Salt-Rising Bread and Some Comparisons With Bread Made With Yeast.

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[Reprinted from the Journal of Industrial and Engineering Chemistry, Vol. 4, No. 1, January 1912.]

SALT-RISING BREAD AND SOME COMPARISONS WITH BREAD MADE WITH YEAST.[1]

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Received October 12, 1911.

Bread is made light and porous by two methods of aeration. The oldest of these and the one, which is most extensively used at present, involves a process of fermentation, in which various microörganisms produce the gas necessary to raise the bread; by the other method it is given a porous character, either by forcing carbon dioxide under pressure into the dough while it is being mixed, as is done in making aerated bread, or by mixing with the other ingredients certain chemicals, which, when they come in contact with the water used in making the dough, evolve gas, either immediately upon mixing the bread or later when it becomes hot during the process of baking.

The method of preparing bread with the aid of yeast has been extensively investigated and the function of this microörganism in bread is now thoroughly understood. By means of the saccharifying enzyme, diastase, which is present in flour, a part of the starch is converted into sugar, which as well as that normally present in flours and that added in making the dough either as sugar in some form or other, or malt extract, is partly converted by the yeast into alcohol and carbon dioxide which aerates the bread.

Another type of bread, involving a process of fermentation, the so-called salt-rising bread, is made by many housewives and bakers, particularly in the south and is not so thoroughly understood and offers far greater difficulties in its preparation. In its preparation there is added neither yeast nor a portion of fermented dough from the previous baking, as is done in making certain kinds of rye bread and a type of white bread as well. In fact there is nothing added that can be called leaven, and each day's baking is dependent upon a new and independent spontaneous fermentation. The method that is commonly employed is as follows: Cornmeal, salt and soda are thoroughly mixed and stirred into enough hot milk or water,

[1] This work was done under the Fellowship established in the University of Kansas, by the National Association of Master Bakers, and the results have in part been made public in papers read in conventions, and through a series of articles in *The Bakers' Review*.

often boiling, to make a batter of the consistency of corn meal mush. This batter or "emptyings," as it is commonly called, is kept in a warm place 15 to 20 hours or until it becomes light and shows the evolution of gas, and is then mixed with flour and water to make a slag sponge. The sponge is allowed to come up well which may take from one to three hours, and is then mixed with the remainder of the ingredients to make a dough of the usual stiffness. The dough is allowed to stand not longer than an hour but is usually moulded into loaves immediately upon mixing. After it has risen to the degree of lightness desired, it is baked in the usual way.

The literature on the subject indicates that the theory of the leaven in this bread is very incompletely worked out and that there are conflicting views both in regard to the nature of the organism or organisms involved and to its source.

In reference to salt-rising bread Margaret Mitchell[1] says: "When a brew is prepared, but no stack yeast or raw dough is added, it will still be found that in time the mixture will ferment if kept warm. This spontaneous fermentation is due to the fact that yeast spores, when dried, are very light, and are blown about so that they are present almost everywhere. These floating spores may be those of the household yeast, or those of 'wild' yeasts which are common, for instance, in drying fruit, etc., but which are not often cultivated. The spores can enter the brew from the air or from utensils used in mixing. When accidentally planted they grow, as any yeast would, and produce fermentation. Along with the 'wild' yeast obtained in this method of making bread, there are usually also obtained a large number of bacteria, which form bodies of characteristic odor and flavor in the course of their fermentation; and to this is due the peculiar odor and flavor of the salt-rising bread."

She says further: "The uncertainty of this method of making bread is one of its disadvantages, but when it is made, often in the same place the 'wild' yeast most successful in raising this kind of bread is apt to be more abundant in the air, utensils, etc., than other 'wild' yeasts."

Professor F. C. Harrison[2] says the following: "There is another method of making risen bread, that is

[1] *U. S. Department Agricultural Bulletin*, **200**, p. 45.
[2] *Ont. Agricultural Bulletin*, **118**, p. 16.

the method called 'salt-rising.' It is the result of a spontaneous fermentation, and is therefore a matter of chance whether good bread will be produced, although in places where such bread has been made for some time there is less likelihood of failure, as the utensils and air of the rooms in which the bread is made contain large numbers of the desirable germs." Dr. E. H. S. Bailey[1] describes the process as follows: "The salt-rising process depends on preparing a favorable medium in which the yeast germs will grow, and then allowing them to get into the dough from the air, or from the ingredients used in making the sponge. The bread is started by the use of flour, or cornmeal, warm milk, and salt. The meal begins to ferment after a short time, if kept in a warm place, but the fermented material will not have the same taste and odor as the sponge from yeast, as various 'wild' yeasts are sure to be present. It is probable that lactic and butyric fermentation also take place to some extent. Although salt, in any quantities above 1.4 per cent., retards alcoholic fermentation, yet as it even to a greater extent retards the growth of foreign ferments, such as lactic and certain 'wild' ferments, it is probable that its addition is an advantage, on the whole, if this method of fermentation is used. Salt-rising bread is finer grained than yeast bread and has a peculiar and characteristic odor, which is due, no doubt, to the lactic fermentation which has taken place."

Helen W. Atwater[2] describes it thus: "The so-called 'salt-rising' bread is interesting as an illustration of self-raised bread. In it the ferments originally present or acquired from the air produce the fermentation which leavens it. To make it warm milk and cornmeal are mixed together into a stiff batter which is left at blood-heat until the whole mass is sour—that is, until the ferments present have produced fermentation throughout. Next a thick sponge is made of wheat flour and hot water in which a little salt has been dissolved. This sponge and the sour batter are thoroughly kneaded together and set in a warm place for several hours. The leavening action started in the batter spreads through the dough and produces a light, porous loaf, which many persons consider very palatable. Such a bread is comparatively free

[1] "Sanitary and Applied Chemistry," p. 168.
[2] U. S. Dept. Agr., *Farmers' Bulletin*, **389**, p. 21.

from acidity, as the presence of the salt hinders undesirable acid fermentation."

P. G. Heinemann and Mary Hefferan,[1] in an investigation of salt-rising bread, isolated a bacillus which agrees in morphology, staining properties and other cultural characteristics with *B. bulgaricus*. In a stain from the mixture of cornmeal, milk, salt and soda the bacilli present showed a granular appearance, but after growing in milk they stained uniformly. These bacilli, the authors failed utterly to isolate by plating on ordinary laboratory media, but they were readily isolated if cultivated in milk at 37° C. or if plated with milk-agar. In milk the pure culture forms a soft creamy coagulum which does not separate from the whey. After 14 days the milk showed an acidity of 1.65 per cent. and the authors think that the milk is a necessary part as without it the bacillus is unable to produce the acid necessary to liberate the carbon dioxide from the added soda. By inoculating sterile milk with cornmeal it was demonstrated that the origin of the organism was the cornmeal and not the milk.

Erbert J. Clapp,[2] in a paper on salt-rising bread, tells of the extreme difficulties encountered in making it and enumerates the conditions and ingredients that he finds important. It is his experience and that of bakers as well that success depends to a large extent upon the king of cornmeal used. Kiln-dried meal is found to give poor results. Best of all he finds coarse meal, "hulls, shucks and all," as he describes it. The hulls and shucks are strained out, however, in making the sponge. He enumerates potatoes which are not generally used as one of the essential ingredients, but says that river bottom potatoes which are high in moisture and low in starch do not give satisfactory results.

Mr. Clapp's formula differs also from the one ordinarily given in that it includes ginger as one of the essential ingredients; milk, on the other hand, is not used at all and he says its use is to be avoided entirely.

He emphasizes the fact that it is important to scald the vessels used in making the bread and to guard against souring. The evolving gas he observed would burn (explode) when a lighted match is applied which he attributes to the generation of alcohol.

It is evident that while there are conflicting views in

[1] *Science*, June 25, 1909, **29**, No. 756, p. 1011.
[2] *Bakers' Helper*, Nov., 1908, **22**, 1164.

the above references, there are also some points of agreement. All of the writers agree that the methods of making this type of bread are exceedingly uncertain; it is a prevalent opinion, too, that success is dependent very largely upon preparing a favorable medium and allowing the desirable organisms to get into it either from the air or utensils. Dr. Bailey and Margaret Mitchell state definitely that the leavening power is due to the presence of "wild" yeasts and that various bacteria contribute more or less to the odor and flavor of the bread; the bacterial fermentation, in the opinion of Dr. Bailey, is due to lactic and butyric ferments. Professor Harrison and Helen Atwater do not specify what the germs are that cause the spontaneous fermentation.

P. G. Heinemann and Mary Hefferan observed a bacillus which they were able to isolate but they do not say whether yeast was present or not. This bacillus, they maintain, is enabled by the use of milk to produce the acid necessary to liberate the carbon dioxide from the soda.

"SAUERTEIG" METHOD.

There is another type of bread made by the use of the "Sauerteig," which, while it differs from salt-rising bread in many respects, has sufficient bearing upon the subject, as will be seen later, to warrant a brief review of some of the literature on the subject.

Long before the existence of microörganisms was discovered, it was known that when meal or flour and water were made into a paste it would, after a time, begin to ferment and evolve gas. This was early made use of in the preparation of bread and it was soon learned that a portion of the dough could be saved to start the fermentation in the next baking. This portion of dough would continue to ferment and become sour, hence its name "Sauerteig," but when mixed with fresh flour and water it would again become active and raise the bread. This method of making bread was, and in some countries is still, used very extensively, particularly in making whole meal bread ("Schwarzbrot") and rye bread. It is similar to the salt-rising method in that the fermentation in both is spontaneous; they differ, however, in that the former is started with hot water or milk, usually boiling, while the latter is made with tepid water. They differ also in that salt-rising bread is made from fresh meal each time while by the "Sauerteig" method

a portion of fermented dough is saved for the next baking, and when a housewife or baker is out it is usually obtained from a neighbor. This method of making bread, while it is crude and uncertain compared with the methods of to-day which involve the use of compressed yeast, is, as would be expected, more certain than the salt-rising method because each time a portion of dough is saved for the next baking which insures the presence of the essential organisms although they may be badly contaminated with others.

