EFFECT OF ENZYMES ON THE PHYSICAL PROPERTIES OF DOUGH

by

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TABLE OF CONTENTS

INTRODUCTION AND OBJECT

The Milling Industry Department for several years has been conducting research with the Swanson-Working recording dough mixer on flours milled from wheats of many different types. The curve from any variety of wheat has been found to show typical characteristics which are not materially changed by growth conditions of climate and soil, nor by storage or milling conditions unless the wheat is very seriously damaged. It was thought desirable to learn whether the enzymes of yeast or enzymes of the types occurring in sprouted wheat have any effect on the characteristics of these curves.

In particular, it is found that Marquis and related spring wheats give a curve radically different from that given by the hard winter wheat Blackhull. It has been known for a considerable period of time that spring wheats and flours from the Northwest usually exhibit a greater enzyme activity than the varieties generally grown in the Southwest winter wheat belt. Thus it was considered especially valuable to learn whether the typical curve of a spring wheat flour might be in any degree due to the mellowing action on the gluten of the large proportion of enzymes usually present in these flours. The curve typical

of Blackhull wheat builds up rapidly and then breaks down rapidly, indicating a gluten of harsh and brittle nature. By adding different enzymes, mostly of a proteoclastic nature, but also the mixed enzymes obtained from sprouted wheat and sprouted barley, the attempt was made to learn whether the effect of these enzymes on the gluten of the Blackhull would make its curve similar to that of the Marquis.

For comparison with the results obtained by drawing curves, the physical properties of the doughs from the different flours were studied by means of the baking test. Since it was desired to study the effect on strictly the physical condition of the gluten brought about by the addition of the different enzymes, it was necessary to eliminate as far as possible the effect of the sugar produced by those preparations containing diastatic enzymes. The best way known to accomplish this is by the use of a large amount of sugar in the baking formula. Since the salt in the regular baking formula has a considerable effect on the physical properties of the dough, the complete baking formula, or the complete formula with the exception of the yeast, was usually used when curves were drawn as well as when the dough was baked.

REVIEW OF LITERATURE

The effects of enzyme action have been known from time immemorial; since organic matter then as now must have undergone decomposition. Some of the terms still in use and still having much of the same significance were employed by the Greek scientific philosophers. Hippocrates spoke of "digestion" as a mechanical process caused by digestive juices. The exact nature of the processes taking place during digestion, putrefaction, and fermentation were not investigated until the beginning of the 19th. century. As already stated these terms had a meaning, but it remained for Pasteur to link them definitely to the action of organisms or their catalysts rather than to a supernatural mechanical process as the ancients seemed to have thought.

The three terms digestion, putrefaction, and fermentation, still describe more or less the same process carried on by enzymes, bacteria, and fungi, but to the initiated such terms have a greater significance. We may think of the digestion as the tearing down of complex molecules, into simpler forms either for absorption, assimilation or transportation from an organ to another by the

action of some specific enzyme. Such a statement is obviously incomplete when we think of the amylolytic and proteolytic enzymes acting upon a piece of dough. The katabolic action goes on, but either one or any of the other three conditions, digestion, putrefaction, and fermentation, may not.

Complex reactions are always difficult to define when grouped together, and therefore we should view separately, in part at least, the factors that help to bring about these conditions.

The work which has been done with enzymes is tremendous but at the present time an exact method for their determination is not available. According to Willstätter (1922) an enzyme is made up of a colloidal carrier together with other substances which have a purely chemical nature. Bayliss (1925) states that an enzyme is a catalyst produced by living organisms and cells. It has a colloidal carrier and an active chemical group. Enzymes are specific in their action and are differentiated by the end products of their action on specific substances of definite structural and stereoisometric configuration.

Hogounenq and Loiseleur (1925) are more specific in regard to the chemically active group, and state that it is a mineral element held by adsorption by the colloidal

complex, but still preserving its cystalloidal properties and specific chemical activity. The electrolyte which either gives or at least helps to give the different enzymes their peculiar properties is of a definite character for each enzyme, such as chlorine for pepsin. Possibly such conception of the physico-chemical properties of enzymes would help to understand, in part at least; the selectivity of a medium in relation to the pH concentration, affecting thereby their response to an electric current, acting thus either positive, neutral, or negative according to the pH of the medium. Their chemical valence, although possibly definite for a certain enzyme such as trypsin which is monovalent, may be progressively augmentative on either side of the electrodes according to the electrolyte adsorbed by the different enzymes.

The amphoteric properties of many enzymes have been established, and for some of them the optimal activity is at their isoelectric point. For invertase this covers a wide zone. The effect that temperature produces on the action of an enzyme upon its substrate is of tremendous importance. Anybody who has attempted to ferment a piece of dough for bread making, or a considerable amount of water, sugar, malt, yeast, and other ingredients for alcohol does not need to be reminded of this fact. Notwithstanding such statement, it should be added that the

optimum temperatures for most of the enzymes with which we are concerned in the baking industry are different for the various enzymes. Swanson and Calvin (1913) in their work with amyloclastic enzymes acting upon a flour suspension, found not only that the activity of the enzymes was greater in the first hour as measured by the amount of reducible sugars produced, but that the optimum temperature for these enzymes was near 65°C. The tryptic enzyme papain is rather inactive at temperatures such as 40°C., but in a favorable medium reaches its optimum at temperatures as high as 90°C., a temperature at which most other enzymes are almost totally destroyed.

The existence of considerable amounts of enzymes in wheat, and its transmission to flour in the milling process; has been known definitely for a considerable period of time. Since the literature studied covered only a very small portion of the thousands of volumes that have been devoted to this subject, I will not even attempt to state who was the first person who noticed some radical changes in the physical properties of a dough, and at the same time had the ingenuity, or the maliciousness according to some contemporaries, to attribute these changes to causes other than miraculous power.

At the beginning of the present century we already had a considerable number of scientists working on the unsolved problem of determining and defining "strength" and "weakness" of flour in terms of one or two simple tests that could be performed without much effort and expenditure of time either in the laboratory or bakeshop.

Fleurent (1896) thought that he had found a very satisfactory method to measure the quality of flour in relation to its baking qualities. These experiments and conclusions had nothing to do with enzyme action, or gas production. The notes transmitted to his colleagues under the auspices of the French Academy, stated that the quantity, as well as the quality of gliadin was the main factor in flour "strength". His evidence was based on the fact that some of the other cereals, such as rice and rye did not contain a large amount of gliadin. He introduced the gliadin-glutenin ratio which could be easily determined by extraction of the gliadin, and the quality of the gluten be determined by its water holding capacity. His conclusions are that a ratio of 25 per cent glutenin and 75 per cent gliadin gives well piled loaves which are easily digested. Therefore this ratio should represent the optimum "strength" for flour, since any other ratio

such as 2 to 2 or 3 to 1 of the two proteins will not produce good bread due to poor fermentation caused by this lack of balance.

