavor-not salt-rising.
7/28/11	27 28	" "	" " " "	"	" " " " "	" " " " " " "
7/28/11	29 30	" "	" " " "	"	" " " " "	" " " " " " "
7/28/11	31 32	" "	" " " "	Putrefactive	" " " " "	" " " " " " "
7/29/11	25 26 31	" "	" " " "	"	" " " " "	" " " " " " "
7/29/11	27 31	" "	" " " "	"	" " " " "	" " " " " " "
7/29/11	29 30	" "	" " " "	Good	" " " " "	" " " " " " "
7/29/11	31 32	" "	" " " "	Putrefactive	" " " " "	Good flavor probably most like salt-rising bread.

As the bread heretofore had been made with cultures of the organisms grown in sterile milk, and as it tasted rather flat from the fact that sterile milk has a characteristic flat taste, the bread in these baking tests was made with a non-sterile milk. The organisms were grown in test tubes of sterile milk for a time until a strong growth was assured, then these milk cultures were put into flasks containing 50 cc. of milk which had been boiled one minute then cooled.

This mixture was incubated over night at 38°C. To the 50 cc. of milk in the flasks were added 1 gm. of salt and 2 gms. of sugar. By this procedure the flat taste due to the sterile milk is done away with and we have a fairly pure culture in the end. Milk which has not been inoculated shows very little change when subjected to the same conditions as the inoculated milk, for a like length of time.

The fact that the cornmeal in ordinarily made salt-rising bread gives a different texture to the finished bread than that which we have in the pure culture breads where no cornmeal is used, made it necessary to put in cornmeal when making the bread into a loaf. An unsterilized meal was used because it darkens when heated sufficiently to sterilize it and so darkens the bread.

When the meal was added no one who tasted the pure culture breads could tell them from ordinarily made salt-rising bread.

Conclusions Drawn from the Experiments Shown in Table V.

Organisms No. 6, 9, and 32 seem to be the best gas producers isolated thus far. They produce about equal amounts of

gas in breadmaking, and this gas production is not noticeably increased by combinations of these organisms or by combinations with other gas producing organisms. The maximum production in all cases was that of the best gas producing organism used.

The flavor and odor of the bread was not materially affected by any combination of the gas producing organisms or by combinations with the non-gas producing organisms except in one case. This exception is organism No.31, a non-gas producer, which gives a decided flavor and odor to bread in which it is used. This flavor and odor is quite similar to that of the usual home-made salt-rising bread.

Sterile milk and sterile special medium act alike in breadmaking. Both give a flat taste to the bread, which is p perhaps due to the fact that the flavor of milk is changed by being boiled, rather than to the fact that in non-sterile milk organisms are present which develop the flavor and are not present in sterile media. Bread made with non-sterile milk has a less flat taste than the others. The milk had been heated sufficiently high to kill many of the micro-organisms but not high enough to materially change its flavor. Raw milk has a tendency to produce sour bread. In this a great many organisms besides the ones introduced by inoculation are present, and the result is that the bread becomes sour.

In breadmaking the period of incubation of the organisms in sterile milk should not be longer than 24 hours, as putrefaction is apt to begin, and this gives the bread an unpleasant odor. When scalded milk is used, 16 hours seems to be a satisfactory period for incubation. Only a small amount of

putrefaction takes place in this time.

Two tablespoons of cornmeal in a pound loaf of bread gives to the finished product the texture which is characteristic of salt-rising bread.

An attempt was next made to verify the results previously obtained. Salt-rising bread was made in accordance with the usual household method. The results were not altogether satisfactory. This process even under favorable conditions is uncertain as is shown by the following table.

Table VI.

Date	Material	Lightness in Sponge.
11/24/11	Cornmeal	Light
12/13/11	" " freshly purchased.	"
12/13/11	" " 2 months old.	Not very light.
1/23/12	" "freshly purchased	Light
1/24/12	" " " "	Not light.
1/26/12	" " " "	" "
2/7/12	" " " "	Light
2/12/12	" " " "	"
2/21/12	" " " "	Not light.
3/2/12	" " " "	Light
2/21/12	" " 9 samples from U.S.government.	One sample showed a little lightness.
2/22/12	" " " " "	Not Light.
3/2/12	" " " " "	" "
3/6/12	Milkpoured on meal very hot.	Two samples slightly light.
3/9/12	Graham flour (Run in duplicate)	light
3/9/12	Hard wheat flour	Not light.
3/9/12	Whole " "	light.