A microscopic examination of a "Sauerteig" reveals both yeast cells and various bacteria in great numbers; at times the former and at times the latter exceed in numbers, and it has been the subject of many investigations to determine the rôle of each in the preparation of bread by this method.

The earliest view was that this bread was leavened by means of an alcoholic fermentation due to the presence of yeast.[1] This view was supported by various analyses that showed the presence of both sugars and saccharifying enzymes in flour, and later the alcohol in the fermented dough. Further, microscopic examination revealed the presence of yeast which was shown to have the cultural characteristics of *Saccharomyces minor* Engel.

In the year 1883 Chicandard[2] advanced a new theory, according to which the fermentation in this bread is due to a bacterium, which he claims produces gaseous products from the albuminous substances in flour.

In support of this theory he says that diastase does not saccharify starch at ordinary temperatures and that there is no decrease in the sugar content of dough during fermentation. As a matter of fact, however, sugar is a normal constituent of flours, and diastase has been shown to be active even at the temperatures at which doughs are ordinarily fermented.

Further, he maintains that there is no alcohol in dough which also has been proved to be contrary to fact. As a further support of his theory he gives an analysis of the evolving gases which consist of about 70 per cent. of carbon dioxide and the remainder of hydrogen and nitrogen. These gases are similar to those evolved from decaying albumin, which he gives as additional evidence in favor of his theory.

[1] *Botanische Zeitung*, **47**, 404 (1889).
[2] *Ibid*, **47**, 407 (1889).

Peters[1] thinks, however, that the carbon dioxide may have been produced by yeast, that the nitrogen may have come from enclosed air and the hydrogen may have been produced by a butyric ferment.

Laurent[2] found that an organism which he called *Bacillus panificans* is normally present in flours. This organism is killed only after heating to 100° C. for 10 minutes and is probably not killed in the baking of the bread. When growing in dough, it produces acetic, lactic and butyric acid, together with CO_2 but no hydrogen nor nitrogen. According to Laurent it is the cause of ropiness in bread, and in the opinion of Maurizio is identical with *Bacillus mesentericus vulgatus*.

An investigation by Dünnenberger[3] leads him to believe that the essential organism in the preparation of bread by this method is yeast and that the presence of bacteria is, at least, unnecessary if not even harmful.

In three papers on "Die Organismen des Sauerteigs und ihre Bedentung für die Brotgährung," Peters[4] reviews the literature on the subject and gives the results of his own exhaustive investigation. He finds numerous yeast cells and many bacteria as well, in a normal "Sauerteig." He succeeds in isolating *Saccharomyces minor*, which has been obtained by previous investigators, another yeast which resembles this species closely, *Mycoderma vini* (*Sacchar. mycoderma*) and *Saccharomyces cerevisiae*. In addition to these four species of yeast he isolated five different bacteria which he describes minutely. He concludes from the results of his investigation that the fermentation of bread by means of the "Sauerteig" is complex in its nature and that no single organism is responsible for all the changes that take place. The Saccharomyces are important in that they produce the alcoholic fermentation which aerates the bread and the bacteria render soluble a portion of the constituents of the flour and produce a certain percentage of acidity which checks various other bacteria that cause diseases in bread.

According to Lehmann's[5] investigation there is present in the "Sauerteig" together with numerous

[1] *Ibid.*, **47**, 408 (1889).
[2] "Getreide Mehl und Brot. Maurizio," p. 238.
[3] *Botanische Zeitung*, **47**, 410 (1889); (*Botanische Centralblatt*, 1889).
[4] *Ibid.*, **47**, 405, 420 and 435 (1898).
[5] *Centralblatt für Bacteriologie*, **1894**, p. 350.

yeast cells a gas-forming bacterium. Upon a gelatin plate made from the dough numerous yeast colonies develop, which upon further examination agree in morphology and cultural characteristics with *Saccharomyces minor* Engel. Among the yeast colonies there usually appear comparatively few colonies of bacteria. If, however, an agar plate is made and incubated at about 37° C. the yeast colonies do not appear, but numerous colonies of bacteria develop, among which one form greatly predominates, others appearing far less plentifully and regularly. This predominating organism which he calls *Bacillus levans* grows on gelatine plates in white water colonies which are spherical in shape with a rather darker zone in the center. It is facultative anäerobe and grows in an atmosphere of CO_2. It is a gas former and now and then bubbles will appear even in sugar-free bouillon gelatine media. In the presence of sugar it produces gas in gelatine and agar media both on plates and in stab cultures. In bouillon media it produces cloudiness while if sugar is added gas is evolved. The organism is motile; does not form spores.

The gases that evolve from sugar bouillon, outside of a little nitrogen, consist of approximately $^1/_3$ hydrogen and $^2/_3$ carbon dioxide. Hydrocarbons were not detected. In sugar-free bouillon only very small quantities of hydrogen were produced and no carbon dioxide. A more complete analysis of the gases produced by this organism is given by Maurizio. It was grown in sugar-bouillon and the results of the gas analysis follow:

	Beerwort.	Diluted beerwort.	Sugar-free bouillon.
	1.	2.	3.
Carbon dioxide	68.9	66.8	63.7
Hydrogen	25 4	28.7	31.8
Nitrogen	5.7	4.5	5.5

The variation indicated by the figures above he attributes to the fact that carbon dioxide is absorbed to some extent by the medium.

When grown in different media the gases produced by this bacillus show considerable variation.

	Beerwort	Diluted beerwort.	Sugar-free bouillon.
	4.	5.	6.
Carbon dioxide	68.4	63.8	0
Hydrogen	22.1	28.7	67.1
Nitrogen	9.2	7.5	32.9

There were obtained from 600 cc. 1 per cent. sugar-bouillon 300 cc. of gas, while in sugar-free bouillon

[1] "Getreide Mehl und Brot. Maurizio," p. 235.

only 30 cc. of gas were obtained from the same amount of media.

The acids that this organism normally produces in media containing sugar are designated as acetic, lactic and oxalic.

Lehmann succeeded in setting up a gaseous fermentation in flour, which had been sterilized by submerging in ether for several days and regained from the ether by evaporation, by subsequent moistening with sterile water and inoculating with this *Bacillus levans*. The evolving gases were the same as those from sugar-bouillon fermented by this bacillus, and the odor the same as that of the "Sauerteig." A similar portion of the sterile flour inoculated from the "Sauerteig" fermented in apparently the same way but an analysis of the gases showed, however, that hydrogen was invariably absent, which would indicate that this is due to the presence of yeast and that the gas in the "Sauerteig" is not produced by *Bacillus levans*.

A control in which the sterile flour was inoculated with both *Bacillus levans* and yeast also failed to yield hydrogen in the evolving gases, which would perhaps indicate that the bacillus is not active in the presence of the yeast. Also when both organisms were inoculated into the media the maximum evolution of gas was obtained much sooner and with a smaller degree of acidity than when the fermentation was due to *Bacillus levans* alone.

Outside of the rôle that *Bacillus levans* plays in bread-making, whether it be desirable or not, it is of interest from another point of view. It was observed by Wolffin and Lehmann that it strikingly resembles *Bacillus coli commune* especially in its morphology. Also it was possible to set up the same gaseous fermentation in a paste made from sterile flour and water with *Bacillus coli commune*, as occurs when the same material is inoculated with *Bacillus evans*. Both ferment maltose, grape sugar and lactose. *Bacillus coli* differs, however, in that it coagulates milk and also in the composition of the gases it produces.

When bouillon with 1 per cent. of grape sugar added was fermented with *Bacillus levans* and *Bacillus coli commune* the following gases were obtained:

	Bacillus levans.	*Bacillus coli commune.*
Carbon dioxide	66.5	22.3
Hydrogen	28.6	75.6
Nitrogen	4.9	2.1

The gases collected from a paste of sterile flour and water exhibit no marked variation from the above table as may be seen from the following:

	Bacillus levans.	*Bacillus coli commune.*
Carbon dioxide	66.5	23.3
Hydrogen	27.7	74.0
Nitrogen	5.8	2.7

Lehmann questions whether these differences in the composition of the gases are sufficient to distinguish this bacillus from the coli group, particularly when the variability of these bacteria is taken into account. Members of this group have been known to lose their ability to coagulate milk, or to ferment sugar after having been grown on sugar-free media for some time. Maurizio also is of the opinion that *Bacillus levans* belongs to the coli group and he, too, concludes, as Peters, that it is the function of the yeast to produce the gas necessary to aerate the bread, and further that various lactic bacteria are desirable in that they check the action of objectional forms such as butyric bacteria and members of the coli group.

The disagreement in regard to the theories concerning the leavening agent in salt-rising bread and the necessary ingredients, the uncertainty of manufacture, and the lack of uniformity in the finished product lead the author to investigate the subject, in order to ascertain definitely, if possible, what the leavening agent really is: whether "wild" yeasts, as the majority of writers on the subject claim; or the interaction of lactic acid formed by bacterial fermentation, with soda as others claim; and then perhaps to obtain the desirable organism in pure culture and propagate it for the production of bread of uniform quality, thus substituting more scientific methods for making this type of bread.

OBSERVATIONS.

The first step in the investigation was to start the fermentation from cornmeal, as is regularly done, and then also to make the bread in order to be certain that the fermenting mass contained the essential organisms. The first experiences of the author in making this bread were in harmony with the references

on the subject in regard to the uncertainty of the method, and even after the bread had been made successfully a number of times, failures were of frequent occurrence, although care was taken to control temperatures, proportions of ingredients, etc.