The enzyme activity of flour seemed to have been better recognized at the turn of the century, as of more importance than a mere chemical or mechanical determination as suggested by Fleurent in measuring flour "strength". Vines, quoted by Baker and Hulton, (1908) suggested that yeast possessed an enzyme capable of dissolving fibrin. The author concluded that if such was the case, this peptic enzyme must be a very important factor in determining "strength" of flour.

Before continuing with the experimental evidence in regard to the existence of the peptic enzyme in yeast, we should return to the subject of the enzymes found in wheat. Vines (1903) has shown that wheat contained an ereptic enzyme, and what is more, that this type of enzyme in seeds is always associated with a peptic enzyme. Baker and Hulton (1908) proceeded to ascertain whether the ereptic enzyme was preserved in the flour, or whether it was separated in the milling process as the peptic

enzyme, which is mostly found in the germ. Water extracts of flour were used on Witte's peptone and after digestion for 20 hours at 37°C., the tryptophane reaction was obtained when bromine water was added, confirming the presence of the ereptic enzyme in flour. In previous experiments with flour suspensions, after incubation for the same period of time and at 37°C. the tryptophane reaction had been negative, showing therefore that the gluten splitting enzyme was not present in flour, at least in the samples tested. The work of Willard and Swanson (1913) is helpful in substantiating the evidence of Baker and Hulton. Various amino compounds, extracts from bran, scourings, and ammonium salts among other things were used to determine their effects on the baking qualities of flour. The use of peptones in small quantities did not injure the loaf in any appreciable way, but when the quantity of the peptones was increased to 1.6 g. the effect was very noticeable. The total expansion of dough was decreased, and the length of proofing was shortened. The oven spring was decreased and the texture was very materially injured. With the larger quantities the dough became difficult to handle because of its physical properties. As has already been stated from the work of Vines,

and the experiments of Baker and Hulton; the ereptic enzyme is preserved in the flour, and its activity in the process of fermentation and proofing is mostly limited by the quantity of peptones present in the flour.

In this work of Willard and Swanson (1913) three risings were allowed during the fermentation of the dough. In the use of bran extracts several procedures were followed: such as trying the different extracts without filtration, after filtration, boiling before using, and not boiled. The filtered and unfiltered extracts had more or less the same beneficial effects. The rising period was shortened, and the oven spring and loaf volume were increased. The only injurious effect was to the texture. These effects are attributed to increased food supplied the yeast by the extracts, but since filtering had no effect whatsoever, the reason must be due to other factors. The authors suggest that there might have been some increase in the amount of enzyme. In the boiled and unboiled extracts used for baking, the latter extracts in every case decreased the period of fermentation, while the heated extracts in general had the opposite effect.

The most interesting part of these experiments showing the rapid effect of the peptic enzyme on gluten, and its almost total elimination in milling is found in their experiments with water extracts of wheat scourings. The action produced on the dough and bread by these extracts is very similar to the effect produced by germinated wheat flour. It was found that 3 per cent had an accelerating effect on fermentation and proofing. Thus it is indicated that the distribution of the peptic enzymes from the embryo to the endosperm is very rapid and general. The bran extracts when obtained from the bran of germinated wheat, had nearly the same effects as the extracts of scourings containing parts of the germ. These scouring extracts had such an effect on the molecular structure of the gluten, than when relatively small amounts were used, the dough was so soft and sticky that it was difficult to handle. Besides this the period of fermentation was shortened, the oven spring and loaf volume were decreased, and the crumb was very much injured. In other words, the gluten splitting enzymes must be found chiefly in the germ, and its elimination in milling is fortunate for the baking industry, at least if the present system is to continue.

Some proteolytic enzymes are also present in baker's yeast. The quantitative amount seemingly depends on the

origin, age, and methods of preparation of the yeast. Waksman and Davison (1926) quoting the works of many authors list the following specific enzymes found in yeast: in comparatively large quantities; carboxydase, catalase, invertase, maltase, melobiase, and zymase. In small quantities; endotryptase, oxidase, and reductase are usually present, and also arginase, emulsin, rennet, raffinase, and trehalase. In one experiment carried out by Baker and Hulton (1908) the authors added 5 per cent of baker's yeast to their dough, and fermented it for 4 hours at 37°C. The amount of soluble nitrogen obtained after corrections for possible autodigestion of the yeast was found to be 2.7 per cent. The same quality of flour treated as above, but without the addition of yeast contained 1.9 per cent of soluble nitrogen as protein. The difference of nearly 1 per cent is attributed by the authors to the presence of an ereptic enzyme in yeast.

The word fermentation is used rather freely in this paper, and as was stated at the beginning, this generic term has a very ancient origin. Fermentation may be defined as a phenomenon brought about by the action of certion organisms on specific substrates, whose end products are usually alcohol, carbon dioxide, and water. As was

pointed out at the beginning of this work on enzymes, the action of the amyloclastic enzymes is not only rapid but easy to measure. (Swanson and Calvin 1913). Recently it has become one of the important laboratory determinations in testing flour quality. That the relative amounts of amyloclastic and proteolytic enzymes varies with different wheats from different areas, will be shown later when the author presents his personal investigations.

Physically and quantitatively the wheat kernel can be divided into three main parts: the endosperm comprising about 84 per cent of the seed, the bran or outer covering about 14 per cent, and the embryo or germ about 2 per cent. These quantities obviously vary because the seeds are not uniform but heterogeneous. According to Osborne and Mendel (1919) the percentage composition of wheat, flour, and bran on a moisture free basis is as follows:

	Flour per cent	Wheat per cent	Bran per cent
Sucrose	0.24	0.61	1.92
Dextrin	3.63	2.01	5.03
Starch	77.52	63.80	0.00
Pentosans	1.80	6.27	32.77
Protein (5.7 x N.) . .	11.44	11.25	17.60
Fiber	0.34	1.75	11.18
Fat	1.15	2.18	3.26
Ash	0.43	2.03	8.61
Undetermined	3.45	10.10	14.23
	100.00	100.00	100.00

These figures given for bran are what Osborne calls pure bran, and are slightly different from the figures given for the commercial samples.

