LII. AN EXPERIMENTAL STUDY OF THE EFFECT OF CERTAIN ORGANIC AND INORGANIC SUBSTANCES ON THE BREAD-MAKING PROPERTIES OF FLOUR AND ON THE FERMENTATION OF YEAST.

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DURING the war, when it was necessary to conserve the wheat supply of the country, the proportion of the flour extracted from the grain was greater than in normal times, and other cereals were being milled and added to the wheat.

The loaf made from such flours was small in size and close in texture, and complaints were also made that it showed a tendency to become sour. Although numerous suggestions for improving the breadmaking properties of the flour were forthcoming, the problem thus raised was an extremely complex one.

The factors on which the breadmaking properties of a flour depend are by no means easy to define and exact data on this subject are difficult to obtain.

It was hoped that these investigations of the effect of certain organic and inorganic substances on the breadmaking properties of flour might yield results which would be of some practical assistance in arriving at a satisfactory solution of the problem. At first the method of investigation adopted was that of making baking experiments. Later, in order to check the behaviour of the various ingredients used in breadmaking, and to obtain some indication of the changes which occur during the different stages of the process, measurements were made of the amount of fermentation produced by the yeast in the absence of flour and also of the expansion of the dough whilst "proving" or "rising" prior to baking.

BAKING EXPERIMENTS.

Loaves were cooked in batches of four, each loaf containing the same weight of the necessary ingredients, *i.e.* flour, raising agent, salt and water, to which might be added different substances which varied with the nature of the experiment.

In order to minimise experimental errors which are almost inevitable in work of this character, one loaf of each batch was prepared as a control and contained the same ingredients in each experiment. In all cases where it was possible, the experiments were repeated two or three times, and an average of the results taken.

Ingredients of doughs raised with yeast.

1. 200 grams of flour. The flours used in these experiments were kindly supplied by Messrs J. and R. Robinson, Deptford Bridge Mills, Greenwich, and their composition was as follows:

I.	Flour containing 80 % of the wheat grain.				
II.	" "	85 %	" "	" "	and barley.
III.	" "	75 %	" "	" "	which was a weak English wheat.
IV.	" "	90 %	" "	" "	and rice, maize, barley and rye.
V.	" "	85 %	" "	" "	and rice, maize, barley and rye.
VI.	" "	75 %	" "	" "	which was a weak English wheat.
VII.	" "	83 %	" "	" "	and barley, rice and rye.
VIII.	" "	85.9 %	" "	" "	and maize, rice and barley.
IX.	" "	85.9 %	" "	" "	and maize, rice and barley.
X.	" "	86 %	" "	" "	and barley, rice and rye.

In addition some white flour similar to the pre-war flour was obtained by a special permit.

2. 5 grams of yeast supplied by a wholesale firm. The age of the samples was known.

3. 2 grams of sugar (or 10 cc. of a 20 % solution).

4. 2 grams of salt.

5. Liquid for mixing. The volume of liquid required for mixing the dough to the necessary consistency varied from 95–120 cc. according to the nature of the flour. The liquid was chiefly tap water, but the total volume included any other liquid, *e.g.* sugar solution, serum, etc., added for the purpose of the experiment.

Method of making bread raised with yeast.

The yeast was mixed to a thin cream with the sugar solution, and added to the flour and salt which had previously been mixed and raised to a temperature of 25°. The rest of the liquid (at 25°) was then added to the dough thus formed and kneaded for five minutes. Longer time could not be allowed as the dough cooled rapidly during this process.

First rise. The dough was allowed to rise in an incubator kept at 25° until the surface skin cracked. The time required varied with the nature of the flour. A dough made from a "strong flour" (I) required one hour and that from a "weak flour" (II) forty-five minutes. The addition of cereals other than wheat reduced the time necessary for rising.

The dough was then kneaded and put into a greased tin which had previously been weighed.

The tins were cylindrical so that the approximate volume of the loaf could be readily calculated. The dough was weighed in the tins. This weight served as a check on the manipulation of the mixing.

Second rise. The dough was put back into the incubator and allowed to remain there until it had about doubled its size. The time necessary for this second rise varied with the flour in the same manner as for the first rise.

Baking of the dough. The dough was put into a hot oven, the temperature raised for the first ten minutes and then decreased at intervals. At first a gas oven was used, but later an electric oven was substituted so that the temperature could be more easily controlled. In some of the later work the second rise was omitted, *i.e.* the dough was mixed, put into the tin, allowed to rise for the necessary time and baked. The same range of results was obtained by this latter method as by the longer process, and any possible error introduced by further handling of the dough was eliminated. Some bakings involving the longer process were carried out in all cases in order to check the results obtained by the shorter method.