After having made the bread successfully a number of times, a microscopic examination of the "emptyings" prepared in the customary way was made. This was found to be literally teeming with bacteria while yeast cells were not to be found. Since this was contrary to what was found in the literature, the examination was repeated again and again; each time, however, bacteria were present in great numbers while yeast cells were invariably absent. This indicates that yeasts play no part in this bread, and the question arises as to how these bacteria function in the dough, whether by producing acid which liberates carbon dioxide from the soda or by decomposing some of the constituents of the flour into gaseous products. It was observed that if a portion of these "emptyings" was transferred to sterile milk by means of the platinum loop, gas began to be evolved after 8 or 10 hours, and that a curd which was broken and full of holes, due to gas bubbles, would form. It was possible to transfer these bacteria from tube to tube and each time the same gaseous fermentation would be set up, which would indicate that the evolving gas is produced by the bacteria from the milk itself, and it becomes of interest to determine the relationship of the bacteria present in the fermenting batter.

It is only in exceptional cases, if ever, that a sample of natural media contains but a single species of microörganism when in a state of fermentation. Often the activity of two or more species in the same media produce changes which neither could do of itself. When several species are simultaneously engaged in the consumption of the same medium, their association is termed symbiosis; as an example of this we may refer to the kefir grains which contain at least one species of yeast and two species of lactic bacteria, the simultaneous growth of which produces from milk the beverage known as kefir. Another common association of microörganisms is styled metabiosis, in which one species by its activities renders the medium suitable for the growth and development of another. A good example of this is the formation of vinegar from cider, due to the presence of yeasts which form alcohol from the sugar,

and acetic bacteria which, after the alcoholic fermentation has ceased, transform the alcohol into acetic acid. The "Sauerteig" is perhaps an example of both symbiosis and metabiosis, inasmuch as there is the simultaneous consumption of sugar by yeasts and lactic bacteria, which relationship is that of the former, and later the conversion of part of the alcohol formed by the yeast into acetic acid, which, as it occurs in the manufacture of vinegar, has already been referred to as an example of metabiosis.

The microscopic examinations having shown that active microörganisms in salt-rising bread are bacteria, it becomes a question whether or not a pure culture can be obtained from the "emptyings" with which alone the bread can be made, or whether the necessary gaseous products of fermentation owe their origin to the combined activity of several species growing side by side.

After transferring the bacteria from one milk tube to another for several times and incubating at 40° C., it was observed that there was exhibited no great variety of forms, indeed the cells were strikingly alike except for slight differences in the length of rods.

It was therefore assumed that the fermentation is due to a single species and plates were made to obtain it in pure culture. The first set of plates was made with an agar-bouillon media and incubated at 40° C. After about 15 hours a good growth had appeared and a number of milk tubes were inoculated from those colonies which seemed to show differences and incubate at 40° C. The next day all the tubes showed a growth, for a soft curd had formed in the milk, but none of them showed any signs of gas production while the one inoculated from the tube from which the plating was made was giving off gas. Since the first plating resulted in a failure, it was repeated. Again numerous colonies appeared in the usual time and this time a larger number of tubes were inoculated from them and incubated at 40° C. Again a curd formed in each of the tubes but there were no signs of gas. After making numerous plates with this agar bouillon medium and isolating many cultures without obtaining any that would produce gas it was thought that perhaps the medium was ton favorable for the gas forming bacteria and that it either did not appear upon the plates at all or became attenuated

during the planting and lost its ability to produce gas.

Since soda is commonly used in making the bread it was thought that an alkaline medium might be more suitable. Therefore a portion of nutrient agar medium with 1.0 per cent. added lactose was titrated with phenolphthalein to an alkalinity of 1 per cent. and another to an acidity of 1 per cent. With each of these plates were made, both directly from the "emptyings," and a tube that was the result of several transfers, and numerous tubes inoculated and incubated at the usual temperature of 40° C., but again all efforts to obtain a gas-forming culture were in vain.

An effort was made to make a medium as similar as possible to the original "emptyings" by adding a portion of cooked flour to the agar medium. This medium, however, was so opaque that it could not be used successfully and was abandoned. As milk is commonly used in making this bread, plates were made with a milk-agar medium and a number of milk tubes inoculated and incubated. Upon observing them the next day one of them showed the characteristic gaseous fermentation. From this tube another set of plates was made and several tubes inoculated. These again were giving off gas after incubating over night. Since milk-agar plates are always rather opaque it was thought advisable to plate this culture upon clear media so as to be more certain that it was pure. This was done but not a tube produced any gas after incubating. This then was either not a pure culture or it lost its ability to produce gas during the plating on clear media. Further attempts to obtain another gas-forming culture by plating with milk-agar failed utterly.

It was observed that when the predominating culture from the fermenting batter was isolated and grown in milk that after a curd had formed the milk began to be peptonized near the surface and after standing a few days a large part of the curd was peptonized. The same effect was observed when milk-agar tubes were inoculated with this culture, only the peptonization proceeded downward in the tube much slower. In milk-agar tubes, inoculated direct from the fermenting batter, the peptonization of the milk was also observed near the surface while farther down gas was formed which at times pushed part of the media together with the cotton plug out of the tube. This latter effect was observed in broth-agar tubes also when the

media was boiled to expel air. Plates made from the organisms after having been grown under these anaerobic conditions yielded no culture that would produce gas.

When milk-agar plates are poured in Petri dishes about $^3/_4$ of an inch deep and 6 inches in diameter, the milk begins to peptonize from the top downward until after several days the medium loses its opaque character entirely and becomes yellowish in color. In these deep plates gas bubbles at times form near the bottom of the plates which may be due either to a single organism or to several. To obtain cultures of these, the plate was inverted to allow the medium to drop out, when tubes could be inoculated by touching the gas bubble with a platinum loop. Milk tubes inoculated in this way showed the characteristic gaseous fermentation after incubating over night, but when plates were made from them and tubes inoculated from colonies, no gas appeared.

Single cells isolated from the fermenting batter by Dr. M. A. Barber's pipette method reacted in milk as those obtained by plating. A soft curd was first formed which later was peptonized from the top downward. When allowed to grow under a cover glass in an incubator at 40° C. a good growth was observed both in milk and broth. Spores formed readily. Single spores put into sterile milk and incubated produced the same reaction in milk as the cells themselves. No culture was obtained by this method that produced gas.

After all these failures to obtain a culture that was pure without a doubt and would retain its gas-producing power, it was thought that perhaps this gas formation was due to a mixture of cultures—perhaps a case of symbiosis or metabiosis. Upon this assumption plates were made and a number of tubes inoculated and incubated. After a good growth had set in, the tubes were numbered and fresh tubes inoculated from them by means of a platinum loop, mixing the various cultures. For example one tube was inoculated from Nos. 1 and 2, another from 1, 2 and 3, etc. The results of this mixing of cultures was as fruitless as all previous efforts, not a single tube showed any gas.

When sterile milk is inoculated from the fermenting batter, gas is driven off after incubating 6 to 10 hours and a curd is formed which is carried to the top by

the gas bubbles and then assumes a tough leathery character. If after incubating for three or four days, sometimes less, a transfer to sterile milk is made, no gas appears. The same loss of gas production occurs in three or four bays if transfers are made at intervals of either 12 or 24 hours. A falling off of gas production is also observed in the fermenting batter; after gas bubbles begin to form the rate of gas production increases for about three or four hours and then gradually decreases. After fermenting for 8 to 10 hours, it becomes very much weaker and does not regain its strength if used in making the sponge and dough. In the latter the weakness is even more apparent than in the former, and when once the batter has fermented too long, the loaves are apt to be only about one-half the normal size. This gradual weakening of the batter has been observed by bakers, as well, and is popularly termed "working itself out." They also observed that when once it has become weakened and lost its gas-producing power that it will not regain its strength by the addition of fresh food material as is done in making the sponge and dough. While it was not possible to propagate the bacteria in liquid media from time to time to be used in making bread, a dried product was prepared which could be used at will with good results.

The product was used by the author in making salt-rising bread in numerous experiments and was also given out to a number of housewives who used it successfully. That the artificial cultivation of bacteria can be of value to the manufacturing baker as well was demonstrated by giving this product a thorough trial in a modern baking plant where the bread was not uniform and it was necessary at times to add compressed yeast to insure proper aeration. From 800 to 1000 loaves were made daily for a month and the bread was uniform in quality and was ready for the bench at the desired time every day while formerly it was often ready sooner or later than desired and consequently upset the system of the plant. The batter made from the product begins to ferment not only more regularly but sooner than if made with meal. One pound is sufficient for the production of 400 to 500 loaves of bread.

The fact that the fermenting "emptyings" made from this product reveal cells which are strikingly alike, a large sporebearing motile rod greatly pre-

dominating, and that once a gas-forming organism was obtained by plating, and this one lost its ability to produce gas after incubating several days, indicates that the gas formation owes its origin to a single organism, which becomes attenuated during the plating. This is corroborated by the fact that a falling off of gas formation is observed both in the batter and when transfers are made to sterile milk, and is further supported by the fact that from either broth or milk-agar plates, in which the colonies were extremely thick, portions of media so large that all organisms should be included could be transferred to sterile milk and no gas would be evolved, which seems clearly to indicate attenuation.

Another instance in which the loss of gas production was observed may be referred to here. In a modern bakery bread is mixed with huge mixers in lots of about 800 to 1500 pounds of dough. A certain make of these mixers is run at very high speed making about 60 revolutions per minute, which is several times that of the average mixer. This extreme treatment is considered very desirable in making ordinary bread as it develops the elasticity of the gluten and improves the color of the bread. A batch of salt-rising bread was tried in one of these mixers, taking out a portion of dough after mixing 6, 15 and 30 minutes, respectively. The loaves made from the dough that was mixed 6 minutes rose very slowly, and were poor in quality, while the loaves from the portions of dough that were mixed 15 to 30 minutes, respectively, failed utterly to rise, even after standing several hours, while when mixed in a slow-speed mixer they rise in 30 to 45 minutes.