It should be stated that the greatest percentage of wheat flours, even those of relatively inferior quality for bread baking purposes, are made up of the endosperm. The properties of the wheat and therefore its main product, flour are due to varietal, climatic, and soil conditions. It seems that one of the first persons to recognize this fact was Voelcker. As quoted by Baker and Hulton (1908) and based on Voelcker's experiments at Woburn Experimental Farm, his statement is thus: "I am more inclined to go further back and to lay emphasis upon those external conditions which were known to exercise an influence on the grain, and to, in a great measure, determine its characteristics." As has already been stated, the two principal constituents of wheat and wheat flour are starch and protein. The quantities of other substances present in flour besides enzymes, sugars, and water, and the two already mentioned should not be overlooked. Salamon in an unpublished work in 1908 quoted by Baker

and Hulton (1908) states that according to his experiments the fatty matter found in flour exercised an enormous influence on the size of the loaves obtained from wheat flour. The viscosity of the fatty substances was a very important factor in determining very materially the resistance offered to the passage of gas, and the size of the vesicles. The influence that the electrolytes found in wheat flour exert on determining the baking qualities is considerable. It is known that the type of electrolyte, and also its quantity will affect the pH of the dough, and consequently the enzyme activity of any particular sample of flour. Sodium chloride for instance when added to a mass of dough which has been overmixed, has the property of bringing the dough back to its original tenacity and consistency. A better illustration of the effect that the amount of electrolytes found in flour was obtained in the work of Swanson and Calvin (1913) in which sulfuric acid and sodium hydroxide of various concentrations were used in testing the inhibiting action of these two substances on the production of sugar by amyloclastic enzymes. A high grade flour and a low grade flour were used in testing the enzyme action per unit time when these two substances were added. In both cases the enzyme activity was greatest in the low grade flour, showing the existence of different

chemical substances capable of absorbing or combining with the electrolytes.

The proteins can be defined as nitrogenous compounds of a very complex nature containing in their molecules the elements: nitrogen, carbon, hydrogen, oxygen, and sometimes sulphur and phosphorus. Due either to the complexity of these important chemical substances, or else to our inadequate methods of analysis; we could say in a very general way that the classification of proteins is not only founded on rather uncertain principles, but that those proteins falling within such classification are not necessarily so because of inherent constitution, but rather such by investigational procedure. It is true that some of the simpler proteins can both be molecularly determined, and have actually been synthesized. In this review a complete classification of the proteins cannot be given. Anybody interested in this subject may consult a text devoted to this subject such as Proteins and the Theory of Colloidal Behavior by Jacques Loeb, or Physiological Chemistry by A.P. Mathews.

The "simple" proteins are classified according to their solubilities and their response to heat. Since some of these simple proteins are always found in wheat and flour, the nomenclature adopted by the American Society of Biochemists is given:

A. Albumins - Simple proteins, coagulated by heat, soluble in water and dilute salt solutions. (leucosin).

B. Globulins - Simple proteins, heat coagulable, insoluble in water, but soluble in dilute solution of salts of strong bases and acids.

C. Glutelins - Simple proteins, heat coagulable, insoluble in water or dilute salt, but soluble in very dilute acids or alkalies. (glutenin).

D. Prolamines - Simple proteins, insoluble in water, soluble in 70 per cent alcohol. (gliadin).

E. Albuminoids - Simple proteins, insoluble in dilute acid, akali, water, or salt solutions.

F. Histones - Simple proteins, not coagulable by heat, soluble in water and in dilute acid, strongly basic, and insoluble in ammonia.

G. Protamines - Simple proteins, strongly basic, non-coagulable by heat, soluble in ammonia, and yielding large amounts of diamino-acids on decomposition.

The most comprehensive exposition of the proteins of the wheat kernel possibly up to the present is that of Osborne (1907). The main constituents of the wheat proteins are gliadin and glutenin. Osborne states that about 80 per cent of the total nitrogenous matter found in the wheat kernel is made up of these two substances. The total

percentage of these two proteins may be slightly variable in different wheats, and particularly flour, but the ratio of these two proteins in wheat and wheat flour is surprizing constant. The other two proteins, leucosin which is soluble in water and coagulable at from 50 to 60°C., and globulin soluble in salt solutions of strong bases, makes the greatest part of the remaining 20 per cent of the proteins found in wheat and wheat flour. A comparative analysis of the hydrolysis of gliadin and glutenin is given by Osborne as follows:

	Gliadin per cent	Glutenin per cent
Glycosoll	0.00	0.89
Alanine	2.00	4.65
Amino-valerianic acid	0.21	0.24
Leucine	5.61	5.95
Alpha Proline	7.06	4.23
Phenylalanine	2.35	1.97
Aspartic acid	0.58	0.97
Glutaminic acid	37.33	23.42
Serine	0.13	0.74
Tyrosine	1.20	4.25
Cystine	0.45	0.02
Lysine		1.92
Histidine	0.61	1.76

Arginine	3.16	4.72
Ammonia	5.11	4.01
Tryptophane	Present	Present
Total	65.85	59.66

Glutenin which is a simple protein and grouped according to its solubility with the glutelins, is coagulable by heat, and soluble in very dilute acids or alkalies, from which it can be precipitated on neutralization.

Because of the statement made concerning the proteins as entities by definition, or at least incomplete definitions, some of the evidence presented by Gortner, Hoffman and Sinclair (1929) will be reviewed. In their experiments 14 different flours were obtained for analysis, and "purity" salts employed for the analysis. The definition given above for globulins, that they are "simple" proteins, heat coagulable, insoluble in water, but soluble in dilute solutions of salts of strong acids and bases", obviously assumes that all salt solutions of strong bases and acids have an equal ionization constant, and equal peptization properties on proteins from different origins. A 5 per cent solution of KCL for example is not ionically equivalent to a 5 per cent solution of Na CL. Even when different salt solutions of equivalent ionizations constants are

used in protein extractions, the amounts of protein extracted from the same flours are not identical. Quoting some of the statements made by Gortner, Hoffman, and Sinclair (1929) they have the following to say in their summary concerning their experiments with the proteins of various flours, using different salts, but ionically equivalent solutions.

1. There is a great variability in the amounts of protein that can be extracted from a given wheat flour by various salt solutions of equivalent ionic concentration.

2. There is an equally striking variability of the proteins of individual flours towards a single salt solution. The amount of protein peptized by a single salt solution will vary 100 per cent in the extreme ranges for the various flours studied.

3. The differences are not dependent upon the pH, but are determined by: (1) the ease of peptization of the proteins in a particular flour, and (2) the specific properties of the particular anions and cations present in the salt solution used.

4. The salt soluble protein fraction does not represent a mixture of albumen and globulin, nor does it represent the non gluten proteins. Some salts extract only a part of the non-gluten proteins. Thus 1 N. potassium

fluoride extracts an average of 13 per cent of the non-gluten proteins, whereas an equivalent concentration of KI extracts 64 per cent.

5. The peptization of the wheat flour proteins by inorganic salt solutions reveals the same sort of differences as does peptization with acid or alkaline solutions. These differences in behavior are undoubtedly associated with the colloidal properties of the wheat flour proteins, which in turn are dependent upon heritable differences of the wheat varieties and upon environmental conditions under which the wheat was grown or that are involved in the subsequent harvesting and storage of the grain.