Weight and volume of the loaf. When cold the loaf was weighed, and after keeping twenty-four hours, it was cut through the centre and the greatest and least heights measured.

The volume of the loaf was calculated from the mean height and the area of the base.

The volume of the control was taken as 1000, and the volumes of the other loaves calculated relatively to this. By this means a comparison between experiments carried out at different times could be obtained.

Doughs raised by a chemical raising agent.

The ingredients of these doughs were as follows:

1. 200 grams of flour.
2. 1 gram of sodium bicarbonate and 2 grams of potassium bitartrate.
3. 2 grams of salt.
4. Sufficient liquid to make a dough.

The raising agent was mixed with the flour and salt, and the mixture passed through a sieve. After adding the liquid the dough was kneaded, placed in a weighed tin, weighed and baked as described above.

The addition of lime-water in breadmaking.

Bread made from "Government controlled" flour showed a tendency to become sour, which was not observed in the case of pre-war white flour. Jago [1911] states that bread made with lime-water is quite free from sourness.

In order to investigate the power of lime-water to correct the tendency to sourness, baking experiments were carried out with doughs mixed with lime-water, some being raised with yeast and others with a chemical raising agent.

Experiments were made with whole-meal flour, Government controlled flour and white flour.

The loaves made with lime-water were in all cases smaller than their controls. The doughs mixed with lime-water appeared drier than the controls, and whilst rising formed a thick skin.

Some determinations were made of the acidity and also of the moisture content of the lime-water loaves and of their controls, determinations being made both on the freshly baked bread and on bread which had been kept for several days.

The results showed that whilst the lime-water neutralised any acidity in the flour it did not prevent the production of acid.

There was no appreciable difference in the rate of loss of moisture on keeping between the lime-water loaves and the controls. The acidity of the bread decreased as it became drier.

Bywaters [1918] states that the use of lime-water has been largely discontinued as the action of the yeast is thereby delayed. Lapigne and Legendre [1917] and also Le Roy [1917] make reference to this subject.

Observations based on practical experience show that bread which is made on a large scale is more liable to become sour than bread which is made in smaller quantities.

This difference it was thought might be due to the fact that when bread is made on a large scale a longer time is usually allowed for the fermentation or "proving" process than is allowed in a small scale baking.

Some determinations which were made of the acidity of the dough during fermentation showed that the acidity increased slightly as fermentation proceeded. In a typical case the acidity expressed as lactic acid was 0.46 % on the freshly prepared dough and increased to 0.66 % after five hours. This shows that the acidity of the dough is dependent to some extent on the length of time allowed for proving.

It thus appears there is little to be gained by the use of lime-water in breadmaking except, perhaps, in the case of a very acid flour or if the process of fermentation is prolonged.

Experiments on the physical properties of gluten.

A study of the technology of breadmaking indicates that the size of loaf is partially dependent on the physical properties of the gluten and that to produce a good loaf the gluten must be moderately elastic.

Experiments were made to see if the elasticity of the gluten could be increased and a larger loaf obtained by stretching the dough by some physical process prior to the addition of the yeast. The dough was placed in a vacuum desiccator and the pressure alternately reduced and then increased again to atmospheric pressure three times.

Some expansion of the dough was produced by this process, but after baking the loaf was not appreciably larger than the control.

THE ADDITION OF OX-SERUM IN BREADMAKING.

At the suggestion of Professor Leonard Hill some experiments were made in which a proportion of ox-serum was added to the liquid used in mixing the dough.

The ox-serum used was separated at the Metropolitan Cattle Market, Islington, and obtained forty-eight hours after the animal had been slaughtered. In cold weather it was possible to keep the serum for three days before it became unfit for use, and experiments were carried out with the serum on the first, second and third day.

Baking experiments were made to ascertain the effect of the serum on the size of the loaf and to find how its action varied with the amount of serum added and the length of time the sample had been kept. The serum was used in proportions varying from 1 to 7 %, expressed by weight in relation to the flour. The results obtained on the addition of fresh serum (first day) in concentrations of 1-3 % with four different flours are shown in Table I.

Table I.