Such variation is not without parallel as may be seen from observations made by Klein,[1] on *Bacillus enteriditis sporogenes*. If deep liquid sugar-gelatine tubes (8 cm.) are inoculated with the bacilli or spores of this organism and incubated at 20° C., the medium is liquefied after 48 hours, with the formation of very few indeed, if any, gas bubbles and the organism sporulates freely. If, on the other hand, a stab culture is made from the same culture into a similar medium, gas formation begins within 24 hours and the medium is torn and rent by the evolving bubbles. In such a culture in which gas is abundantly given off, complete liquefaction takes 8, 14, or even 20 days, instead of

[1] *Centralblatt für Bakteriologie*, **18**, I Abteilung, No. 24, p. 737 (1895).

48 hours, and spore formation is not observed. The gas is principally methane.

A violent evolution of gas, after incubating from 1 to 2 days which breaks the layer of cream, accompanied with the formation of a curd which separates from the clear acid whey, is given by Klein[1] as the typical characteristic of this organism in milk.

If two gelatin tubes are inoculated with spores and the bacilli of the organisms, respectively, and incubated for several days, the results are so different that it is difficult to believe that the two cultures are identical, for the former shows the typical spherical liquefying colonies, while in the latter only small non-liquefying spots appear.

By transferring spores of the organism to fresh sugar-gelatine for a number of times, it undergoes marked changes in its cultural characteristics. It soon acquires greater liquefying powers, a tendency to form threads and the ability to rapidly form spores. In milk these variations are quite pronounced, for it no longer produces the changes which have been described as typical. Instead of the violent evolution of gas which disturbs the layer of cream, the formation of a curd and the separation of a clear whey, the milk undergoes an entirely different transformation, in which there is no gas whatever evolved and the cream is not disturbed. After several days the milk immediately underneath the cream begins to be decomposed into a yellowish solution which remains separate from the white coagulum underneath. The whey in the typical milk culture smells of butyric acid and reacts acid while the yellow solution of the atypical culture is weakly alkaline and has a foul odor. The bacilli in the typical milk culture are short rods without spores, while those of the atypical culture often appear in threads which as well as the short rods sporulate within a few days. When once the organism has changed so that spores when repeatedly transferred to milk continue to give the atypical culture characteristic, the change is permanent and it does not revert to the original form. The virulence in the atypical is almost or entirely lost.

On the suggestion of Professor Stephens some "emptyings" were obtained from a lady who makes salt-rising bread which he at one time found to be teeming with both yeast cells and bacteria. This

[1] *Centralblatt für Bakteriologie*, **22**, I Abteilung, p. 577.

was contrary to what had been observed, but as it agreed with a number of the references a microscopic examination was made, but again many bacteria were present and no yeast cells. From these "emptyings" a set of plates was made and a number of tubes inoculated. On removing them from the incubator the next day several of them were evolving gas. One of these cultures was plated repeatedly on both milk and broth-agar media and each time all the colonies appeared alike and all the tubes inoculated were evolving gas after standing in the incubator for about 15 hours, which is sufficient evidence that it is a pure culture, and it becomes a question as to whether it is capable by itself to properly ferment bread. The mere fact that it will produce gas when growing in milk is not sufficient evidence that bread can be made with it, for often the "emptyings" will apparently be fermenting normally, but when made into a sponge by the addition of water and flour the production of gas almost ceases, and when the dough is made it fails utterly to rise. Indeed at times both the "emptyings" and sponge will appear normal and the dough will fail to rise, and it has happened in practice that a batch of about 1000 pounds of dough was run through the mixer, divider and moulder, and after being put into pans failed to rise and had to be taken from the pans and mixed with compressed yeast.

In order to determine whether this bacterium is of itself capable of aerating bread, it is of course desirable to use sterile flour; this unfortunately can be obtained only with difficulty and perhaps not at all without altering the character of the flour and destroying to a certain extent its bread-making qualities.

One of the two methods that have been employed is by heating the flour to a temperature of 115° to 120° C. This temperature is not sufficiently high to completely sterilize the flour for spore-forming organisms certainly survive, and besides it darkens the flour and, according to Peters, destroys its ability to form a dough. The other method of submerging the flour under ether for several days is more satisfactory, as it is less injurious to the flour, but it is a difficult matter to sterilize enough to be used in baking tests.

In these experiments it was thought best not to sterilize the flour but to grow the bacteria in sterile media up to the time the sponge was made, which would give the bacteria in the flour only from two to four hours to become active, for it was never more

than four hours from the time the sponge was made until the bread was put into the oven and usually between two and three hours. When a dough was made without the addition of either bacteria or yeast, no marked changes were apparent within three or four hours, and if yeast was then worked into it, it would begin to rise and after fermenting properly, nearly normal bread could be made, which would indicate that the bacteria in flour do not have a marked effect upon a dough within three or four hours.

As already stated, milk is a common ingredient in salt-rising bread, and as it can be sterilized conveniently, it was used in the following baking experiments. With this pure culture bread was made from the following ingredients: 800 grams of flour, 100 cc. sterile milk, 410 cc. water, 12 grams salt and 15 grams sugar. The sterile milk was inoculated with the pure culture obtained and incubated 17 hours, when it was made into a sponge with 175 grams of flour and 200 cc. water. After the sponge began to drop, which took about two hours, the dough was made by mixing the sponge with the remainder of the ingredients and moulded into loaves and put into pans at once. The bread rose well and was baked in the usual way and had the characteristic odor and taste of salt-rising bread. This experiment was repeated a number of times, which indicates that this bacterium is able by itself to properly aerate bread. Bread was also made by the "straight dough" process in which all the ingredients are mixed at once leaving out the sponge stage. In this experiment the whole of the liquid used was milk fermented by this bacterium, which was made into a dough by mixing with the other ingredients. The dough was at once moulded into loaves and put into pans. It rose rather slower, than when a sponge was made, and produced bread of poorer quality. "Straight dough" bread was also made by using as the liquid $^1/_2$ fermented milk and $^1/_2$ water; also by allowing the bread to rise, as is always done in making yeast bread, previous to moulding into loaves. This bread rose faster in the pans and the loaves had more of a tendency to crack. The bread was coarser in texture and when cut crumbled easily.

This bacterium could be propagated by transferring to fresh milk occasionally. After standing two months, however, it had become much weaker, and while it would still produce gas when grown in milk, it

would no longer raise bread. Even by transferring to fresh milk a number of times it failed to regain its strength. After 200 cc. of milk fermented with this organism had been kept for 9 months in a German flask closed with a cotton plug, it was impossible to abtain any growth whatever by inoculating sterile milk from it.

An effort was made to prepare a dry product containing this organism to be used in making bread. This was done by growing the pure culture in sterile milk and then mixing flour to make a sponge. After the sponge had risen and fallen it was spread on panes of glass in thin layers and allowed to dry. Some of this dried product was mixed with meal and was used in starting the bread in the usual way. When the liquid used in starting the "emptyings" was heated to the boiling point the addition of some of this dried material seemed to make no appreciable difference either in the regularity or the time it would take to show the formation of gas bubbles, as was observed by setting at different times, a number of emptyings in Erlenmeyer flasks plugged with cotton, both with and without any added material. When the meal was stirred into the boiling milk a temperature of 85° to 90° C. was obtained. When the milk was heated to 90° C., it seemed rather doubtful whether the addition of some of this product was noticeable; if heated to 80° or 70°, however, it was observed that gas began to form both sooner and more regularly if a portion of the dried product was added, which would indicate the possibility of preserving this organism in the dry state for its economic use.

The source of the organisms involved, especially when the bread is made without adding them by artificial means, is one of the important considerations in making this kind of bread. It is the opinion of Dr. Bailey, Professor Harrison and Margaret Mitchell that they get into the prepared medium either from the air or utensils and that failures are far less frequent in places where the bread has been made for some time. To determine whether or not the desired bacteria get into the medium from the air, a number of Erlenmeyer flasks were partially filled with the usual ingredients in the proportions that they are used in the "emptyings." These flasks were plugged with cotton and sterilized by steaming for about 45 minutes on three or four consecutive days. After incubating for a few days to make certain that all life in the

flasks was destroyed, the plugs were pulled and the flasks set in a gas oven which had been repeatedly used as a place to keep the "emptyings" and sponges as well while they were fermenting. If these bacteria are propagated from one baking to another by means of the air then this oven should prove a particularly fertile source, and as the media was properly prepared and the temperature carefully regulated, these flasks should soon show the characteristic fermentation. As a matter of fact, however, it was only occasionally that a gaseous fermentation would develop in these flasks and then with great irregularity; the gas bubbles would first appear near the top of the media and later they would form farther down and never did they occur uniformly throughout the media as is the case when the "emptyings" are set in the usual way. Often an abnormal fermentation would occur in these flasks: at times this would become manifest in a souring of the media without any gas formation, and at other times various moulds would appear on the surface of the media. This would indicate that the air is not the true source of the gas-forming organism, and although it may get into the media from the air at times it cannot be relied upon to produce the desired fermentation with any certainty or regularity.

In how much the utensils serve as a means of propagating the bacteria evidently depends to some extent upon the operator; if they are thoroughly cleaned each time after use, the chances are that the bacteria are nearly all removed with the ingredients; on the other hand if carelessly cleaned, it is possible that enough bacteria will be retained to again start the fermentation. At best, however, this cannot be a reliable source, and as the bacteria would be only on the sides of the vessel, some time would elapse before they would get into the center of a non-liquid medium. It is the experience of the author that it matters little, if any, whether the "emptyings" were made in vessels that had been previously used or not.

Evidently the milk is not the source of the organisms for the bread can be made with water, also it is possible to obtain the gaseous fermentation by inoculating sterile milk with cornmeal. The true source seems to be the cornmeal, and it is the experience of bakers and housewives that the more highly cleaned meals do not give as good results, which would indicate that it is associated in some way with the exterior of the grain of corn. It is the experience

of housewives, and canning factories as well, that it is a difficult matter to can corn so that it will keep; often the ends of the cans bulge out, owing to the formation of gas which may be due to the survival of the salt-rising organisms.