Table VI.

Finished Bread.	Source of Salt-rising bread Organism. Organism plated.
Good	6
"	2
A little sour.	Not plated.
Rose slowly . Very sour	" "
Not baked	" "
" "	" "
Good	2
Slow to rise, sour	Not plated.
Not baked	" "
Good	5 organisms.
Not baked	Not plated.
" "	" "
" "	" "
" "	" "
Slow to raise. Not typical salt-rising bread.	" "
	" "
Raised well. " " " " "	" "

Several organisms were isolated from these trials and were used in breadmaking as in the previous experiments. Comparisons were made with the bread in which these organisms were used and the bread made with organisms previously isolated as well as bread made by the usual household process. The newly isolated organisms differed from the older ones in cultural characteristics and their microscopic appearance; both were equally capable of producing good bread.

From table VI. we see that both Graham and whole wheat flour can be used as a source of lightness in breadmaking, but these are not so generally used and are not considered as reliable as cornmeal by the housewife. The results obtained with the meal vary somewhat--the fresh meal perhaps being the better. Roller process meal and buhr stone process meal were used in the salt-rising starter. A fermenting mixture was not obtained from either kind except in a very few cases. This meal was rather old being some samples which were sent out from Washington, D.C., which may account for the fact that starters made from it did not become light.

It was found that twenty eight of the organisms which were isolated do not produce gas, while fifteen are gas producers. Each of the twenty eight organisms which do not produce gas was used with gas producing organisms in subsequent trials. Only one of the non-gas producing organisms(No.31) had a marked effect on the quality of bread produced. This bread had a noticeable flavor and lacked the flat taste which is so characteristic of breads made with gas producing or-

ganisms alone. No. 31 was studied in detail. Some of its characteristics are recorded in Table VII. As the other twenty seven non-gas producing organisms had no marked effect on the quality of bread produced, they were not further considered. The more rapid gas producing organisms, however, were studied in detail. These results are given in the following table. (table VII.) .

Characteristics of some of the Organisms Isolated.

Organism	Size	Motility	Spore Formation	Gas Production	Gram's stain	Relation to Oxygen	Liquefication of Gelatin	Peptonization of milk	In Glucose Hydrogen sulphide	Ammonia	Reduction of nitrates	Gas. Dextrose Bouillon	Reaction	Gas - Lactose	Reaction	Gas - Sucrose	Reaction
2	1X2	+	+	+	−	+	−	−?	+?	+?	−	+	+	acid	+	acid	+	acid
6	1X1.4	+	+	+	−	+	+	+	+?	+	−	−	+	"	+	"	+	"
9	1.4X2	+	+	+	−	+	−	−?	+?	+?	+?	+?	+	"	+	"	+	"
31	2X3	+	+	−	−	+	+	+	+	+	+	−	−	"	−	"	−	"
32	1.4X2	+	+	+	−	+	+	+	+?	+?	+	−	+	"	+	"	+	"
36	1X1	+	+	+	−	+	+	+	+?	−	+	−	+	"	+	"	+	"
38	1.4X1.4	+	+	+	−	+	+	+	+?	+	+	−	+	"	+	"	+	"
39	1.4X1.4	+	+	+	−	+	−	−?	−	+?	−	+	+	"	+	"	+	"
42	1X1.4	+	+	+	−	+	−	−?	−	−	−	+	+	"	+	"	+	"
47	1.4X1.4	−	+	+	−	+	−	−?	−	+?	+?	+	+	"	+	"	+	"

There is much similarity among these organisms. With one exception they are bacilli, which form spores, are negative to Gram's stain, and are facultative anaerobes. Their greatest dissimilarity lies in their cultural characteristics. The following data have been obtained from cultures grown at room temperature.

I. No. 2 curdles milk only after ten day's growth. The gelatin stab growth is filiform. In bouillon it produces a rather uniform cloudiness with a white granular sediment and no pellicle. The growth on agar and gelatin is soft, glistening and of a grey white color. The colonies are small and circular with well defined edges. The potato growth is somewhat darkened, soft and spreading.