Concentration of serum	Flour	Weight of loaf in g.	Height of loaf in cm.			Volume of loaf	
			Maximum	Minimum	Mean	Calculated	Comparative
First day 1 %	I	281.0	11.25	7.25	9.25	726.8	1156
" " 2 %		282.5	11.25	7.0	9.12	716.9	1124
" " 3 %		279.5	10.25	6.25	8.25	643.2	1023
Control		280.9	9.75	6.25	8.0	628.6	1000
First day 1 %	II	269.9	9.5	7.0	8.25	648.2	1048
" " 3 %		274.9	9.75	6.25	8.0	628.6	1016
Control		274.9	9.5	6.25	7.87	618.7	1000
First day 1 %	IV	290.6	9.0	6.5	7.75	608.9	1051
" " 2 %		287.6	9.0	6.5	7.75	608.9	1051
" " 3 %		292.4	8.75	6.5	7.62	599.1	1034
Control		290.2	8.5	6.25	7.37	579.4	1000
*First day 1 %	III	251.0	8.5	6.5	7.75	589.3	1053
Control		246.0	8.25	6.0	7.12	559.8	1000

A marked increase in the volume of the loaf resulted from the addition of 1 % fresh serum.

With concentrations greater than 1 %, the increase on the whole was less marked, although with flour IV the same result was obtained with 2 % as with 1 % of serum. The increase obtained varied with the flour, the maximum increase being 15.6 % with flour I.

It should be noted that the addition of the serum affects only the volume, not the weight of the loaf; the slight differences in weight observed are to be attributed to experimental errors.

Further experiments showed that the increase obtained with 1 % of serum was greater than that obtained with concentrations less than 1 % and also that on the addition of 1 % serum to pre-war white flour a decrease of 1.5 % in the volume of the loaf was obtained.

When the serum was used on the second and third days, the loaf obtained with the addition of 1 % serum was considerably smaller than on the first day. The size of the loaf increased with the proportion of serum used, but even when 7 % of serum was added the increase in volume was not so great as that obtained with the addition of 1 % of serum on the first day.

The maximum increase on the different days obtained with flour I were as follows:

1st day an increase of 15.6 % with 1 % of serum.				
2nd	"	12.5 %	"	7 % "
3rd	"	12.5 %	"	7 % "

It thus appears that the active principle in serum, which produces an increase in the size of the loaf, is destroyed gradually on keeping, and although the effect of this destruction could to some extent be rectified by increasing the amount of serum used, the maximum effect obtained with the fresh serum could not be produced on subsequent days.

The effect of heat and preservatives on the properties of serum.

Some experiments made with serum which had been kept at a temperature of 50–60° for an hour and a half, showed that this treatment had practically no effect on the action of the serum.

Samples of serum, which were heated on arrival and used for baking experiments on the following days, gave a typical first day action on the second day, *i.e.* the maximum increase was obtained with the addition of 1 % of serum, but the increase in the volume of the loaf was not so great as on the first day. Not until the fourth day did the serum heated on the first day give a typical second day reaction, *i.e.* the volume of the loaf increased with the concentration of the serum. Serum heated in this manner did not keep appreciably longer than raw serum. Thus the active principle in the serum is not destroyed by being heated to 50–60°, but it is to some extent protected although not preserved by this treatment.

Serum heated to 100° coagulated and on standing a pale coloured liquid separated out which could be expressed from the solid mass. Baking experiments made with this boiled serum showed that it had very little effect in increasing the volume of the loaf and in some cases the loaf obtained was smaller than the control. The liquid portion was more effective than the solid, but in neither case was a marked increase in volume obtained.

Since the power of the serum to produce an increase in the size of the loaf deteriorates rapidly on keeping, and the maximum effect can only be obtained with fresh serum, some experiments were made to see if it were possible to preserve the serum either by some process of desiccation at low temperatures, or by the addition of chemical preservatives.

An attempt was made to evaporate the serum under reduced pressure at the room temperature, but the process was found to be too slow to be of any practical use.

The protein material in the serum was precipitated by the addition of a saturated solution of ammonium sulphate. The precipitate was filtered and then dried in a vacuum desiccator at the room temperature.

This dried preparation when added to the flour gave similar results to the fresh serum, and did not deteriorate on keeping for a considerable time. Unfortunately the serum obtainable at this stage of the investigations was poor in quality and results yielded were not comparable with those obtained earlier.

The addition of small quantities of formaldehyde (1 part of 40 % formaldehyde to 1000–2000 parts of serum) preserved the serum for several days and in baking on a small scale gave a loaf as large as that obtained with fresh serum; but in one or two experiments which were made on a large scale (see baking on a large scale) a slight decrease was observed.

This may be due to some inhibitory action of the formaldehyde on the yeast owing to the longer time allowed for rising on the large scale, but further experimental work is needed before any definite statement on this point can be made.