EXPERIMENTAL

Three different flours were obtained. One was a patent made from hard spring wheat grown in the Northwest. The other two were patent flours milled from hard winter wheat, chiefly Blackhull. The absorption of each was determined by the centrifugal method. Samples of 250 g. were used both for baking and for the curve determinations. The baking formula contained 3 per cent yeast, 6 per cent sugar, and 1.75 per cent salt when fermentation was desired. Doughs without yeast were incubated for various periods.

Various amounts of commercially pure pepsin, papain, and malt extract were added to the flours before mixing.

Several attempts were made to obtain uniform results with the Wallace and Tiernan viscosimeter, using a flour suspension shaken at frequent intervals for 1 hour. The suspension was placed in the viscosimeter cup, the plunger was inserted and placed at zero and the motor turned on. Different amounts of 80 per cent lactic acid were added but even a fraction of a second would change the readings very materially, and therefore this work was abandoned.

A detailed description of the Swanson-Working recording dough mixer (1933) will not be attempted. The main parts of the apparatus, at least those with which we are mostly concerned in these experiments, are the mixing bowl and the recording mechanism. The mixing bowl is of circular form with a flat bottom to which four pins are securely attached. The force offered by the dough to the rotation of the mixing pins which are attached to a movable head is transmitted to a lever under the mixing bowl, which is also connected with the recording apparatus.

Procedure

A sample of 250 g. of flour was carefully weighed and placed in the bowl. The predetermined amount of water

was added, the sample mixed for one minute at 81 r.p.m. and the diagramatic curve shown in figure 1 was the result. This flour is a patent made from spring wheat. The rise of the curve was constant in a sort of oscillating form, and not until the third minute of mixing did its curve become rather flat, beginning to give away rather slowly. In order to determine the effect that fermentation had on the curve, several samples of the same flour were mixed for one minute using the baking formula already given. The resultant dough was placed in a pan and fermented at 32° for 2 hours. The dough was transferred to the mixing bowl, and its curve is shown in figure 2. The height of the curve was slightly greater which may be accounted for by the salt added. It broke sooner, but the general configuration of the curve was not changed by the fermentation. A similar experiment was made with the addition of 1 mg. of the proteoclastic enzyme pepsin, and after 2½ hours fermentation the curve was determined. As shown in figure 3 the height of the curve is about equal to that in figure 2, but the breaking down of the dough is very materially accelerated by the small amount of proteoclastic enzyme.

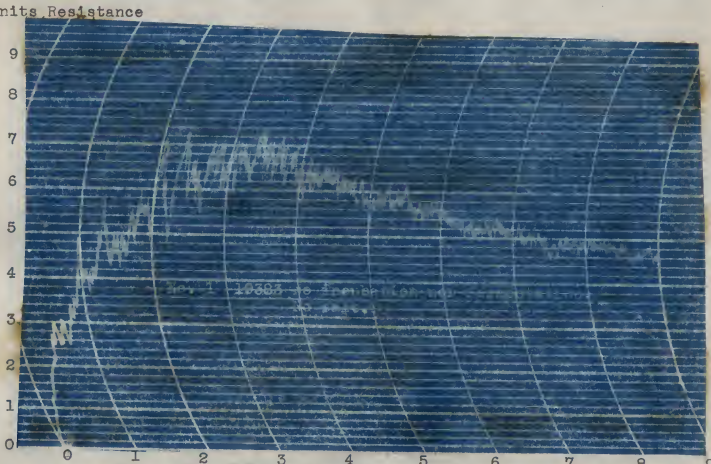

Figure 1. 19383 No incubation nor fermentation.
No salt.

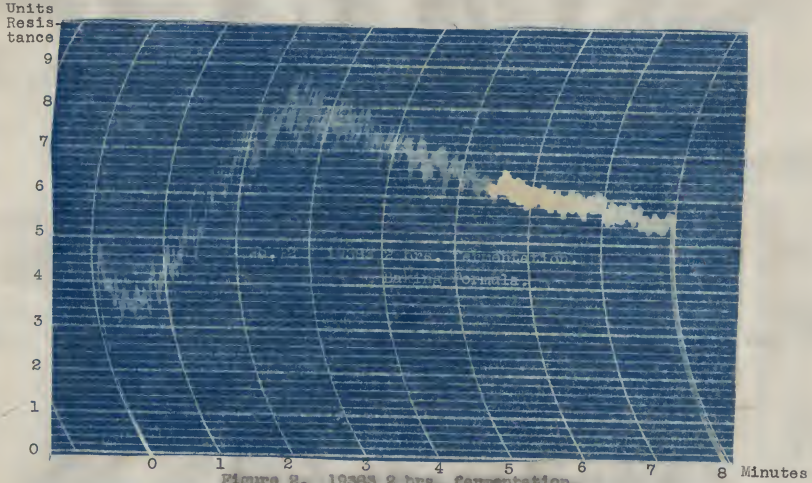

Figure 2. 19363 2 hrs. fermentation.
Baking formula.

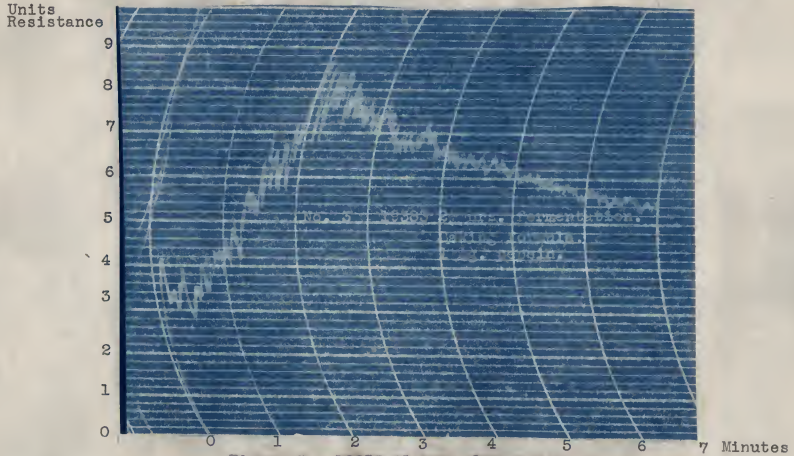

Figure 3. 19383 2½ hrs. fermentation.
Baking formula.
1 mg. pepsin.

Since the simple fermentation curve shown in figure 2 was not much different from that obtained without fermentation, another set of samples was prepared as before. The only difference in the procedure was that after 2 hours fermentation the dough was punched, and placed back in the pan to raise for one half hour more, then the curve shown in figure 4 was drawn. As can be seen by comparing figures 2 and 4, the punching and the additional half hour of fermentation did not change the configuration of the curve to any appreciable extent.