II. No. 6 coagulates milk in forty eight hours and completely digests it in three weeks, leaving a cloudy yellow whey. In seven days the gelatin is entirely liquid. In bouillon cloudiness is apparent with a white granular sediment and a very thin white pellicle . The growth on agar is soft yellowish and glistening. The colonies are small and circular with a well defined edge. On potato the growth is darkened.

III. No.9 coagulates and digests milk slowly. In eighteen days the top part begins to be digested, leaving a cloudy yellow whey. The gelatin stab growth is filiform. The bouillon remains clear, with a white granular sediment and a very thin white pellicle. On agar and gelatin the growth is white, soft and glistening. The colonies are circular with very definite edges. The potato growth is not darkened.

IV. No. 31 forms a very soft curd in milk and digests it rapidly. Digestion of practically all the curd takes

place in seven days, leaving a cloudy yellow whey. This organism liquefies gelatin quickly in a stratiform shape. The bouillon is clear with a white granular sediment and no pellicle. On agar the growth has a slightly rough appearance with a tendency to dryness. It is grey white in color. The colonies are circular with a rather indeterminate edge. The potato growth is not discolored.

V. No. 32 coagulates milk in forty eight hours and digests it rather slowly, leaving a clear yellow whey. It liquefies gelatin slowly--after eighteen days there was only a small stratiform liquefication. The bouillon becomes slightly cloudy with a white flocculent sediment and no pellicle. On agar the growth is slightly rough looking with occasional transverse creases. It appears dry and comes off in flakes with the needle. It is grey in color. The colonies are small and circular with fairly well defined edges. The growth on potato is very heavy, smooth and not discolored.

VI. No.36. coagulates milk in forty eight hours and digestion takes place slowly, one third of the coagulum being digested in three weeks, leaving a cloudy yellow whey. It liquefies gelatin quickly in an infundibuliform shape. The bouillon becomes cloudy with a white granular sediment--no pellicle is formed. The growth on agar is slightly rough and of a grey white color. The colonies show well defined edges. The potato growth is not darkened.

VII. No. 38 coagulates milk in forty eight hours. Digestion takes place slowly leaving a cloudy yellow whey. Gelatin becomes liquified quickly in an infundibuliform shape. The bouillon becomes cloudy with a white granular sediment and

no pellicle. On agar the growth is of a grey color, very spreading with a flat glistening surface. The colonies are small and circular and have well defined edges. The potato growth is soft, abundant and slightly darkened.

VIII. No. 39 coagulates milk very slowly and the coagulum shows a very slight digestion in three weeks. The gelatin stab growth is filiform. The bouillon becomes cloudy with a white granular sediment and no pellicle . On agar the growth is abundant, raised, glistening and grey. The colonies have a granular appearance. They are small and circular with well defined edges. The potato shows a heavy growth---soft and not discolored. Gas bubbles are noticed in this growth.

IX. No. 42 coagulates milk very slowly and shows that a little digestion has taken place in three weeks. The gelatin stab growth is beaded. The bouillon becomes cloudy with a white floceulent sediment and no pellicle. On agar the growth is spreading, soft glistening and bullate. This growth is of a brownish color. The colonies are circular, rough in appearance and have an indefinite edge. The potato growth is abundant, smooth and not darkened.

X. No. 47 coagulates milk slowly and shows some digestion after three weeks. The whey remaining is yellow and cloudy. The gelatin stab growth is filiform. The bouillon becomes cloudy with a white flocculent sediment and no pellicle. On agar the growth is heavy with a raised center. This growth is soft, glistening and of a grey white col-

or. The colonies are circular, granular in appearance and have no well defined edge. The growth on potato is abundant and slightly discolored.

All of these organisms grow well on the nutrient media used; agar, gelatin, bouillon, milk, potato, and sugar media. The best and strongest growth is made at a temperature of 37°C, although a good growth takes place at 20° C. These stain well with methylen blue and gentian violet. Some of the organisms have been kept growing for a year on agar by making frequent transfers to fresh agar; those which were kept longer than three weeks were sealed to prevent drying.

Since Kohman found that the organisms which he isolated, lose their gas producing power after several transfers have been made in milk, the best gasproducing organisms in this present study were tested to see if these also lost their gasproducing power under these conditions. Sterile milk tubes were inoculated with these organisms and transfers were made to another set of milk tubes twenty four hours later; similar transfers were made for five successive days. The organisms from the third and fifth transfers were tested for gas production in lactose agar shake cultures. They all showed strong gas production. Bread was made from the third transfer and compared with bread made from fresh inoculations of these organisms. The results are summarized in Table VIII.