Boric acid was found to be ineffective in preserving serum.

Possible substitutes for serum.

Defibrinated blood was tried as a substitute for serum; its action was found to be similar to that of second day serum but the loaf was dark in colour.

Solutions of the following substances were also used: gelatin, egg and blood albumin, caseinogen in lime-water.

With caseinogen in lime-water the loaf obtained was smaller than the control. (Comp. the addition of lime-water.)

Gelatin gave an increase of 10 % with flour I, but with the other flours the increase was only from 1–2 %.

A slight increase only (1–2 %) was obtained with albumin and it was not considered that any of these substances could be regarded as a satisfactory substitute for serum.

The addition of serum to bread raised with a chemical raising agent.

Serum appeared to have no action on doughs raised with a chemical raising agent; the loaves obtained were the same size as the controls.

Baking on a large scale.

In order to decide whether the action of the serum would be the same if the bread were made in larger quantities, one or two experiments were made in which doughs were prepared with 10 lb. of flour, which, after mixing, were divided into eight loaves.

The relative increase in the size of the loaf was less than in the small scale bakings, but the serum was at this time very poor in quality and the experiments were not continued.

Further work is needed to confirm these results and to find whether the optimum concentration of the serum is the same on the large as on the small scale.

The action of serum in the presence of phosphate and of potato is dealt with later.

THE ADDITION OF PHOSPHATES IN BREADMAKING.

Jago [1911] states that the addition of mineral phosphates to the flour results in a marked improvement of the loaf, but that the addition of such salts is illegal.

Harden [1914] has shown that the addition of phosphates increases the total fermentation produced by a given volume of yeast juice.

Baking experiments were made using monosodium dihydrogen phosphate, $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$, and monohydrogen disodium phosphate, $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, and also acid calcium phosphate, $\text{CaH}_4(\text{PO}_4)_2$.

The quantities of phosphate used varied from 0.25–3.0 % of the flour.

The average increase in the volume of the loaf obtained on the addition of 1 % of each of these salts to flour was as follows:

Monosodium hydrogen phosphate	+3.5 %
Disodium " "	+2.6 %
Acid calcium phosphate	–0.75 %

Although the calcium salt produced a slight decrease in the size of the loaf, it gave a bread which was whiter in colour than the control or that containing the other phosphates. Further experiments showed that the maximum increase in the size of the loaf was obtained on the addition of 0.5 % of monosodium hydrogen phosphate, and that its effect was more pronounced with "weak" than with "strong" flours.

Thus an increase of 7.4 % was obtained with flour III and an increase of only 1.6 % with flour IV.

With disodium hydrogen phosphate the size of the loaf increased with increasing concentrations of the salt up to 3 %, and it was not considered advisable to exceed this amount. The maximum increase obtained with the latter salt was less than that obtained with the monosodium salt.

Judging by the experimental results obtained, there appeared to be no advantage in using a mixture of the salts in the proportion of five molecules of the disodium salt to one of the monosodium salt, although such a mixture was found by Harden [1914] to produce the maximum effect on the fermentation of yeast juice, or in using a mixture of the two salts in the proportions of the concentrations found to produce the maximum effect, *i.e.* 0.5 % of monosodium hydrogen phosphate, and 3 % of disodium hydrogen phosphate.

The addition of phosphates to doughs raised with a chemical raising agent.

The addition of phosphates to doughs raised with a chemical raising agent did not produce any increase in the size of the loaf.

The addition of phosphate and serum in breadmaking.

A comparison of the action of phosphate with that of serum showed that in the case of a weak flour a greater increase in the size of the loaf was obtained by the addition of phosphate than by the addition of serum.

With flour III the addition of 0.5 % of monosodium dihydrogen phosphate produced an increase in the size of the loaf of 7.4 %, and the addition of 1 % of fresh serum an increase of 5.3 %, but with some of the "stronger flours," *e.g.* V and VI, the results were in the reverse order.

If both phosphate and serum were added the increase in the volume of the loaf was greater than that obtained with phosphate alone, but the relative increase in the size of the loaf as compared with serum alone depended on the nature of the flour, *i.e.* the serum and phosphate in most cases produced a larger loaf than the serum alone, but in some cases a smaller one.

THE ADDITION OF POTATO IN BREADMAKING.

In order to economise wheat flour and other cereals, the addition of potato to the flour used for breadmaking has been extensively advocated.

As the production of potato flour involves special plant for drying the potatoes, it was thought that it would be more convenient, providing that equally good results could be obtained, to use either raw or cooked potato instead of potato flour.