Another series of experiments with the same flour from spring wheats was performed, but using 300 grams of flour instead of 250 grams. In the first experiment whose curve is shown in figure 5, the flour was mixed for 1 minute at 81 r.p.m. with water only instead of the baking formula, and left in the same bowl for 1 hour, after which the curve was drawn in the usual manner. The increase in the amount of flour obviously increased the height of the curve, but there was no appreciable change in the configuration of the curve even when compared to the graph made from the flour with no fermentation whatsoever as shown in figure 1. In experiment No. 6 of (fig.6)

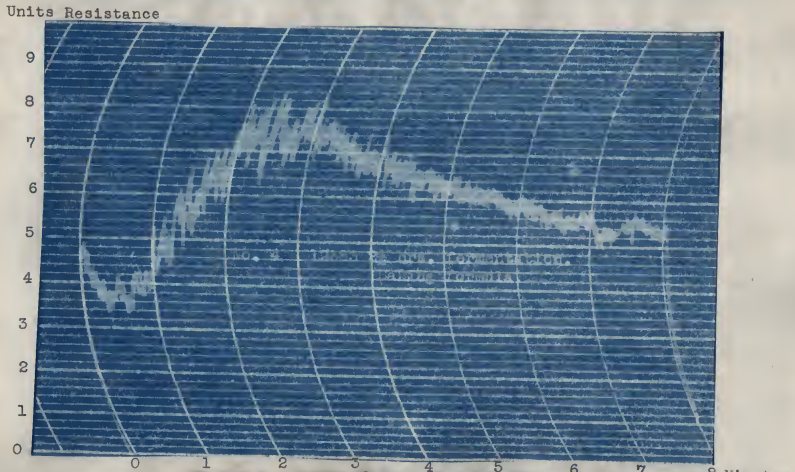

Figure 4. 19503 2½ hrs. fermentation.
Baking formula.

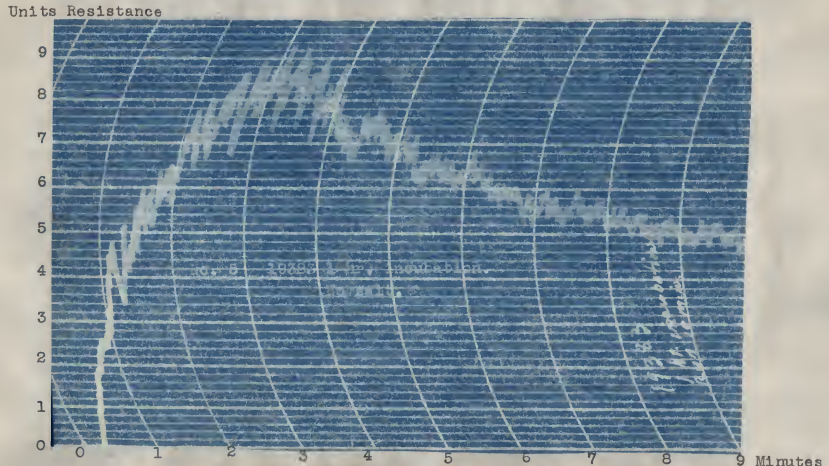

Figure 8. 19383 1 hr. incubation.
No salt.

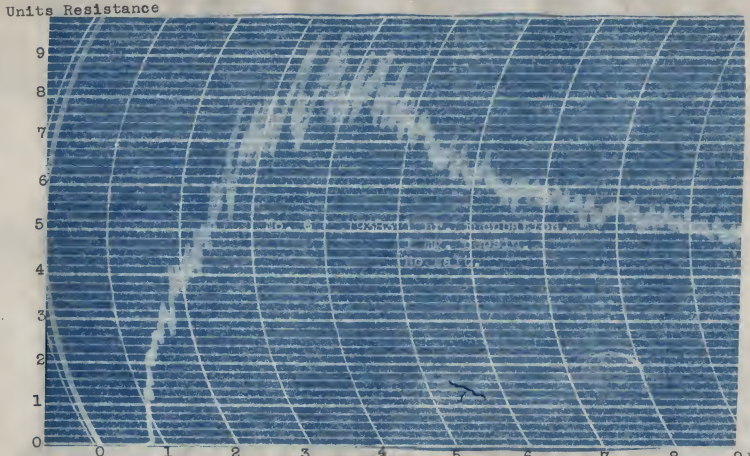

Figure 6. 19383 1 hr. incubation.
1 mg. pepsin.
No salt.

the series the same procedure as in figure 5 was followed, except that 1 mg. pepsin was added to the flour-water immediately before mixing. The period of development of the gluten was not affected as far as the rise in the curve was concerned. But once the maximum rise was obtained, the downward slope was more steep and the oscillations smaller and more uniform, indicating that the enzyme was beginning to have some splitting effect on the gluten. The effect of the proteoclastic enzyme can be better illustrated for comparison in the fermented doughs shown in figures 7 and 8 in which the dough for figure 8 had 1 mg. pepsin. The fermentation with yeast decreased the length of the development period and the small amount of pepsin affected rather visibly the maximum height of the curve.

It should be stated that the addition of the proteoclastic enzyme to the dough in the small quantities mentioned did not affect the general physical appearance of the dough even after two and one half hours fermentation. Neither was the feeling of the dough affected within the limits of observation.

The following series of experiments was made on two flours milled from Southwest wheats. These experiments were as carefully performed as the ones already described.

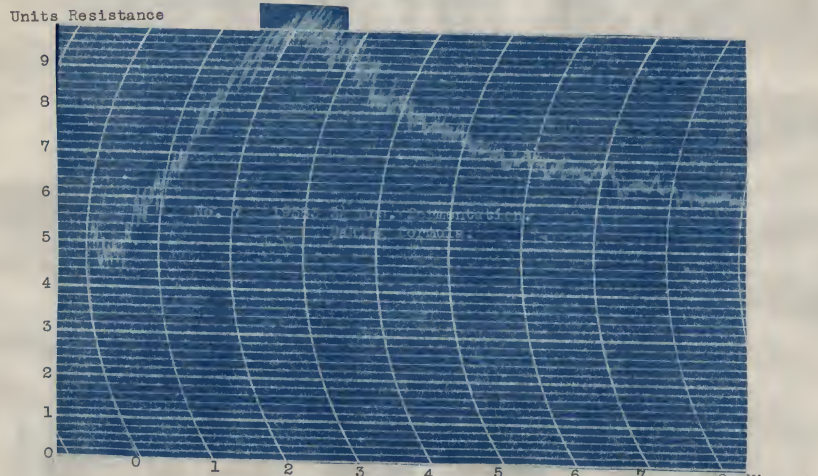

Figure 7. 19383 2½ hrs. fermentation.
Baking formula.