To Determine if Organisms Lose their Gas Producing Power by Transferring.

Table VIII.

To Determine if Organisms Lose their Gas Producing Power by Transferring.

Organism	Medium	Transfer	Growth in milk	Lightness in dough.	Bread.
32	Milk	3	Coagulated in 24 hrs	Light	Good
42	"	3	Not Coagulated	"	"
47	"	3	Not Coagulated	Not very	Not good.

It should be noted that in two cases(No.32 and 42) the results are not in agreement with those obtained by Kohman. The ability of these organisms to produce gas after several successive transfers is not decreased . The organism(No.47) is slightly weakened by transferring, however, the gas producing power is not altogether lost.

Kohman states that many of the organisms which he isolated lost their power to produce gas when propagated in liquid media. Therefore, a series of experiments were undertaken to determine if similar phenomena take place in the organisms isolated by the author. Inoculations of these were made in milk and bouillon; after a period of six and nine days bread was made from these cultures. The results are summarized in Table LX.

Table IX.

To Determine if Bacteria can be Propagated in Liquid Media from Time to Time.

Organism	Medium	Age of Culture	Lightness in Batter.	Lightness in Dough	Finished Bread
32	Milk	6 da.	Light	Light	Good
32	Bouillon	" "	"	"	"
42	Milk	" "	"	"	"
42	Bouillon	" "	"	"	"
47	Milk	" "	"	"	"
47	Bouillon	" "	Not Light	———	———
32	Milk	9 da.	Light	Light	Good
32	Bouillon	" "	Not Light	———	———
42	Milk	" "	Light	Light	Good
42	Bouillon	" "	Not Light	———	———
47	Milk	" "	Light	Light	Slightly sour.
47	Bouillon	" "	Not Light	———	———

The milk cultures taken after six and nine days produced as much gas in bread as the freshly inoculated cultures with the exception of No. 47. The organisms which were grown in bouillon when transferred to bread dough did not produce as much gas as did the same organisms when grown in milk. This may be explained by the fact that, either all conditions necessary for growth were not present in the bouillon or, substances were produced by the organisms which retarded their development. Comparable phenomena have been observed in the case of lactic acid organisms. It must be concluded, therefore, that some of the organisms that are concerned with the gas production in salt-rising bread can be propagated in liquid media.

An attempt was made to determine if the organisms (No. 2,6,31 and 32) which were found to be most efficient in the making of salt-rising bread, could be preserved in a dried form. They were grown in sterile milk until a vigorous culture had been obtained; they were then mixed with cornmeal, pressed into cakes, and dried. Three months later these dried cakes were used for breadmaking. They produced a light loaf which was, however, not as well flavored as bread made from fresh cultures. The slightly unpleasant flavor produced was probably due to changes which had taken place in the cornmeal as the result of long standing. A dried product was made with other organisms, using flour as the dessicating agent. The results were quite satisfactory. Two months later bread was made from this dried material. It is obvious, therefore, that the market can be supplied with a commercial preparation of these organisms for the making of salt-rising bread.

The bacteria which have been isolated seem to belong to the subtilis or the vulgatus group, although none of them have been identified with any of the organisms given in Chester's Manual of Determinative Bacteriology.

The bread which is made with pure cultures of organisms, such as (No. 31 and 32 or 31 and 42) is in texture and flavor very similar to that of the homemade product. The strong odor characteristic of much of this is lacking. However, the bread made with these organisms may be called typical salt-rising bread.

Conclusions.

Since the writer was unable to obtain the same or-

ganisms from a large number of starters for salt-rising bread, it is obvious that there are a number of organisms capable of producing this bread.

When an organism such as (No.31) is incubated in milk ,changes which are probably putrefactive take place, giving the characteristic salt-rising odor and flavor to the bread.

The gas producing organisms do not lose their power to produce gas, by growing in milk, or by transferring them repeatedly from one milk tube to another.

The organisms producing salt-rising bread can be kept in a dried state without injury to them.

Good salt-rising bread can be produced with pure cultures of certain organisms by using proper technique and the right ingredients.

The odor, flavor and texture of bread made with these organisms are more uniform than those of bread made by the usual home process.

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Approved May 15, 1912.
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