The potato was substituted for a portion of the flour, due allowance being made with raw and cooked potato for the water-content of the material, *e.g.* 70 g. of raw potato were taken as equivalent to 19 g. of flour and a mixture of 181 g. of wheat flour and 70 g. of raw potato as equivalent to the 200 g. of flour used in the other baking experiments.

The average results obtained were as follows:

Raw potato, increase in the volume of the loaf	1.2 %
Boiled " " " " " "	4.2 %
Potato flour " " " " " "	1.25 %

Steamed potato had the same effect as boiled potato.

Baking experiments were also made with potato which had been boiled twice as it has been suggested that all enzymes are destroyed by this treatment. This potato had the same effect as ordinary boiled potato.

Experiments made to determine the best proportions of potato to use showed that the size of the loaf decreased with increasing proportions of raw potato, but increased with increasing proportions of boiled potato.

Thus when the potato and flour were in the proportion of 1 : 2 the increase in the volume of the loaf was 3.6 % with raw potato and 10.7 % with boiled potato, and when the potato and flour were in the proportion of 2 : 1 the increase was 1.8 % with raw potato and 14.5 % with boiled potato.

In the latter case, however, the mixture was quite unsuitable for bread-making as the upper part of the cooked dough was hollow and the lower part

had the appearance and consistency of cooked batter. On the average the best results were obtained with a mixture of wheat flour and potato, containing boiled potato equivalent to from 25–33 % of the total weight.

The volume of the loaf decreased with increasing proportions of potato flour, but the flour being dry it was possible by this means to introduce a larger proportion of potato and still obtain a loaf which was satisfactory as regards texture and appearance, although smaller than the control. This bread, however, became stale and dry very rapidly. It was observed that the addition of potato made the dough less spongy. Owing to its high water-content raw potato, which was grated before adding to the dough, tended to make the dough moist and considerably less water was required for mixing than was required by the control. The doughs containing raw potato were greyish in colour owing to changes due to enzyme action which take place when raw potato is exposed to the air and this made the bread a bad colour.

In mixing doughs containing boiled potato the amount of water used was in most cases the same as for the control.

The addition of potato and serum in breadmaking.

Baking experiments made with the addition of serum to bread containing cooked potato showed that the maximum effect was produced, as in the case of flour alone with the addition of 1 % of fresh serum.

The increase in volume of the loaf obtained with fresh serum in the presence of potato was slightly less than when the potato was omitted, the average difference in the results being about 1 %. Further, when the serum was used on the second day the addition of 2 % of serum gave the maximum increase and the same result was obtained on the third day.

During a spell of cold weather it was possible to bake with one sample of serum on the fourth day. The volume of loaves containing potato and serum then increased with the amount of serum added, *i.e.* the action of the serum was then the same as that observed with second day serum when flour alone was used.

The addition of potato and malt extract in breadmaking.

The addition of malt extract to dough containing potato did not increase the size of the loaf, and if the proportion of potato to flour was more than 1 : 2 the loaf became smaller on the addition of malt.

It was previously noted that with boiled potato a larger loaf was obtained when the proportion of potato was increased, but on the addition of malt and potato the size of the loaf decreased as the proportion of potato increased.

Further, it was found that the amount of boiled potato which could be introduced into the bread was less in the presence of malt, as the bread showed a tendency to become extremely heavy.

EXPERIMENTS ON THE FERMENTATION OF YEAST AND MEASUREMENTS
OF THE EXPANSION OF THE DOUGH DURING RISING.

In order to check, as far as possible, the behaviour of the various substances used in making the bread, and to obtain some indication of the changes which occur at the different stages of the process, measurements were made of the amount of fermentation produced by the yeast with the various ingredients in the absence of flour and also of the expansion of the dough prior to baking.

Experiments on the fermentation of yeast.

The fermentation experiments were carried out in 100 cc. flasks, each flask being attached to a nitrometer, filled with brine, in which the carbon dioxide evolved could be collected and measured. The flasks were placed in a thermostat kept at a temperature of 25°.

The weights of yeast, sugar, and any additional substance were exactly one-tenth of the weights used in the baking experiments, and, except where otherwise stated, the total volume of liquid was 10 cc.

The experiments were carried out in batches of four. One of each batch contained yeast, sugar, and water only, and was used as a control, and each experiment was done in duplicate.

The mixture to be fermented was placed in a flask and the volume of carbon dioxide evolved read off at regular intervals.

Experiments on the expansion of dough.