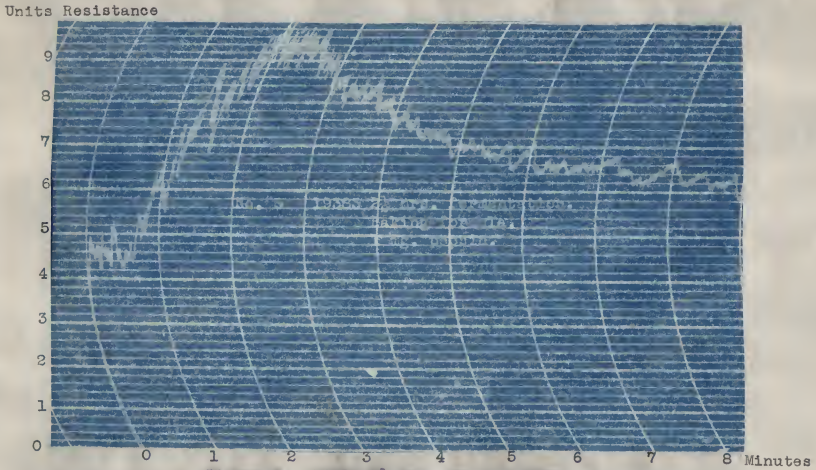

Figure 8. 10393 2½ hrs. fermentation.
Baking formula.
1 mg. papain.

In this series not only was the proteoclastic enzyme pepsin used, but also the enzyme papain (extracted from the tropical fruit Carica papaya), as well as the mixture of enzymes found in germinated wheat, and that found in malt extract. The last two groups of enzymes named were used in larger quantities as stated below. These experiments were strictly uniform, including the amount of flour, which in every case was 250 g.

For comparison two sets of doughs were fermented at 32° for 2 hours. The only difference between these two sets of dough was that one of them contained 1 mg. papain, plus the baking formula already described which was added to both. The maximum height in the curve produced by these two doughs was identical, but while the one with papain broke down as soon as the peak was reached (fig. 10), and its downward resistance produced a practically smooth curve, the one with the yeast alone acted more like the straight flour without anything except water added (fig. 9). One gram of powdered malt extract shows the greatest effect on the curve of this winter wheat flour (fig. 11) of any material tried. The general configuration of the curve for this same flour is still apparent. There was no

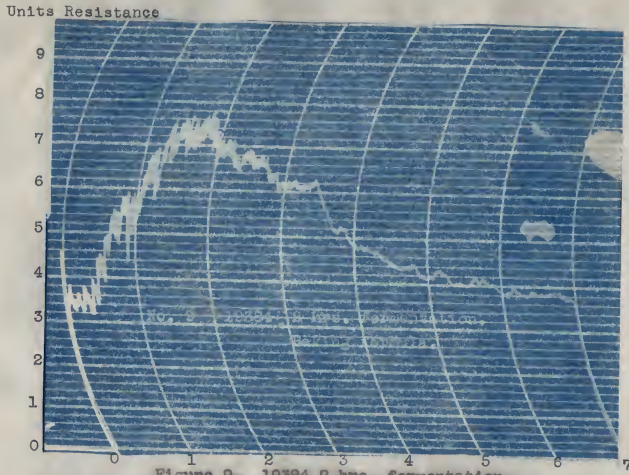

Figure 9. 19384 2 hrs. fermentation.
Baking formula.

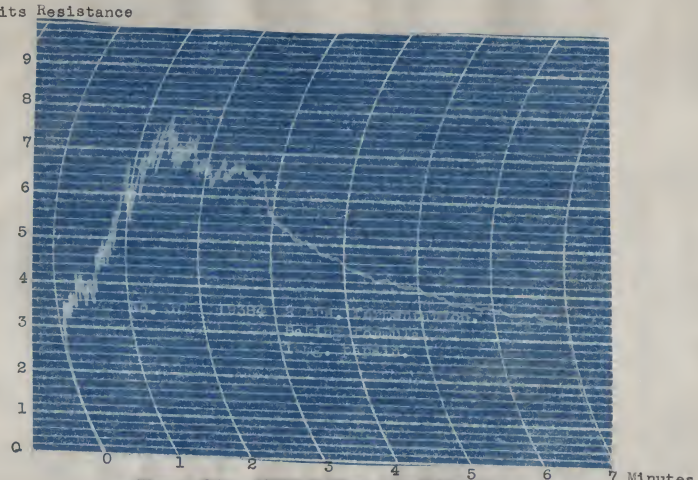

Figure 10. 19384 2 hrs. fermentation.
Baking formula.
1 mg. papain.

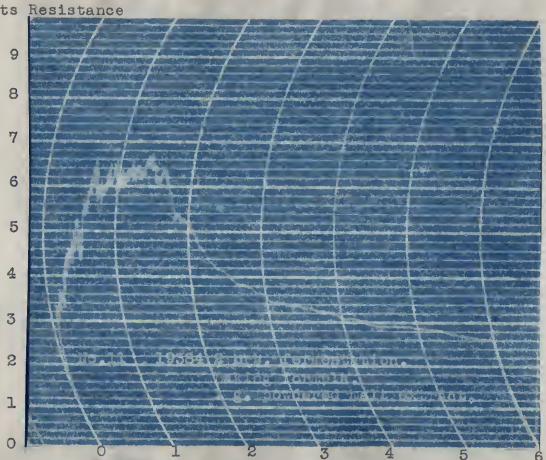

Figure 11. 19384 2 hrs. fermentation.
Baking formula.
1 g. powdered malt extract.

way to compare numerically the amount of enzyme present in the powdered malt extract and the sprouted wheat with that of the commercial papain, except by their action on the dough. It can be seen by figure 13 that the action of yeast does not have much effect in the modification of the dough as far as this machine can record it. Therefore the action in the previous three curves (figs. 10, 11, and 12) must have been due to the proteoclastic enzymes almost entirely, and their concentration can be more or less measured by comparison of the effect produced in the height and oscillations in the curves. Fermentation with yeast, even with the relatively large amounts used, does not have any appreciable effect on the qualities of the dough even after three hours fermentation. Figures 13 and 14 look practically identical even though there was a considerable difference in the length of fermentation under identical conditions.

If the last 10 figures are closely studied, a great similarity in the curves can be immediately observed. Although the last 4, and the previous 6 were from two different flours, and varying amounts of different enzymes were added, the basic similarity is still apparent,

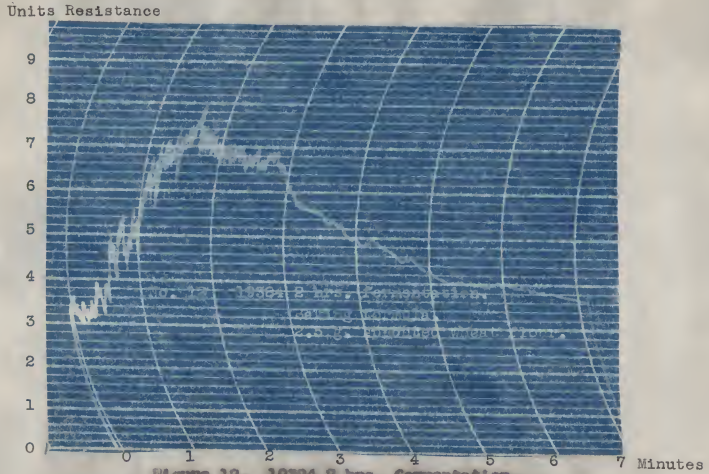

Figure 12. 10304 2 hrs. fermentation.
Baking formula.
2.5 G. sprouted wheat flour.