As already stated, it has been a matter of much dispute as to what ingredients are necessary in making salt-rising bread, and something over two hundred baking experiments were made to determine the necessary ingredients, the proportions in which they would be used, the most favorable temperature, etc. These baking experiments were taken up as systematically as possible, observing the effect of changing one thing at a time both upon the rate of the evolution of the gas and the character of the bread produced, and keeping a careful tabulated record of the results, some of which will be given here. One series of these experiments was made to determine the effect of milk, and the following table shows the proportions of the ingredients used, when they were mixed in "emptyings," sponge and dough, and the temperature of each (see Table I). The ingredients in Nos. 101, 102 and 103 are precisely the same in quantity, differing only in that No. 101 had the milk added in the "emptying," No. 102 in the sponge and 103 in the dough. It is evident from the table that the emptyings in 101 were light and ready to be made into the sponge by 8:50, while 102 and 103 required 40 minutes longer; in the sponge it was observed that 101 was more active than 102 and 103 still more sluggish. In the dough the advantages of adding the milk in the early stages were still more apparent. The bread from 101 was largest in volume and best in every way, No. 102 was rather heavy and 103 failed to rise more than about one-half the usual height. From these experiments it is evident that milk has a marked influence upon the growth of the bacteria and consequently upon the character of the bread produced. Various baking experiments were made to determine to which of the constituents of the milk to attribute this.

It was found that whole milk did not give appreciably better results than skimmed, which would indicate that the butter fat, while certainly it has a "shortening" effect, as all fats do, has no bearing upon the gas production of the organism. The other two important constituents of milk, namely the casein and milk sugar, were used separately in making the bread. Four different samples of casein were obtained:

	1909.									1910
Batter	Date	11/3	11/3	11/3	11/14	11/14	11 14	11 14	11/10	11/10	5/9
	No.	101	102	103	132	133	134	135	112	115	378
	H_2O	150	150	150	150	150	150	150	150	150	150
	Dried milk	10	10	10	10	5A[1]	10A[1]	5B[2]	10M[3]	3C[4]	5
	Cornmeal	20	20	20	20	20	20	20	20	20	22
	Sugar	4	4	4	0	0	0	0	0	0	...
	Starter	2	2	2	2	2	2	2	2	2	2
	Soda	2½	2½	2½	2½	2½	2½	2½	2½	2½	2
	Time	9.30	9.30	9.30	10.30	10.30	10.30	10.30	10.30	10.30	8.30
Sponge	H_2O	200	200	200	200	200	200	200	200	200	200
	Dried milk	0	10	0	0	0	0	0	0	0	0
	Flour	150	150	150	150	150	150	150	150	..	150
	$NaHCO_3$	0	0	0	0	0	0	0	0		
	Time	8.50	9.30	9.30	12.05	9.30	9.30	9.30	8.30	..	2.20
	Temp.	40	41	41	43	42	40	40	40	..	41
Dough	H_2O	200	200	200	200	200	200	200	200	...	200
	Flour	650	650	650	650	650	650	650	650	...	650
	Sugar	0	0	0	0	0	0	0	0	..	0
	Salt	12	12	12	12	12	12	12	12	..	12
	Oil	0	0	0	0	0	0	0	12	..	0
	Time	10.00	11.00	11.40	1.05	10.50	10.45	10.35	9.55	..	3.30
	Temp.	37	37	37	38	39	38	38	39	..	41
	Oven	11.00	12.10	..	2.00	10.15	12.00	12.00	11.05	...	4.25
	Out	11.45	..	..	..	..	..	..	11.35		

[1] A in 133 and 134 is casein from skim-milk.

[2] B in 135 is casein from another sample of skim-milk casein.

[3] M in 112 is malt extract.

[4] C in 115 is asparagin.

Note.—All batters, except in No. 378, were set in the evening at the time given and the sponges in the morning of the next day. No. 378 was finished in one day.

two made from the buttermilk and two from skim milk.

It was found that when the "emptyings" were set as in No. 101 with 10 grams of casein instead of 10 grams of powdered skim-milk, gas began to form several hours sooner, especially when the buttermilk caseins were used, as may be seeen from bakings 132 and 134. Even five grams of casein which is about the equivalent of casein in 10 grams of powdered skimmed milk was more effective than the latter and almost as effective as 10 grams of the same material. In baking Nos. 133, 134, 135, all of which were made with casein, the gas began to be evolved about 2½ hours sooner than in No. 132 which was made with powdered skimmed milk. No. 131 showed signs of gas about ½ hour sooner than 133 and 135 but it was made into a sponge about the same time.

When lactose was used instead of milk in the above formula the gas began to form little sooner than if it was not used. Both lactose and casein used together were apparently no more effective than the same amount of casein alone. These experiments would indicate that the essential constituent of the milk is the casein rather than the milk sugar or butterfat. The two samples of butter milk casein, as stated above, favored the gas production more than those made from skim-milk and also more than the powdered milk itself. This may perhaps be partly explained by the fact that the casein from buttermilk undergoes a partial hydrolysis during the fermentative process of buttermaking, which may render it more assimilable by the bacteria. Also it was observed later that 2½ grams of soda in the above formula made the media rather alkaline and checked the fermentation somewhat, and as the caseins, especially those made from buttermilk, reacted acid, neutralized a part of this alkali and made the medium more favorable to the growth of the bacteria.

Malt extract which is used extensively in making ordinary bread also proved to be of value in making salt-rising. By the use of 10 grams of malt extract as in baking No. 112 it was possible to make good salt-rising bread. The value of malt extract is perhaps to be attributed both to its content of maltose and various soluble nitrogenous substances, and it is a question whether the enzymes have the same value that they do in making bread with yeast, especially

those that have a proteolytic action, for the bacteria themselves have a marked action upon the gluten.

The addition of three grams of asparagin, as in No. 115, was detrimental and there was no gas production even after standing for several days. Ammonium nitrate was added to the formula in quantities varying from $^1/_2$ to 5 grams, and it was found to retard the production of gas in all cases and when more than about 2 grams were used, gas failed to be produced at all. Potatoes and ginger, which are at times used, were tried but no beneficial effects were observed.

A series of "emptyings" were set in the same way using different sugars, *i. e.*, maltose, cane sugar, dextrose and lactose, and it was found that the gas began to evolve sooner than if no sugar was added to the meal. These sugars, as near as could be observed from the time bubbles began to form, were all fermented equally well and also as far as the production of bread is concerned.

These results were confirmed by adding 1 per cent. of cane sugar, maltose lactose, dextrose and dextrin respectively to separate portions of a nutrient agar medium and inoculating with a small loop full direct from the fermenting batter. The agar medium to which the carbohydrates, named above, were added contained 10 grams peptone and 5 grams beef extract to a liter of water. Seven hours after inoculating the tubes containing carbohydrates were all rent with gas bubbles and the medium in each tube was raised to about twice the initial volume. The tube containing no sugar showed only a few small bubbles which did not increase appreciably with longer incubation, while in a control no gas bubbles were observed. The dextrin and sugars were all fermented equally well.

The addition of milk accelerates both the production of gases and acids. It was repeatedly observed that those "emptyings" which were set with milk or casein began to throw off gas sooner and more rapidly and, too, that the evolution of gas ceased sooner and the medium became sour. For example on one occasion the "emptyings" made without milk were allowed to stand at a temperature of 40° C. for 31 hours or 25 hours after gas bubbles appeared and it was still alkaline and gas was given off, while when milk is added it becomes sour in a very much shorter time and the evolution of gas ceases. This has been

observed by bakers as well, and the author has been told that the addition of milk causes the fermenting material to "work itself out" much sooner. When once the evolution of gas has ceased the "emptyings" no longer work well, if at all, when used in making the sponge. This is quite different in fermenting either a batter or a clear wort with yeast, for after the fermentation has ceased and the yeast settled to the bottom, it can be saved for a week or more and used in making a sponge. This also accounts for the fact that a portion of salt-rising dough cannot be saved for the next baking with the same success as can a portion of dough made with "Sauerteig," for the ferments in the former rapidly lose their gas-producing power and fail to regain it with the addition of fresh flour and water. The "emptyings" as well fall off in gas production after fermenting for some time. After the first bubbles appear, the rate of evolution of gas increases to a maximum and then gradually decreases. When once this maximum has been passed they no longer give good results when used to make bread, for the weakening in gas production becomes even more pronounced in sponge and dough.

It is well known that, in all fermentative processes, the temperature is of great importance, and in a modern baking plant the temperature of the dough is under almost perfect control, varying only a degree or two from day to day during the four seasons of the year. Bakers who make salt-rising bread have found that 26.6° (80° F.), the temperature at which ordinary bread is fermented, is far too low for this type of bread. To determine at what temperature the salt-rising organism is most active a number of "emptyings" were made in the usual way and placed in ovens at a temperature of 35° C., 40° C., 45° C., 50° C., 55° C. and 60° C., respectively. It was found that the optimum temperature lies somewhere between 40° and 50° and that it is possible to ferment bread of about equal quality anywhere between these two temperatures. At 35° the gas is evolved very much slower and when the bread is put into pans it takes considerably longer for it to rise to the usual height. When set at 55° C. the gas is formed slower than at lower temperatures while at 60° C. only a few bubbles of gas are formed in 24 hours. From these results it is apparent that the bacteria grow and can be used

in making bread through as wide a range of temperature as yeast although it is about 15° C. higher.

It is the experience of the author that best results are obtained by fermenting the "emptyings" and sponge at a temperature rather lower than the optimum and then taking the water used in making the dough hot enough to bring it to a temperature of 42° to 46° C. In bread made with yeast as well it is in accordance with the best bakery practice to increase the temperature toward the latter stages of the fermentation.