The proportions of flour, yeast, etc., used in these experiments were exactly half those used in the baking experiments; it was thus possible to get a direct comparison between the baking experiments, the expansion of the dough, and the fermentation experiments.

The dough was introduced into wide-necked glass bottles fitted with rubber bungs through which delivery tubes were passed. The volume of the air displaced by the rising of the dough was measured by the displacement of water. The water was kept alkaline by the addition of sodium hydroxide, so that any carbon dioxide which escaped through the dough should be absorbed.

It was felt that the experiments would be more complete if some arrangement could be devised by which the expansion of the dough during baking as well as during the fermentation process could be measured.

An apparatus for this purpose was constructed of tinned copper, but although several different types of washer have been tried it has so far not been possible to make the screw top absolutely air-tight at the baking temperature, and reliable results have not yet been obtained.

Results of experiments on the fermentation of the yeast and the expansion of the dough.

Experiments were first made to compare the amount of carbon dioxide produced by the yeast, sugar, and water only with the expansion of the dough during fermentation.

The carbon dioxide evolved by the fermentation of the yeast was measured at the end of one hour and at the end of four hours.

It was found that the volume of the gas produced varied with the age of the yeast, and the maximum amount of fermentation was produced, not with fresh yeast, but with yeast that was two to three days old.

The amount of fermentation produced varied considerably with the different samples of yeast; the worst sample produced 2.3 cc. during the first hour and a good sample 18.4 cc. in the same time.

These results were confirmed by measurements of the expansion of the dough (see Table II).

Table II.

Expansion of the dough during fermentation.

Age of yeast. Days	Increase in volume of the dough.			
	20 mins. cc.	40 mins. cc.	60 mins. cc.	80 mins. cc.
1	10.0	52.0	121	—
2	—	62.0	136.6	230
3	15.0	61.5	124	202
4	12.0	54.0	123	210

Fermentation experiments, measurements of the expansion of the dough and baking experiments were carried out on the same day with the same sample of yeast.

The fermentation produced by the yeast and the expansion of the dough were measured as described above, and the results which would be obtained with the quantities used in the baking experiments were then calculated. A dough was also prepared and baked without the addition of the yeast to act as a control, and it was assumed that the volume of the loaf less the volume of this cooked dough (422.3 cc., see Table III) and less the expansion of dough during fermentation, might be taken as a measure of the expansion on baking.

Thus in Table III the figures in column VI are obtained by subtracting the volume of the baked control (422.3 cc.) and the expansion of the dough (column III) from the volume of the loaf (column V).

As might be expected the amount of fermentation produced in the dough is greater than with the yeast alone, this probably being due to the presence of substances in the flour, *e.g.* gluten, etc., which act as yeast foods.

Further there is a maximum increase on baking which is obtained (under the conditions of the experiment) after the dough has risen for 40 minutes.

Table III.

I	II	III	IV	V	VI	VII
Age of yeast Days	Time of fermentation Minutes	Fermentation of yeast (5 g.) cc.	Expansion of dough during fermentation (5 g. yeast) cc.	Volume of loaf (5 g. yeast) cc.	Expansion on baking cc.	Ratio of fermentation to expansion of dough (III : IV)
—	nil	—	—	422.3	—	—
1	20	6	10	510.7	78.4	1 : 1.7
	40	28	52	579.5	105.2	1 : 1.9
	60	64	121	628.6	85.3	1 : 1.9
2	nil	—	—	422.3	—	—
	40	31	62	599.1	114.8	1 : 2
	60	72	136	628.6	70.3	1 : 1.9
	80	115	230	658.1	5.8	1 : 2
3	20	8	15	500.9	63.6	1 : 1.9
	40	31	61	569.1	85.8	1 : 1.9
	60	71	124	617.1	70.8	1 : 1.8
4	20	6	12	520.5	86.2	1 : 2
	40	31	54	579.5	103.2	1 : 1.7
	60	68	123	628.6	83.3	1 : 1.8
	80	110	210	658.0	25.7	1 : 1.9

These results show that the ratio of the fermentation of the yeast to the expansion of the dough is fairly constant.

This fact may be of practical significance, for if the maximum increase on baking could be ensured the loaf would be increased in volume by a physical process instead of by further fermentation which utilises and wastes some of the ingredients of the dough.

The action of serum and albumin during fermentation.

At the time when these fermentation experiments were made, satisfactory samples of fresh serum were not obtainable, but some measurements were made of the fermentation produced by yeast and the expansion of the dough in the presence of serum which had been "salted out" of solution and dried in the manner previously described. This serum contained a small amount of ammonium sulphate, but control experiments made with this salt showed that in small quantities it was without action on the yeast.