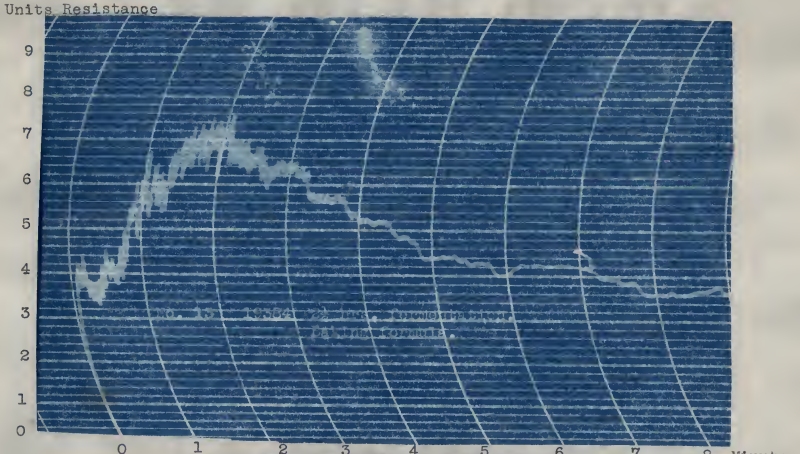

Figure 13. 19304 2½ hrs. fermentation.
Baking formula.

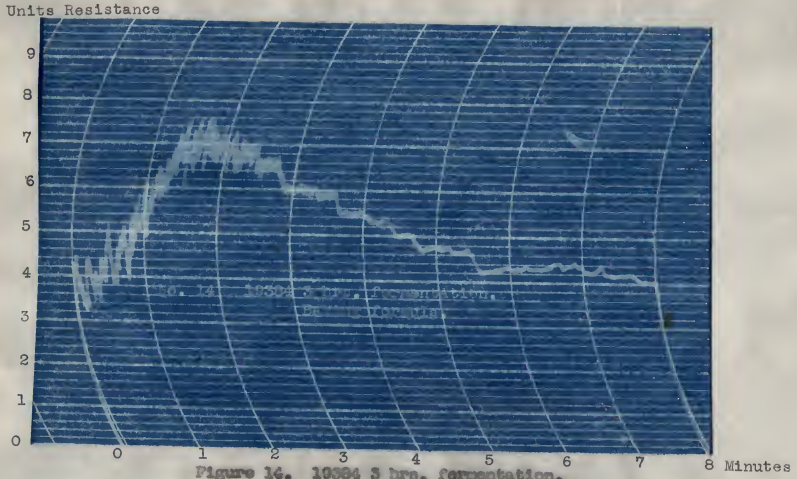

Figure 14. 10384 3 hrs. fermentation.
Baking formula.

and the curves are quite different from those produced by the Northwestern spring wheat flour.

The enzymes used with the flour whose curves are shown in figures 15, 16, 17 and 18 were old. For experimental purposes the quantities added were double the amount of the fresh stock employed elsewhere. Even with this quantity the effect was less than with the fresh enzyme.

Baking Data

In preparing the dough for baking the endeavor was to keep the procedures as uniform as possible. Up to the time of molding the dough, the conditions and handling were identical to those given the dough whose curves were determined in the recording dough mixer. Only two flours were used in the baking experiments. One was the spring wheat flour, and the other the commercial flour milled from Southwest wheat already mentioned, and whose curves have been recorded and described.

The baker's marks given to the bread according to its texture and general appearance are more or less arbitrary, and are based on the operator's experience and judgement.

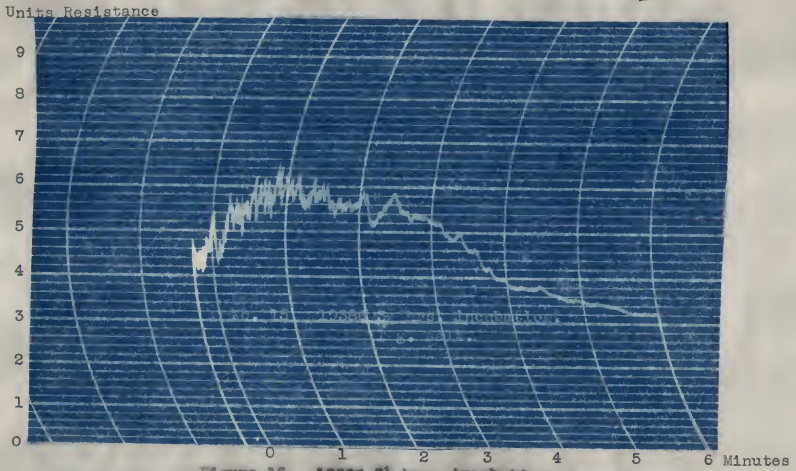

Figure 15. 19386 5½ hrs. incubation.
1 p. salt.

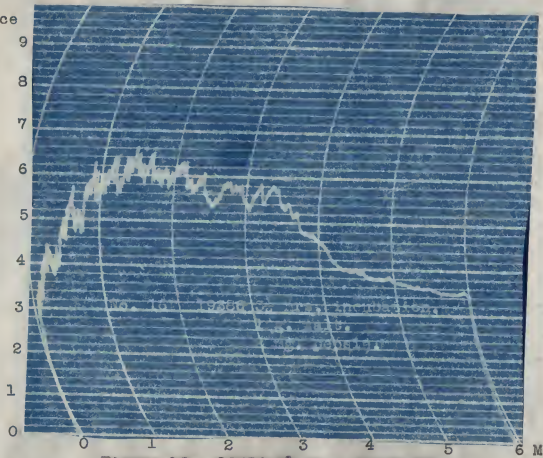

Figure 16. 19306 3½ hrs. incubation.
1 g. salt.
2 mg. pepsin.