Sudden changes of temperature seem to have no marked influence upon the bacteria other than accelerating or retarding the rate of fermentation, for good bread was made from "emptyings" that had cooled to 22°, by making the sponge in the usual way at 39° C. and the dough at 42° C. This is of practical importance, for often in practice the "emptyings" are not kept at constant temperature. In the later stages, however, especially in the dough, it is essential that the temperature should be up to 40° C. or over.

GASES EVOLVED.

It was observed by Clapp, as already mentioned, that when a lighted match is applied to the bubbles which form on the "emptyings" the escaping gaseous products burn. This he attributed to the evolution of alcohol. The evolving gases were determined as follows: 46 grams of cornmeal, 20 grams of powdered milk and 4 grams of starter were stirred into sufficient hot water to make a batter. After 8 to 10 hours when gas was being given off rapidly, the fermenting mass was stirred into a mixture of 1000 cc. water and 120 cc. powdered skim-milk. The meal settled to the bottom and the decanted milk was used for collecting the gas; glass cylinders of a capacity of 500 to 750 cc. were filled with this milk and inverted in a porcelain dish over a portion of the same milk. Gas began to form at once and after standing for an hour or two the cylinder was completely filled. The cylinder was half filled in about 20 to 30 minutes but as the column of liquid became shorter, the increase in volume of gas was proportionally slower. The gas obtained by this method, it was assumed, is similar to that produced in the bread.

The collected gas was taken into a 100 cc. Hempel gas burette by displacing water. The CO_2 was determined in a KOH pipette, the oxygen in a phosphorus pipette and the hydrogen by passing the gas

through a short capillary tube filled with palladiumized asbestos, the tube being kept at a temperature of 100° C. In Series 1 and 2 methane was tested for by passing the gas into an explosion pipette but none was detected. The following table shows the composition of the gases.

SERIES 1.—PRELIMINARY.

	Gas taken. cc.	Vol. after CO_2 removed. cc.	Oxygen added cc.	Contraction. cc.	CO_2. Per cent.	H. Per cent.
	56.6	36.2	22.2	48.2	**36.1**	**57**
			SERIES 2.			
A.....	64.9	39.7	39.7	56.8	**38.8**	**52.2**
B.....	73.3	44.7	33.5	62.7	**39.8**	**57.0**
			SERIES 3.			
A.....	74.6	48.2	33.4	66.8	**35.4**	**60.0**
B.....	68.6	44.5	26.3	63.4	35.1	61.7

SERIES 4.

	Gas taken cc.	Vol. after CO_2 removed. cc.	After over P. cc	Oxygen added. cc	After explosion cc.	Decrease. cc
A.....	78.0	48.5	48.0	81.6	13.3	68.3
B.....	79.7	49.8	49.3	78.8	9.6	69.2

	CO_2 found. Per cent.	Oxygen found. cc.	Air. Per cent.	H. Per cent.	Total CO_2 + H + air. Per cent.
A.....	37.8	0.5	3.2	**58.3**	**99.3**
B.....	37.6	0.5	3.2	**58.7**	**99.5**

In Series 1 and 2 the gas used in the analyses was collected immediately upon mixing the milk and the fermenting mass. It was observed that after the cylinder was filled with gas and removed from the milk that a rennet curd had formed which was carried to the top by the evolving gas and could readily be skimmed off. In Series 3 and 4 the milk used was boiled to expel carbon dioxide and air and cooled to 45° C. before mixing with the fermenting mass, also the first cylinder of gas evolved was discarded and that used for analysis was collected over the clear whey after the curd was skimmed off. By discarding the first cylinder of gas, it was assumed that the whey became saturated with the evolving gases. The Hempel burette was filled by displacing this clear whey.

In Series 1, 2 and 3 it will be seen that the CO_2 and hydrogen never exceeds 97 per cent., and in Series 4 the oxygen was also determined after removing the CO_2. This oxygen was calculated to its equivalent

of air and added to the percentages of CO_2 and hydrogen and brings the total to nearly 100 per cent. Since no methane was found, the hydrogen in Series 4 was determined by means of an explosion pipette. Each series was collected from a separate lot of the fermenting milk, and as some air is sure to be dissolved when the cylinder is filled this may account for the discrepancies in the different series.

LOSSES IN BREAD-MAKING.

In making bread with yeast it is found that best results are obtained when the dough is allowed to rise from 1 to 4 times, depending upon the "strength" of the flour, previous to moulding into loaves. During this period of fermentation which generally ranges from 5 to 8 hours in the "straight dough" process of making bread, various chemical and physical changes take place which render the gluten more elastic and better suited for the production of light bread. These changes are chiefly: (1) The formation of alcohol and CO_2 from the sugars; (2) the production of soluble carbohydrates, as sugars and dextrins, from insoluble forms as starch; (3) the production of various organic acids such as lactic, acetic and at times butyric; (4) a partial solution of the proteid compounds in the flour; (5) the formation of amid and ammonium compounds from insoluble proteins; and various other changes that are only incompletely understood. Inasmuch as many of the substances formed during the processes of fermentation are either gases or are volatile, appreciable losses of dry matter occur during the fermentation and baking of bread.

These losses as determined by various investigators are as follows: Voorhees,[1] 4.3 per cent.; Heeren,[1] 1.57 per cent.; Fehling,[1] 4.21 per cent.; Craeger,[1] 2.14 per cent.; Jago,[2] 2.5 per cent.; Danglish,[1] 3 to 6 per cent.; Snyder and Voorhees,[3] 2 to 6 per cent. or even 11 per cent. in cases of long fermentation processes.

While, with the use of yeast better bread can be made by permitting it to rise previous to moulding it into loaves, it is best to put salt-rising bread into pans at once upon mixing the dough; indeed if allowed to stand only an hour or two, marked changes occur in the physical nature of the dough and the bread becomes inferior in quality. This no doubt is to be explained by the fact that the bacteria in salt-rising

[1] U. S. Dept. Agr., Office of Experiment Stations, *Bull.* **35.**

[2] Jago, "The Science and Art of Bread-Making," p. 361.

[3] U. S. Department Agriculture, *Bull.* **67,** pp. 11 and 28.

bread have a greater proteolytic power than yeast in proportion to their amylolytic power. Hence the gluten is sufficiently ameliorated as soon as the gas necessary for aeration is produced. While with the use of yeast, it is found necessary to allow the dough to rise to double its original bulk several times before moulding into loaves, in order to bring about the necessary degradation in the gluten.

The difference in the methods of fermentation, the difference in the gases produced, and the absence of alcohol in salt-rising bread was suspected to make an appreciable difference in the losses of dry material in the two kinds of bread. It was observed, too, both by the author and practical bakers, that the yield in salt-rising bread is invariably somewhat larger than in yeast bread.

With the object in view of detecting any differences that normally occur in the losses of these two types of bread, these were determined. The flour used was a Kansas patent and the bread was fermented as nearly as possible in accordance with the best bakery practice. All determinations were made from the same sample of flour. This was made uniform by putting about equal portions in eight separate dishes and then partly filling a large glass stoppered bottle from these by taking in rotation a tablespoonful at a time from each dish. When the large bottle was about two-thirds filled, it was rolled back and forth over the floor so as to insure perfect uniformity.

The yeast bread was made by the straight dough process which is used most extensively by manufacturing bakers at the present time.

The bread was mixed in a small universal bread mixer as it was found that there was less mechanical loss than if mixed by hand. The yeast was dissolved in a small portion of the water and the salt and sugar were put into the mixer and dissolved in a part of the water. The yeast was then added and the containing vessel rinsed with the remainder of the water. The flour was then put in and the mixer turned, until the dough was uniform, when it was carefully removed and put into a large porcelain evaporating dish and allowed to ferment as long as had previously been determined best for this particular flour. It was kneaded down three times. After having been properly fermented the dough was moulded into 2 loaves, without the use of flour, allowed to rise and baked regularly in an electric oven at a temperature of 220°

to 230° C. It was thought best to use as large quantities as given above, for it was a convenient amount for the mixer and can be handled with a smaller percentage error, due to mechanical losses than a smaller amount. It was found that a little material remained both in the mixer and the evaporating dish in which the dough was fermented. That in the mixer was estimated by allowing it to dry in the air and then weighing the mixer on a torsion balance both before and after the mixer was washed out and dried thoroughly. The difference was small, less than $^1/_{10}$ of 1 per cent. of the total quantity of flour used and was deduced from this amount. That in the evaporating dish was dried in a large copper water-jacketed oven and then calculated from the difference in weight of the dish before and after washing and drying. This amount was also small and was deduced from the total amount of dry material used.

In these experiments no attempts were made to determine the nature of the losses in bread-making but merely the absolute amounts. The two loaves were baked side by side in a single pan, and in sampling they were broken apart and each loaf was cut into four nearly equal pieces. An end piece of one loaf and the diametrically opposite center piece of the other loaf were taken as an aliquot part of the total weight of the bread. These were dried sufficiently to be crumbed by means of a small coffee mill and separate small portions of 2 to 3 grams were used in drying completely in a water-jacketed oven. It was found difficult to get concordant results by drying small portions of the crumbs although they were thoroughly mixed; and also that the pieces as chosen above did not represent a true sample of the baking and this method of determining the moisture in the bread was abandoned. The losses as determined by this method varied as widely as 4.88 per cent. to 6.79 per cent. in two different bakings made as nearly alike as possible. On another occasion a variation of from 7.3 per cent. to 9.7 per cent. was obtained. As it was impossible to obtain concordant results by drying samples, the whole quantity of bread in each baking was dried in a large water-jacketed oven. The bread was cut in thin slices, care being taken that no crumbs were lost, and placed loosely in large porcelain dishes and dried. After drying it was cooled in large desiccators over sulphuric acid and weighed on a torsion balance, upon which all the ingredients as well, were

weighed. By this method a constant weight could be obtained and the results obtained from separate bakings by the same method were concordant.