The serum used had been kept for twelve months. The addition of this serum increased the volume of carbon dioxide produced during fermentation and also the amount of expansion in the dough.

Thus the increase in volume of the loaf observed on the addition of serum must to some extent at least be attributed to the action of the serum as a yeast stimulant.

Some measurements made with albumin showed that it had some stimulating action on the yeast, but that this action was less marked in the dough.

The action of phosphates during fermentation.

Fermentation experiments were made with yeast, sugar and phosphates in the proportions found by the baking experiments to give the best results.

In the first series of experiments the phosphate appeared to exert an inhibitory action on the yeast, as the volume of carbon dioxide evolved was less than from the control.

This it was thought might be due to the fact that although the phosphate and yeast were in the same proportions as in the baking experiments, they were used in a more concentrated form.

Harden [1914] found that when phosphate is added to fermenting yeast juice and sugar, the effect varies with the concentration and that with a mixture of 25 cc. of yeast juice and 4.5 g. of sugar, 5–10 cc. of 0.3 molar solution of phosphate in a total volume of 45 cc. produced the maximum effect.

Fermentation experiments were therefore made using 0.5 g. of yeast, 0.125 g. of cane sugar with from 2 to 40 cc. of 0.3 molar solution of monosodium dihydrogen phosphate in a total volume of 45 cc. Table IV shows the results of a typical experiment.

Table IV.

Volume of 0.3 molar solution of phosphate cc.	Percentage increase in fermentation of yeast on the addition of phosphate.			
	1 hour	2 hours	3 hours	4 hours
2	0	3.6	4.3	1.5
5	0	14.3	14.9	11.0
10	8.3	17.9	17.0	13.2
20	33.3	44.9	40.4	29.4
30	41.7	50.0	44.7	35.3
40	41.7	46.4	40.4	29.4

These results show that the maximum increase was obtained with 30 cc. of the 0.3 molar solution (= 0.12 g. of $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$), *i.e.* with 0.12 g. of phosphate and 0.5 g. of yeast in 45 cc., which corresponds to 1.2 g. of phosphate and 5 g. of yeast in 450 cc.

The optimum increase on baking was obtained with 1 g. of phosphate and 5 g. of yeast in about 330 g. of dough, so that the results of fermentation experiments confirm those obtained in the baking experiments.

The action of potato during fermentation.

In order to study the effect of the addition of potato on the fermentation of yeast, experiments were made with the following preparations of potato:

I. *Raw Potato*: (a) peeled potato, (b) peeled potato washed, (c) juice expressed from potato, (d) potato peel, (e) washed potato peel, (f) juice extracted from potato peel.

The solid matter was finely grated before use.

II. *Cooked Potato*: (a) boiled potato (mashed), (b) steamed potato, (c) boiled potato (washed), (d) potato which had been boiled twice. (These potatoes were boiled for 20 minutes, left over night and boiled again until cooked.)

III. *Potato Flour*.

In the first experiments 4 g. of potato were used with 0.5 g. of yeast, but later it was found that a more marked effect was obtained when the same weight of yeast was used with 10 g. of potato.

Table V.

Condition of potato	Weight of potato g.	Average percentage increase in the fermentation of yeast with sugar and water produced by addition of potato				
		1 hour	2 hours	3 hours	4 hours	5 hours
Raw potato peeled	4	68.9	—	—	31.2	—
" " " and washed	4	48.2	—	—	8.9	—
Potato peel	4	78.1	—	—	29.7	—
" " washed	4	48.8	—	—	12.0	—
Juice of potato	4	-29.4	—	—	12.2	—
Juice from potato peel	4	-59.8	—	—	-25.8	—
Raw potato	10	92	84	69	64	64
Juice of potato	10	-28.7	5.5	22	28	—
Boiled potato	10	82	61	35	21	21
Steamed potato	10	93	84	60	35	33
Potato flour	equivalent to 10 g. of potato (i.e. 2 g.)	34	25	20	—	—
Juice of potato filtered and boiled	10	16	36	54	64	77
Juice of potato filtered, boiled and filtered	10	9	24	39	48	58

These results show that raw potato produced a marked increase in fermentation, but that if the potato were washed until the liquid filtering through was clear this increase was appreciably diminished.

The juice extracted from the potato inhibited fermentation at first, but later produced a slight increase.

The increase produced by raw potato was greater than the sum of those produced by the washed potato and the juice of the potato.