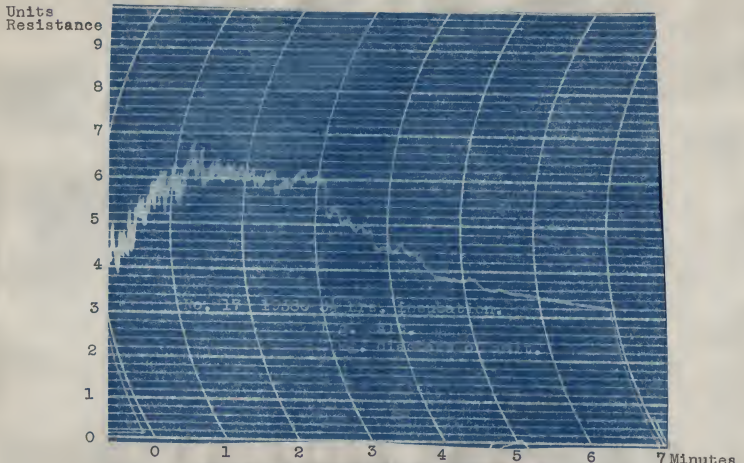

Figure 17. 19386 3½ hrs. incubation.
1 c.c. salt.
2 c.c. diastase of salt.

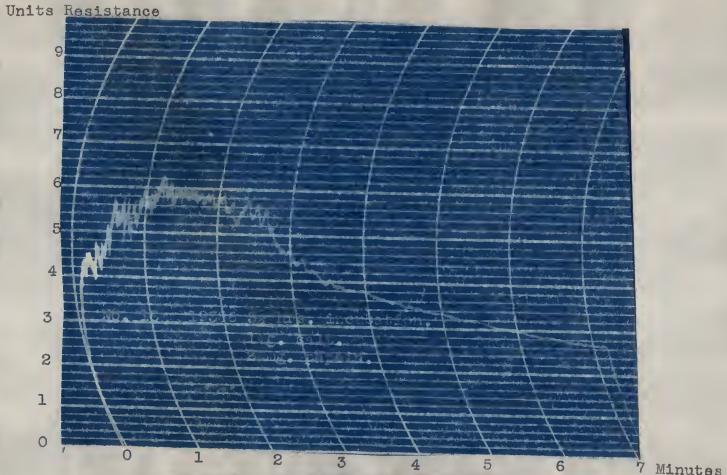

Figure 18. 19386 3½ hrs. incubation.
1 c. salt.
2 mg. pepsin.

The volume of the dough is easily measured since it is fermented in graduated cylinders, but this volume is not a very good indication of what it will be when baked. The baking was done in cylindrical pans used in the Department of Milling Industry,(1) producing very convenient loaves for volumetric measurement by displacement of seeds in a standard can.

The total fermentation in all cases was 2½ hours, and the proofing time about 30 minutes, depending on the rate of rise of the dough. The flour from the Southwest will be designated as A, and that milled from spring wheat as B. The absorption for flour A was 62 per cent, and that for B was 64 per cent. The protein and ash for these flours were: A, 12.8 per cent protein, and .448 per cent ash; and for B, 13.9 per cent protein, and .468 per cent ash. It can be seen that the routine chemical properties of these two flours were very closely alike.

For Experiment No. 1 four loaves of each variety were baked, and the proteoclastic enzyme papain was used in two of them. The data obtained in adding 1 mg. papain to the

(1) Kansas Agric. Exp. Sta. Bulletin 202, p. 13-14.

winter and spring wheat flours is given in Table I. The volume of the bread made from the Southwest flour was increased from an average of 1620 cc. for the controls to an average of 1658 cc. for the loaves having 1 mg. of papain. The texture was also slightly improved. The spring wheat flour was affected by the enzyme decreasing the volume of the loaves. The controls had an average loaf volume of 1760 cc. compared to an average of 1727 cc. for the loaves with 1 mg. papain.

The data obtained in experiment No. 2 in which pepsin was added to half of the loaves baked from winter wheat flour, and papain to the same number of loaves baked from spring wheat flour, is shown in Table II. The addition of 1 mg. pepsin to the Southwestern flour increased the loaf volume from an average of 1448 cc. to 1518 cc., and the texture from 90 to 94 per cent. To the spring wheat flour 1 mg. of papain was added, and this affected the dough to such an extent that two out of three loaves were molded with difficulty, and the third became almost a liquid mass.

The comparative effect of malt extract and papain on both varieties of flour is shown in Table III. In every case the spring wheat flour was affected adversely in texture of the loaf by the addition of enzyme compared to the controls which had no enzyme. The proteoclastic

Table I The effect of adding 1 mg. papain to hard winter and spring wheat flours.

Loaf	No.	Treatment	Dough volume cc.	Loaf volume cc.	Texture per cent
A	1	Control	1700	1610	96
	2	Control	1700	1630	96
	3	1 mg. papain	1700	1650	98
	4	1 mg. papain	1700	1665	98
B	1	Control	1700	1770	98
	2	Control	1700	1750	98
	3	1 mg. papain	1700	1735	98
	4	1 mg. papain	1700	1720	96

Table II Effect of the addition of proteoclastic enzymes to hard winter and spring wheat flours.

Loaf	No.	Treatment	Dough volume cc.	Loaf volume cc.	Texture per cent
A	1	No enzyme	900	1460	94
	2	1 mg. pepsin	1000	1505	94
	3	No enzyme	900	1445	90
	4	No enzyme	900	1436	90
	5	1 mg. pepsin	1000	1525	94
	6	1 mg. pepsin	1100	1525	94
B	1	Control	1400	1720	98
	2	1 mg. papain	1400	1730	98
	3	Control	1800	1775	98
	4	Control	1800	1770	98
	5	1 mg. papain	1100	1120	50
	6	1 mg. papain	1100	--	--

Table III The effect of adding malt extract in comparison with proteoclastic enzyme.

Loaf No.:	Treatment	:Dough volume cc.	:Loaf volume cc.	:Texture per cent
A 1	Control	1400	1670	94
2	Control	1400	1715	94
3	1 g. malt extract	1500	1720	98
4	" " "	1500	1770	98
5	1 mg. papain	1300	1755	97
6	1 mg. papain	1300	1710	95
B 1	Control	1500	1710	98
2	Control	1500	1760	98
3	1 g. malt extract	1500	1710	96
4	1 g. " "	1600	1660	96
5	1 mg. papain	1400	1710	96
6	1 mg. papain	1300	1670	96

enzyme and the powdered malt extract helped both the volume and the texture of the Southwest flours.

SUMMARY

Three different flours were selected for this work. One milled from Northwestern spring wheat, and the other two from Southwestern winter wheats. Proteoclastic enzymes and malt extract were added in varying quantities to test their effect on the flour in two respects: alteration in physical characteristics as shown by the recording dough

mixer, and the influence on baking qualities. A series of experiments was carried out in which the enzymes were added, also yeast, when fermentation was desired. In these experiments the Swanson-Working (1933) dough mixer was employed to determine the curve of the doughs, and see if the addition of enzymes to the Southwestern flour would tend to make its curves similar to that of the spring wheat flour.

ACKNOWLEDGMENTS

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