In addition to determining the losses in bread fermented normally with yeast, they were also determined when the dough was moulded into loaves and placed into pans at once upon mixing without previously allowing it to rise. When it had risen to the usual height it was baked, dried and weighed as described above. The losses in salt-rising bread were determined in practically the same way except that the bread was made by the sponge and dough method which is commonly used for this type of bread. The amounts of ingredients used are as given in baking No. 378, and the time and temperature of mixing "emptyings" sponge and dough as well. The small portion of the ingredients that remained in the beaker and evaporating dish from the "emptyings" and sponge respectively was determined by drying in the water-jacketed oven and weighing on the torsion balance both before and after washing and drying. This amount was small in proportion to the total quantity of ingredients and was deduced from the total dry material used. What remained in the bread mixer was air dried and then determined as was done with yeast bread and subtracted from the amount of flour used.

The moisture in the ingredients used in these experiments was determined by careful sampling and drying small separate portions in the water-jacketed oven. As the moisture content of the flour the mean of 6 closely agreeing determinations was used, while the moisture content of the other ingredients was determined only in duplicate. The salt was heated until decrepitation ceased and was then assumed to be free from water. Knowing the amounts of ingredients used, the percentage of moisture in each and the weight of dry material recovered the loss of dry material can be readily calculated.

The materials used in making the breads are given in the following table:

MATERIALS IN BREAD MADE WITH YEAST.

Fermented normally.	Flour. Grams.	Water. Grams.	Yeast. Grams.	Sugar. Grams.	Salt. Grams.
Bread No. 99.....	800	490	10	12	12
Bread No. 100.....	800	490	10	12	12
Put into pans at once upon mixing.					
Bread No. 101.....	400	245	5	6	6
Bread No. 102.....	400	245	5	6	6

LOSSES IN BREAD-MAKING

	Materials (water-free).				Material used (dry).	Material recovered.	Material lost.	Loss.
Bread made with yeast:	Flour.	Yeast.	Sugar.	Salt.				
Fermented normally:	Grams.	Grams.	Grams.	Grams.	Grams.	Grams.	Grams.	Per cent.
Bread No. 99	711.34	3.65	14.99	12.00	741.98	704.95	37.03	4.99
Bread No. 100	711.53	3.65	14.99	12.00	742.17	702.75	39.42	5.31
							Mean,	5.15
Put into pans at once upon mixing:								
Bread No. 101	356.30	1.82	7.49	6.00	371.61	365.25	6.36	1.71
Bread No 102	356.29	1.82	7.49	6.00	371.60	364.50	7.10	1.91
							Mean,	1.81
Salt-rising bread:	Flour.	Salt.	Cornmeal.	Soda.				
Bread No. 378	710.84	12.00	19.80	1.95	751.27	748.20	3.07	0.41
Bread No 381	710.34	12.00	19.80	1.95	750.77	747.00	3 77	0.46
							Mean	0.44

	Milk.	Starter.
378	4.82	1.85
381	4.82	1 85

SALT-RISING BREAD.

	Flour.	Water.	Corn-meal.	Salt.	Milk.	Soda.	Starter.
Bread No. 378.....	800	550	22	12	5	2	2
Bread No. 381.....	800	550	22	12	5	2	2

The losses expressed in percentages, as given in the table, are calculated upon the total dry material used in each baking and it will be observed that they are much smaller in salt-rising bread than in bread made with yeast, and that in the latter they depend upon the amount of fermentation the bread is subjected to.

Breads No. 101 and No. 102 were of poor quality, being coarse in texture and dark in color as is always the case when bread made with yeast is not allowed to rise several times previous to putting into pans. Over three hours were required for the bread to rise to the usual height in the pans while ordinarily, when the bread is fermented in the usual way, it rises in 35 to 55 minutes. It was made by this method merely to show that the losses are due largely to the destruction of materials by the yeast. The losses in salt-rising bread were found to be even smaller than in bread made with yeast when allowed to ferment merely enough for aeration.

This difference in the losses in the two types of bread is to be explained by the fact that: (1) yeast produces 1.04 parts of alcohol for every part of CO_2, both of which are largely driven off during the process of baking, while in salt-rising bread there is no alcohol produced; (2) it is necessary to ferment bread with yeast from 5 to 8 hours while it is allowed to rise in the pans only one hour or less, hence only a small part of the total gas produced is actually used in aeration, while salt-rising bread is made into loaves at once upon mixing the dough and very little gas is lost; (3) the gases produced by yeast consist of CO_2, while those produced by the salt-rising bacterium consist of about $^1/_3$ CO_2 and $^2/_3$ hydrogen which is only $^1/_{22}$ as heavy as the former.

The fact that the losses in ordinary bread vary with the amounts of fermentation perhaps in part accounts for the variation in the results obtained by different investigators. Snyder allowed the dough to double its bulk only once and baked it after it rose again, which by practical bakers would be considered sufficient fermentation only for very weak flours such as are generally used in making pastries. Losses of

8.14 per cent. and 10.23 per cent., as reported in *Bull.* **67,** U. S. Dept. Agr., p. 28, it is the opinion of the author, are due to experimental errors, for the flour used in these experiments required and was subjected to more than the average amount of fermentation, and the 5.15 per cent. is considered more than an average loss.

The fact that the bacteria in salt-rising bread by their presence in the dough have such a marked effect upon the gluten and that best results are obtained by placing the loaves into pans at once upon mixing the ingredients is considered significant and an effort was made to produce the same results in yeast bread by adding various agents such as other bacteria, enzymes and organic acids.

Cream of tartar when added to the usual ingredients produced a shortening effect upon the dough and hence it was not necessary to subject it to the same amount of fermentation. It was not possible, however, to produce bread with good color and texture by moulding the loaves as soon as the dough was made even though sufficient acid was used to render the dough short to the feel. In excess acids weaken flour and smaller and poorer loaves are the result.

Pepsin was added to doughs in the proportions of 0.1 gram, 0.2 gram, 0.5 gram and 1.0 gram to 800 grams of flour, and it was found that in all cases it was necessary to give the bread a preliminary rising before putting into pans. When pepsin was added to the extent of $^1/_8$ of 1 per cent. the dough became runny and sticky in a short time and it was impossible to make good bread from it, either when it was moulded into loaves at once or when previously allowed to rise. The bread was extremely coarse and darker in color, and at times the destruction of the gluten was made evident by the formation of a large hole extending almost the whole length of the loaf. In all cases, even when only 1 part to 4,000 parts of flour was used, the pepsin produced a noticeable effect. The dough invariably became more runny and the loaves flattened somewhat, and it was questionable whether it was of any advantage even in extremely small quantities. When used to the extent of 1 part in 1600 parts of flour, its effect was harmful without a doubt.

Five different malt extracts which contain both amylolytic and proteolytic enzymes were used in an effort to hasten the changes that are necessary in

dough to make it suitable for bread-making purposes. The activities of these extracts were found to be as follows: No. 1, 8.56; No. 2, 15.71; No. 3, 8.69; No. 4, 9.69; No. 5, 20.13. The activity is defined as the number of times its weight of maltose a given quantity of extract will produce when allowed to act upon an excess of acid-free soluble starch for one hour at a temperature of 40° C.

Various baking experiments using these extracts in different porportions indicate that the extracts have a marked proteolytic action as well as sugar-producing powers. The stronger extracts, especially when used in quantities exceeding 1/2 or 2 per cent. calculated on the flour, have a decided softening action upon the dough, which seems similar to that produced by pepsin. Flour made from wheat that has germinated has a tendency to soften and become "runny" which would indicate the development of proteolytic enzymes. These experiments with malt extracts indicate that while they have an action upon the gluten and shorten the period of fermentation somewhat, their function in bread-making is mainly amylolytic. When used in bread-making they produce considerable sugar as may be seen from the following results obtained by the author.

SUGAR IN BREAD.

Bread No.	Saccharine material added.	Sugar found. Per cent.
1	2 1/2 cane sugar.	5.37
2	None.	3.64
3	1 per cent. extracted No. 5.	6.00
4	1 per cent. extracted No. 1.	5.68
5.	1/8 of 1 per cent. extracted No. 5 and 3 1/2 per cent. cooked flour.	5.27

The percentages of saccharine material added are calculated on the flour, and the percentages of reducing sugar found are calculated as maltose on the water-free bread.

It is well known that dough is a suitable medium for bacterial growth and as, in these experiments, one culture was isolated that could both aerate bread and produce the necessary transformations in the gluten, an effort was made to couple the gas-producing power of the yeast with a bacterial fermentation that would quickly bring about these later changes and perhaps also have an important bearing upon the flavor of the bread.

Preliminary experiments were made to determine

the number of bacteria normally present in flours. These were found to vary in numbers from about 25,000 to 300,000 cells per gram of flour. Bleached flour from the same sample of wheat was found to contain only about $^1/_2$ to $^3/_4$ as many as the unbleached.

Two of the commoner bacteria found in flour were isolated and the pure cultures used in various experiments in bread-making. For convenience they will be designated as A and B. When grown in milk A produced a soft white curd which did not separate from the whey even after standing several months. B produced more acid and after standing a few weeks the curd was almost completely dissolved, forming a dark colored solution. Bread was made by adding milk in various proportions, which had been fermented from 40 to 72 hours with these bacteria. A had no marked effect upon the feel of the dough and the bread was normal except that it was closer-grained and the loaves had a tendency to crack. The dough made with the addition of B was stickier, softer and more runny than usual, although no more than the usual amount of water was added. The bread was smaller in volume and had still more tendency to crack. In these experiments it was found that the effect of the bacteria depended largely upon the activity of the yeast. If the yeast was used sparingly or if it was weak and the bacteria were added in large numbers the effects enumerated above were quite pronounced, while if plenty of strong yeast was added, even in the presence of many bacteria, the bread was nearly normal. In one series of experiments normal bread was made from a flour containing 26,000 bacteria per gram, by adding enough milk, fermented with A to increase that number 30,000-fold, and with B to increase the number of cells 60,000-fold.