Potato peel increased the fermentation of the yeast to a greater extent than raw potato. This might be due to the higher water-content of the former, i.e. the total solid matter added was greater in the case of the peel.

The juice extracted from the peel had a more marked inhibitory action than the juice from the potato, and the combined effect of the washed peel and the juice of the peel acting separately was less than that of the peel.

Boiled potato did not increase the fermentation to the same extent as raw potato, but steamed potato had the same effect as raw potato for the first two hours and its stimulating power then became less marked.

This difference in the behaviour of boiled and steamed potato is doubtless due to loss of material in the cooking water during boiling and it is possible

that if the potato were boiled in a small volume of water and the cooking water, as well as the potato, used this difference would be eliminated.

The stimulating action of the potato flour was even less than that of washed potato.

When the juice extracted from the potato was filtered and the filtrate boiled, the filtrate became slightly turbid, this being due no doubt to the cooking of minute particles of starch which had passed through the filter. On filtering this boiled liquid a clear green filtrate was obtained.

A comparison of the results obtained with this boiled juice with those obtained with the raw juice indicates that the boiling to some extent destroyed the principle of the raw juice which exerted an inhibitory action on the yeast. Juice which was only filtered once gave a better result than that which was filtered again after boiling.

Observations made on the colour of this boiled juice showed that when grated potato was exposed to the air it became first pink and then rapidly passed through shades of red until it was almost black. When the juice extracted from this potato was boiled and filtered, the filtrate was always green in colour and the depth of colour increased with the length of time the potato was exposed to the air before the juice was extracted.

It has previously been observed that the yeast showed an optimum activity on the second or third day when fermenting with sugar and water, but in the presence of potato it was found that the yeast was more active on the first day.

The results given in Table IV indicate that the increase in fermentation produced by the addition of potato gradually diminished as fermentation proceeded. This effect, which was also observed in the case of other substances which acted as yeast stimulants, may be due to the fact that the apparatus used was such that an equal but limited supply of oxygen was available in each experiment. The substance which stimulated the yeast may thus have been unable to exert its full action towards the end of the experiment owing to the partial exhaustion of the oxygen.

Measurements made of the expansion of dough containing potato showed considerable variations.

Raw potato produced an average decrease in volume of dough as compared with the control of 10.8 %, whilst boiled potato gave a decrease of 5.7 %. The juice from raw potato inhibited the expansion of the dough and washed potato showed the same effect as raw potato but in a greater degree. Steamed potato decreased the expansion of the dough but to a less extent than boiled potato.

The fermentation experiments as a whole form a valuable check on the baking experiments and also demonstrate the necessity of studying the changes involved during the different stages and not the final stage only if any reliable information as to the processes on which breadmaking is based are to be obtained.

Thus, although the fermentation experiments are in general in agreement with the baking experiments this is not always the case. For example, raw potato stimulated the action of yeast to a greater extent than boiled potato, but the loaf obtained with boiled potato was larger than that obtained with raw potato; also the doughs containing potato were smaller than the controls, the difference being less for cooked than for raw potato, but the loaves containing potato were larger than the controls.

SUMMARY.

The amount of fermentation produced by yeast with sugar and water and in the dough varied with the age of the yeast. The maximum effect was obtained on the second or third day after the yeast had been compressed.

The ratio of the fermentation of the yeast with sugar and water to the fermentation in the dough was approximately constant.

The maximum increase in volume on baking was obtained, under the conditions of the experiments, when the dough was allowed to rise for forty minutes, but this factor may vary with the proportions of the different ingredients used. The acidity of the dough increased with the time of fermentation and the acidity of the bread decreased with the moisture content.

The addition of lime-water to bread does not appear to prevent the production of acid, but it neutralises any acid formed; in the presence of lime-water a smaller loaf was obtained.

On the addition of fresh ox-serum the maximum increase in the size of the loaf was produced with 1 part of serum to 100 parts of flour. The power of the serum to increase the size of the loaf diminished rapidly on keeping, but was not destroyed by heating to a temperature of 50–60°.

With phosphates the best results were obtained when monosodium dihydrogen phosphate was added in the proportion of 1 part of phosphate to 200 parts of flour. Neither phosphate nor serum produced any increase in the size of the loaf when the bread was raised with a chemical raising agent.

Raw potato, cooked potato and potato flour, when substituted for an equivalent amount of wheat flour, diminished the expansion of the dough on fermentation, but both raw and cooked potato gave a larger loaf, the increase being greater with cooked than with raw potato.

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