Besides these baking experiments with pure cultures a series of bakings was made using buttermilk as part of the liquid. The buttermilk was used in proportions ranging from $^1/_6$ to $^4/_6$ of the total liquid used and in all cases it was found that it had a softening action upon the dough. The bread was smaller in volume and firmer than when no buttermilk was added and had a tendency to crack near the top edge of the pan. Even though the bacteria exceeded by ten-fold or more the number of yeast cells present in the dough, it was found advantageous to allow the bread to rise and to knead it down previous to molding the loaves.

If the yeast was strong and produced a vigorous fermentation the effect of the bacteria was not marked and these experiments would indicate that, while sour bread was formerly a common occurrence, in the modern short fermentation processes bacteria play a less important role than is supposed and that flavor in bread is to be attributed rather to the activities of the yeast than to bacterial fermentation.

While neither the pure cultures of bacteria obtained from flour nor the bacteria in buttermilk had sufficient action upon the gluten to permit molding into loaves at once, it was possible to make a dough, using a salt-rising sponge and compressed yeast, and put the loaves into pans at once upon mixing with good results; indeed the bread was better in texture than if it was allowed to rise before molding into loaves. With the use of other bacteria, together with compressed yeast, on the other hand, better bread could be made by allowing it to rise once or twice before molding. While the use of compressed yeast in salt-rising bread produces good-appearing bread with good texture it has not met with the approval of the consumer, for it loses more or less of its characteristic taste and acquires another due to the yeast. On theoretical grounds it would hardly be expected that the two organisms can be used to advantage in the same dough for the production of bread, owing to the great difference in temperature at which they work best. It is in accordance with the best bakery practice to-day to ferment doughs with yeast at a temperature of 26° C. while the optimum temperature for the salt-rising organism lies about 15° C. higher.

Some of the bacteria normally present in cornmeal are extremely resistant to heat, so much so that a mixture of meal and milk, after being steamed for two hours or for a half hour on three or four successive days, will at times show the evolution of gas after incubation.

The artificial product prepared in these experiments is even more resistant to heat and shows the evolution of gas much sooner as may be seen from the following experiment: into 250-cc. German flasks containing 150 cc. water, which was brought to a boil, were stirred, in Series 1, 18 grams of cornmeal and 10 grams dried skimmed milk, and in Series 2, 18 grams of the artificial product and 10 grams of the same milk.

Series 1.	Series 2.	Time steamed.
Flask No. 1*a*	Flask No. 1	1 hour
Flask No. 2*a*	Flask No. 2	1 hour and 20 min.
Flask No. 3*a*	Flask No. 3	1 hour and 40 min.
Flask No. 4*a*	Flask No. 4	1 hour and 55 min.

The flasks were plugged with cotton and immediately placed in a steaming autoclave. After being incubated at 40° C. for 18 hours, all flasks in Series 2 were giving off gas while in Series 1 no signs of life were evident within 48 hours when a few gas bubbles began to form in flask 1*a*. No gas appeared in flasks 2*a*, 3*a* and 4*a* even after incubating for 3 days, although putrefaction set in.

In another series 6 flasks were made as in Series 2 and heated respectively 1 hour and 55 minutes, 2 hours and 25 minutes, 2 hours and 55 minutes, 3 hours and 25 minutes, 3 hours and 55 minutes, and 4 hours and 30 minutes. Flasks 1, 2 and 3 were evolving gas after incubating for 18 hours; flask 4, 2 hours later; while flasks 5 and 6 became putrid after three days.

This treatment is far more severe than any the organisms are subjected to in the process of baking the bread, and Dr. Emley raised the question whether or not they survive the heat of the oven and perhaps cause disturbances, due to gas production, in the alimentary tract of the consumer. To throw some light upon this question some of the bread was cut at various times with a sterile knife, and small portions of about 1 gram each were plucked out from the freshly cut surface with a sterile pair of forceps and dropped into test tubes partly filled with sterile milk. This was done repeatedly, and of all the tubes inoculated in this way only about one-half showed any growth. The growth was seldom observed in less than 24 hours and in no case did gas bubbles appear as is always the case when sterile milk is inoculated from the fermenting batter, which would indicate that the gas-forming organisms perish in the oven. The fact that these organisms do not survive in the bread when it is in the oven only about 45 minutes and reaches a temperature of 100° to 103° C. for only a few minutes, and yet in a batter made from meal and milk are not killed by three hours' steaming, can be explained only by differences in the life history of the organisms. In the meal they occur as spores while in the bread they are actively growing and are quickly killed by the heat before they can pass into the more resistant state. The organisms that do sur-

vive are probably introduced as spores by means of the flour rather than the meal, for in the former they have much the shorter time to germinate. This is supported by the fact that salt-rising bread keeps as well as bread made with yeast, and that the latter also is not an absolutely sterile product for certain organisms that cause ropiness in bread are known to survive.

The flora of bacteria present in the fermenting salt-rising batter made from cornmeal varies greatly, depending upon the temperature that obtains when the meal is stirred into the water or milk. If the temperature of the mass does not exceed a temperature of about 65° an organism or perhaps a group of organisms may be isolated that will produce gas readily in milk or broth or even on plates unless they are very shallow. When grown in nutrient agar it produces gas also and causes holes and rents in the medium. On plates it appears in round, raised, semitransparent colonies. In milk gas bubbles are given off and after about two days, sometimes less, a curd will form which is broken and full of gas bubbles. After a week or longer a part of the curd, usually down one side of the tube, will dissolve, leaving a clear light colored whey. In broth gas bubbles are given off and a rather uniform cloudiness appears. In stab cultures the growth spreads over all or nearly all the top of the medium, while farther down gas bubbles appear. The growth along the line of puncture is rather feathery, disturbed or broken by gas bubbles here and there. Stroke cultures show an abundant raised growth semitransparent, with smooth projections extending out. On potato a narrow raised, grayish white growth appears, which does not show a tendency to spread. Gelatin is not liquefied at room temperature, and if incubated at 37° C., will solidify again on cooling. It forms indol. If the temperature of the batter exceeds 75° C. this organism is not found upon plates poured from it, and spore formation has not been observed. These cultural characteristics together with its morphology and staining properties indicate that it is a member of the coli group.

Another colony which was more opaque and slightly ragged also produced gas in milk but produced no curd within four days. The curd was more broken with gas bubbles than when the milk was fermented with the other culture. In their growth in stab and slant cultures the two could not be distinguished, but

on potato this last one described produced a creamy yellowish growth instead of a grayish white. It is probably a member of the same group.

In addition to these, certain flat, white, opaque colonies, with ragged edges, appeared in about equal members. These also produced gas in small quantities and formed a curd when grown in milk. Also certain flat, smooth-edged, opaque colonies were evident. None, or very few indeed, of the colonies that appear upon milk-agar plates poured from batters, the initial temperature of which did not exceed 60 to 65° C., show a peptonization of the milk either upon the plates themselves or when transferred to milk tubes. If the temperature of the batter exceeds 75° C., however, a different flora of bacteria is present, for nearly all the colonies that appear show a peptonization of the milk, which is made apparent by the clear zone that surrounds the colonies, and also by the yellow solution that is formed when the colonies are transferred to milk tubes. The bacteria that survive the higher temperatures differ also in that they, as far as has been determined, liquefy gelatin. If a temperature of 75° C. or over obtains in making the batter, it is only occasionally that gas bubbles appear in plates made from it although the batter itself is vigorously evolving gas, and, as already stated, only once, and then not without some doubt in regard to its purity, was a culture obtained that would produce gas, and this culture soon lost this ability. When the meal is subjected to lower temperatures, however, a gas-forming organism can readily be isolated, and it is the opinion of the author that the pure culture with which bread was made in these experiments was subjected to a temperature lower than 75° C., by the housewife making the bread, and that the organism, although its cultural characteristics were not fully determined, was a member of the coli group. It produced a similar reaction in milk and gelatin media, did not form spores, and had the morphological characteristics.

As would be expected from the variations in the flora of bacteria present in the batter, depending upon the temperature at which it is made, differences are also observed in the character of the bread produced. The batter when not subjected to temperatures that destroy non-spore-bearing organisms has a different and usually a stronger odor which at times becomes very offensive, and it not eliminated during

the process of baking, although it is usually moderated somewhat. It is the experience of the author that better bread is obtained if the liquid used in making the batter is brought to a boil, which subjects the meal to a temperature of 85 to 90° C., than if the batter is set at lower temperatures. Fermentation and the evolution of gas begins sooner, but with less regularity and certainty, when the temperature of the batter is not raised to the point at which non-spore-bearing organisms perish. With the use of the artificial product prepared in these experiments, however, which it was found best to stir directly into the boiling liquid, the evolution of gas begins both sooner and with a much greater degree of regularity than if meal is used and set at the most favorable temperature.

CONCLUSIONS.

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-spore-bearing organisms. The chief source of the bacteria is not the air and utensils, as has been suggested in the literature, but the corn-meal 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 group 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.

Bread made by the "Sauerteig" method differs from salt-rising in that the gaseous fermentation in the latter is due entirely to bacteria, while in the former the leavening power owes its origin primarily to yeasts, and it is a question whether the bacteria present, some of which are gas formers, are desirable or not; they differ also in that the latter is made from fresh material each time, while in the preparation of the former a portion of dough is saved to start the fermentation in the next baking.

The gases produced by the salt-rising bacteria, as found in these experiments, consist of nearly $^2/_3$ hydrogen and rather more than $^1/_3$ carbon dioxide and no hydrocarbons.

The losses of materials, due to decomposition and volatilization of some of the constituents, are much smaller in salt-rising bread than in bread made with yeast, and the losses in the latter vary with the amount of fermentation to which it is subjected. This difference in the losses of materials in the preparation of the two kinds of bread is to be explained by the fact that (1) there is no alcohol found in the former; (2) that owing to inherent difference in the nature of the ferments involved it is subjected to far less fermentation; and (3) the gases are much lighter.

The author wishes to express his gratitude to Robert Kennedy Duncan for his helpful advice and direction in this work.

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